This electronic thesis or dissertation has been downloaded from the King's Research Portal at https://kclpure.kcl.ac.uk/portal/

KING'S College LONDON

# Investigation of copper and cadmium in the food chain of three-spined stickleback populations, Gasterosteus aculeatus L., in the River Wandle, U.K

M-HChen, Unknown

**The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without proper acknowledgement.**

END USER LICENCE AGREEMENT

**Unless another licence is stated on the immediately following page** this work is licensed

under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International

licence. https://creativecommons.org/licenses/by-nc-nd/4.0/

You are free to copy, distribute and transmit the work

Under the following conditions:

- Attribution: You must attribute the work in the manner specified by the author (but not in any way that suggests that they endorse you or your use of the work).
- Non Commercial: You may not use this work for commercial purposes.
- No Derivative Works - You may not alter, transform, or build upon this work.

Any of these conditions can be waived if you receive permission from the author. Your fair dealings and other rights are in no way affected by the above.

**Take down policy**

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 20. Oct. 2023

# INVESTIGATION OF COPPER AND CADMIUM IN THE FOOD CHAIN OF THREE-SPINED STICKLEBACK POPULATIONS, *Gasterosteus aculeatus* L., IN THE RIVER WANDLE, UK

by

Meng-Hsien CHEN

Thesis summitted to King's College London (University of London) in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

August 1992

King's College London (KQC)
University of London
Ecotoxicology and Environmental Health Group
Division of Life Sciences
Kensington Campus
Campden Hill Road
London W8 7AH
United Kingdom

BIBL. LONDIN. UNIV.

# ABSTRACT

This investigation examined Cu and Cd accumulation in field populations of 3-spined sticklebacks, *Gasterosteus aculeatus*. The metal content of the fish was studied in relation to growth, degree of metal exposure and infection by the cestode, *Schistocephalus solidus*. A further objective was to determine the route and rate of supply of metals to the sticklebacks.

From an initial survey three sites were identified in the River Wandle, a tributary of the River Thames, with different sediment loadings of Cu and Cd. Then between July 1989 and April 1990, over 600 non-parasitised and parasitised sticklebacks were collected in four seasons from the three sites. Whole fish (excluding gut contents) were analysed for Cu and Cd, and large sticklebacks were examined for the distribution of metals in the different organs. Copper and cadmium levels were measured in the water, sediments, organic detritus and a range of plants and invertebrates from the sites. Length-frequencies were used to age fish, and their diet was determined from gut content analyses.

All samples showed the same trend between sites, with relatively low metal concentrations at the reference site and elevated values at two contaminated sites. Metal levels in water were very low, but raised in solid phases (sediments, organic detritus, and biota). Strong evidence points to a significant dietary uptake of Cu and Cd as well as uptake from water by sticklebacks. The liver was the main organ of accumulation. Biomagnification was unlikely, since sticklebacks gave some of the lowest metal concentrations measured.

Significant regression models established that body burdens of Cu and Cd accumulated with the age of fish but no such relationships were found between concentration and age. Body burdens were more informative than concentrations for comparing metal levels in different groups of fish. Regression models relating metal body burden and size differed between non-infected and infected sticklebacks, explainable by diversion of part of the burden to the parasite.

This is one of few studies that have shown increased body burdens and concentrations of metal in fish and other organisms in a contaminated hard-water system. If, as indicated by this work, diet is an important route for Cu and Cd entry into fish, then water concentrations for regulatory purposes are of little value. Serious consideration should be given to establishing dose-response relationships based on metal body burdens. This would provide a valuable means of relating laboratory and field data.

## ACKNOWLEDGMENTS

I am very grateful to my supervisors, Dr. Roland G. Bailey, Dr. Steve Smith, and Dr. W. Peter Williams for help throughout this study. Dr. Bailey always generously helped in many ecological aspects such as field survey and analysis of stomach contents, and also in correction of my English writing. Dr. Smith helped me with many aspects of the analytical work and always gave much time particularly with his critical discussion and encouragement and in the writing this thesis. I am indebted to Dr. W. Peter Williams for his support, friendship and the opportunity given to me to accomplish my academic study in this division.

My sincere appreciation to my husband Chiee-young who helps me in many ways in my research including as a technician for my field work, helping with computation, and acting as body guard for my late working in college. Without his help and company for these years this research would not have been fruitful.

I will like to express my appreciation to members in our group who helped me in many ways in my work and in particular Dr. M.K. Kwan and Mr. Tony Cullen.

I am also thankful for the financial support from Ministry of Education, Taiwan and job security of my post in the Department of Marine Resources, National Sun Yet-Sen University, Kaohsiung, Taiwan for these years.

Finally, I am grateful for the support and encouragement from my parents and my parents-in-law.

# TABLE OF CONTENTS


*CONTENTS* *page no.*

**TITLE PAGE** ---------- **i**

**ABSTRACT** ---------- **ii**

**ACKNOWLEDGEMENTS** ---------- **iii**

**TABLE OF CONTENTS** ---------- **iv**

**LIST OF TABLES** ---------- **x**

**LIST OF FIGURES** ---------- **xv**

**LIST OF PLATES** ---------- **xx**

**1** INTRODUCTION

**1.1 Heavy Metals in Aquatic Environment** ---------- **1**

**1.2 The Effects of Pollution to Three-spined Stickleback** ---------- **5**

**1.3 Aims and Objectives** ---------- **8**

**2** DEVELOPMENT OF METHODS TO DETERMINE COPPER AND CADMIUM IN SAMPLES OF FISH, INVERTEBRATES, PLANTS, SEDIMENTS, AND WATERS

**2.1 INTRODUCTION** ---------- **10**

**2.2 METAL ANALYSIS TECHNIQUE** ---------- **12**

2.2.1 Procedures to Minimise Metal Contamination and Loss During Handling and Preparation of Samples ---------- 12

2.2.2 Methods for Decomposition of Samples ---------- 13

2.2.3 Optimisation of AAS Instruments ---------- 16

**2.3 DEVELOPMENT OF GRAPHITE FURNACE PROGRAMMES** ---------- **19**

2.3.1 Design of Graphite Furnace Programmes ---------- 19

2.3.2 Development of Graphite Furnace Programme for Copper ---------- 21

2.3.3 Development of Graphite Furnace Programme for Cadmium ---------- 25

**2.4 DISCUSSION** ---------- **36**

**3 PILOT STUDY AND SAMPLING SCHEME**

**3.1 THE RIVER WANDLE AND ITS FISHES** ---------- **41**

**3.2 A PILOT STUDY** ---------- **42**

3.2.1 Introduction ---------- 42

3.2.2 The Results ---------- 49

**3.3 SAMPLING SCHEME AND PROTOCOL** ---------- **55**

**4 EVALUATION OF THE RELATIONSHIPS BETWEEN GROWTH, *SCHISTOCEPHALUS* INFECTION, AND COPPER AND CADMIUM IN THREE-SPINED STICKLEBACK POPULATIONS IN THE RIVER WANDLE**

**4.1 INTRODUCTION** ---------- **65**

4.1.1 Heavy Metal Accumulation in Fishes ---------- 65

4.1.2 The Infection of *Schitocephalus solidus* in the 3-spined Stickleback ---------- 67

**4.2 MATERIALS AND METHODS ---------- 68**

4.2.1 Fish Sampling and Handling ---------- 68

4.2.2 Estimation of the Degree of *Schistocephalus solidus* infection in the Stickleback Populations ---------- 68

4.2.3 Determination of Age of Age Structure of Stickleback Populations ---------- 69

4.2.4 Estimation of the Growth of Wandle Sticklebacks ---------- 72

4.2.5 Determination of Copper and Cadmium in the Fish and Their Cestode Parasites ---------- 72

**4.3 RESULTS ---------- 73**

4,3.1 The Infestation of *Schistocephalus solidus* in Wandle Sticklebacks ---------- 73

4.3.2 Age Groups of the Sticklebacks at Each Site ---------- 77

4.3.3 The Frequency Distributions of Copper and Cadmium in the Stickleback Population at the Each Site ---------- 84

4.3.4 Fresh Weight-Dry Weight and Length-Weight Relationships and Growth of Stickleback Populations ---------- 84

4.3.5 Copper and Cadmium in Sticklebacks and the Cestode Parasites in Each Site ---------- 94

4.3.6 Copper and Cadmium in the Sticklebacks and the Cestode Parasites and Their Relationships to the Size of the Organisms ---------- 98

4.3.7 Evaluation of the Metal Body Burden-Fish Dry Weight Relationships Between (A) Sites, (B) Age, and (C) Seasonal Surveys ---------- 111

4.3.8 Evaluation of the Concentrations of Cu and Cd in Sticklebacks at the Three Sites ---------- 120

**4.4 DISCUSSION ---------- 127**

4.4.1 The Incidence and Intensity of *Schistocephalus solidus* in the Wandle Sticklebacks ---------- 127

4.4.2 The Age Composition and Growth of the Stickleback ---------- 128

4.4.3 Copper and Cadmium in the Sticklebacks ---------- 130

4.4.4 Assessment of the Influence of Infection by *Schistocephalus solidus* on the Cu and Cd Contents of the Sticklebacks ---------- 134

**5 THE DIET OF THREE-SPINED STICKLEBACK IN THE RIVER WANDLE**

**5.1 INTRODUCTION ---------- 137**

**5.2 MATERIALS AND METHODS ---------- 138**

5.2.1 Fish Sampling and Handling ---------- 138

5.2.2 Methods for Diet Analysis ---------- 139

5.2.3 The Diet Analysis of 3-spined Stickleback ---------- 139

**5.3 RESULTS ---------- 141**

5.3.1 The Diet and Feeding of Three-spined Stickleback in the River Wandle ---------- 141

5.3.2 Comparison of Diet Between Sites ---------- 141

5.3.3 Seasonal Variations in the diet Within Sites ---------- 151

5.3.4 Comparison of the Diet in Different Size Classes at Each Site ---------- 153

5.3.5 Comparison of the Diet Between Males and Females ---------- 155

5.3.6 Comparison of the Diet Between Non-parasitised and Parasitised Fish ---------- 155

**5.4 DISCUSSION ---------- 155**

**6 DISTRIBUTION OF COPPER AND CADMIUM IN THE ABIOTIC AND BIOTIC COMPONENTS IN THE RIVER WANDLE**

**6.1 INTRODUCTION ---------- 160**

**6.2 MATERIALS AND METHODS ---------- 162**

**6.3 RESULTS ---------- 163**

6.3.1 Copper and Cadmium Concentrations in Water, Organic Detritus (Floc) and Sediment ---------- 163

6.3.2 Copper and Cadmium Concentrations in the Aquatic Organisms ---------- 167

**6.4 DISCUSSION ---------- 175**

6.4.1 Relative Distribution of Cu and Cd in Abiotic and Biotic Components of the Habitat of Stickleback Populations ---------- 175

6.4.2 Cu and Cd in the Waters ---------- 178

6.4.3 Cu and Cd in the Sediments and Organic Detritus (Floc) ---------- 179

6.4.4 Cu and Cd in the Aquatic Plants and Algae ---------- 180

6.4.5 Cu and Cd in the Aquatic Invertebrates ---------- 182

6.4.6 Assessment of Metal Exposure in the Diet of Sticklebacks ---------- 182

**7 COPPER AND CADMIUM DISTRIBUTION IN THE ORGANS OF THE 3-SPINED STICKLEBACK**

**7.1 INTRODUCTION ---------- 186**

7.1.1 The Distribution of Metals Within the Fish Body ---------- 186

7.1.2 Metal Binding-Proteins in Fish ---------- 189

**7.2 MATERIALS AND METHODS ---------- 191**

7.2.1 Investigation of Cu and Cd Distribution in the Stickleback ---------- 191

7.2.2 Investigation of Metal Binding Proteins in Liver of Sticklebacks ---------- 191

**7.3 RESULTS ---------- 192**

7.3.1 The Cu and Cd Distribution in the Organs of the Stickleback ---------- 192

7.3.2 Metal Binding Protein in the Liver of Stickleback from High-Metal Content Sites in River Wandle ---------- 196

**7.4 DISCUSSION ---------- 201**

8 A SUMMARY OF DISCUSSIONS AND CONCLUSION

**8.1 Development of Methods for Determining Cu and Cd ---------- 204**

**8.2 General Consideration of the Sampling Sites and the Stickleback Populations ---------- 205**

**8.3 Copper and Cadmium in Stickleback ---------- 207**

**8.4 Influence of Parasite Infection on the Cu and Cd Contents of Sticklebacks ---------- 210**

**8.5 Evaluation of Cu and Cd Exposure to Stickleback Populations ---------- 212**

**REFERENCES ---------- 219**

**APPENDICES ---------- 238**

# LIST OF TABLES

**Table 2.1** The results of validation by various standard materials. (The original data are listed in Appendix A.5). ---------- **40**

**Table 3.1** Major fish species observed in the River Wandle between Jan. 1988 and Jul. 1989. ---------- **50**

**Table 3.2** The frequency of the different morphs at each sampling sites in the River Wandle from Jan. 1988 to Oct. 1988. ---------- **52**

**Table 3.3** The incidence of infestation by the plerocercoid stage of *Schistocephaus solidus* in the 3-spined stickleback from the River Wandle from Jan. 1988 to Oct. 1988. ---------- **54**

**Table 3.4** Sex ratio and male percentage at each sampling sites in the River Wandle from Jan. 1988 to Oct. 1988. Only the fish that total length larger than 25 mm were examined. ---------- **54**

**Table 4.1** An example of length-frequency data used to construct a Cassie curve and to identify the different age groups of sticklebacks at site E in 21st July 1989. ---------- **70**

**Table 4.2** Summary of the infestation of *Schistocephalus solidus* in the parasitised stickleback at the three sampling site in the River Wandle. (and for significance abbreviations). ---------- **74**

**Table 4.3** Cassie curve estimates of the number (No.), percentage of total (%), mean and standard deviation (S.D.) for different age groups of non-parasitised and parasitised sticklebacks. ---------- **78**

**Table 4.4** Results of the Chi-square tests of the various parameters on the fitness of normal and lognormal distributions of the $0^+$ age groups of sticklebacks at the three sites in the River Wandle. --- **85**

**Table 4.5** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total length and dry-weight in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.), as well as t-tests on the parasitic effects. ---------- **86**

**Table 4.6** Results of linear regressions between dry body weights and fresh body weights, and the linearity tests on the non-parasitised and parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle. ---------- **87**

**Table 4.7** The ANOCOVA tables of site effects on the relationships between the dry weights and fresh weights in non-parasitised and parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle. ---------- **87**

**Table 4.8** The ANOCOVA tables of the effects of age on the relationships between logarithmic dry weights and the logarithmic total lengths at the each sites and between sites in the non-parasitised sticklebacks. ---------- **88**

**Table 4.9** The ANOCOVA tables of the effects of age on the relationships between logarithmic dry weights and the logarithmic total lengths at the each sites and between sites in the parasitised sticklebacks. ---------- **91**

**Table 4.10** The ANOCOVA tables of the effects of parasitism on the relationships between logarithmic dry weights and the logarithmic total lengths between the non-parasitised and the parasitised sticklebacks. ---------- **91**

**Table 4.11** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.int.), as well as t-tests on the parasitic effects. ---------- **96**

**Table 4.12** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in cestode parasites of stickleback at the three sites in the River Wandle, with the arithmetic means (AM), their standard deviations and 95% confidence limits (C. Int.). ------- **97**

**Table 4.13** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- **101**

**Table 4.14** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- **102**

**Table 4.15** Results of linear regressions between logarithmic concentrations of copper (logFSCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- **105**

**Table 4.16** Results of linear regressions between logarithmic concentrations of cadmium (logFTCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- **106**

**Table 4.17** Results of linear regressions between total copper burdens (PTCu) or total cadmium burdens (PTCd) and total lengths (PTL) or dry weights (PDW), and the linearity tests for cestode parasites in parasitised sticklebacks at the three sites in the River Wandle. ---------- **109**

**Table 4.18** Results of linear regressions between concentrations of copper (PSCu) or cadmium (PSCd) and total lengths (PTL) or dry weights (PDW), and the linearity tests for cestode parasites in parasitised sticklebacks at the three sites in the River Wandle.---**110**

**Table 4.19** The ANOCOVA tables of the site differences on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weights (logFDW) in non-parasitised and parasitised sticklebacks, and between total burdens of Cu (PCu) or Cd (PCd) and dry weight (PDW) in the cestode parasites at the three sites in the River Wandle. ---------- **112**

**Table 4.20** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic dry weights (logFDW), and the linearity tests in the different age groups of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.---**114**

**Table 4.21** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic dry weights (logFDW), and the linearity tests in the different age groups of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- **115**

**Table 4.22** The ANOCOVA tables of the age effects on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weight (logFDW) in the non-parasitised sticklebacks at the three sites in the River Wandle. ---------- **116**

**Table 4.23** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic dry weights (logFDW), and the linearity tests in the seasonal batches of $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle.--**117**

**Table 4.24** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic dry weights (logFDW), and the linearity tests in the seasonal batches of $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle. ---------- **118**

**Table 4.25** The ANOCOVA tables of the seasonal effects on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weight (logFDW) in the $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle. ---------- **119**

**Table 4.26** Assessment of the differences between age groups by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.), as well as t-tests on the parasitic effects. ---------- **121**

**Table 4.27** Assessment of the differences of the effects of host's age by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in cestode parasites at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.). ---------- **123**

**Table 4.28** Assessment of the differences between different seasonal batches by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in the $0^+$ age groups of non-parasitised at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.). ---------- **125**

**Table 4.29** Summary the age structure and the mean length of age groups in various populations of *Gasterosteus aculeatus*. ---- **129**

**Table 4.30** Comparison of the body burdens of Cu and Cd of non-parasitised and parasitised sticklebacks adjusted to a standard size of 40 mm TL, at each site. ---------- **135**

**Table 5.1** The percentage relative abundance (P%) and frequency of occurrence (0%) of different foods in stomachs of the 3-spined sticklebacks at the three sampling sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **142**

**Table 5.2** The percentage relative abundance of different foods in stomachs of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **143**

**Table 5.3** The percentage occurrence of different foods in stomachs of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **144**

**Table 5.4** The percentage relative abundance of different foods in the 5 size classes (mm TL) of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. **145**

**Table 5.5** The percentage occurrence of different foods in the 5 size classes (mm TL) of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **146**

**Table 5.6** The percentage relative abundance (P%) and occurrence (O%) of different foods in male and female 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **147**

**Table 5.7** The percentage relative abundance (P%) and occurrence (O%) of different foods in non-parasitised (N-PAR) and parasitised (PAR) 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ---------- **148**

**Table 5.8** Summary the percentage of diet composition in stomachs of 3-spined sticklebacks in various literature. ---------- **158**

**Table 6.1** Concentrations of Cu and Cd in samples of water taken during each season (1989-1990) at the three sampling sites along the River Wandle. ---------- **164**

**Table 6.2** Concentrations of Cu and Cd in samples of organic detritus (floc) and sediment taken during each season (1989-1990) at the three sampling sites along the River Wandle. ---------- **165**

**Table 6.3** Concentrations of Cu and Cd in samples of algae and aquatic plants taken during each season (1989-1990) at the three sampling sites along the River Wandle. ---------- **168**

**Table 6.4** Concentrations of Cu and Cd in samples of aquatic invertebrates taken during each season (1989-1990) at the three sampling sites along the River Wandle. ---------- **170**

**Table 6.5** Summary of mean concentrations of Cu and Cd in the various types of aquatic plants collected from contaminated site (Site E). ---------- **173**

**Table 6.6** Summary of mean concentrations of Cu and Cd in the various types of aquatic invertebrates collected from contaminated site (Site E). ---------- **174**

**Table 6.7** Concentrations of Cu and Cd (ug/g dw) in the major food items of the stickleback populations and in the fish from the River Wandle, P% = percentage relative abundance of food item in stomach of stickleback (see Chapter 5). ---------- **184**

**Table 7.1** Summary of published literature showing the influence of the route of exposure of Cu and Cd on the distribution of the metals in the tissues of fish. ---------- **187**

**Table 7.2** Comparison of Cu and Cd distribution in tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- **194**

**Table 7.3** Comparison of Cu and Cd concentrations in the same tissue of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- **195**

**Table 7.4** Comparison of percentages of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- **197**

# LIST OF FIGURES

**Figure 2.1** Copper calibration curve by flame atomic absorption spectrophotometry. ---------- **18**

**Figure 2.2** Cadmium calibration curve by flame atomic absorption spectrophotometry. ---------- **18**

**Figure 2.3** A standard curve for copper by electrothermal atomisation. ---------- **22**

**Figure 2.4** Typical standard curve for copper by electrothermal atomisation. ---------- **22**

**Figure 2.5** Comparison of Cu absorbance values with different drying times. ---------- **23**

**Figure 2.6** Comparison of Cu absorbance values with different ashing temperatures. ---------- **24**

**Figure 2.7** Comparison of Cu absorbance values for various types of samples with different ashing times at 900$^{o}$C of ashing temperature. ---------- **26**

**Figure 2.8** Comparison of Cu absorbance values for various types of samples with different atomisation temperatures. ---------- **27**

**Figure 2.9** Standard addition curves of various types of samples in the copper graphite furnace programme. ---------- **28**

**Figure 2.10** Typical standard curves for cadmium by electrothermal atomisation. ---------- **29**

**Figure 2.11** Comparison of Cd absorbance values with different ashing times at 300$^{o}$C of ashing temperature. ---------- **30**

**Figure 2.12** Comparison of Cd absorbance values for various types of samples with different ashing temperatures. ---------- **31**

**Figure 2.13** Comparison of Cd absorbance values for various types of samples with different ashing times at 400$^{o}$C of ashing temperature. ---------- **33**

**Figure 2.14** Comparison of Cd absorbance values for various types of samples with different atomisation temperatures. ---------- **34**

**Figure 2.15** Standard addition curves of various types of samples in the cadmium graphite furnace programme without chemical modifier. ---------- **35**

**Figure 2.16** Comparison of Cd absorbance values with 0.25% $H_3PO_4$ as chemical modifier at different ashing temperature settings. --- **37**

**Figure 2.17** Comparison of Cd absorbance values with 0.25% $H_3PO_4$ as chemical modifier at different atomisation temperatuers. ----- **37**

**Figure 2.18** Standard addition curves of various types of samples in the cadmium graphite furnace programme with 0.25% $H_3PO_4$ as chemical modifier. ---------- **38**

**Figure 3.1** A map shows the 11 sampling sites in the River Wandle. ---------- **43**

**Figure 3.2** Day-time water temperature, sampled on a monthly basis from December 1987 to July 1989. ---------- **51**

**Figure 3.3** The length frequency distribution of 3-spined sticklebacks on each sampling occasion at the various sampling sites in the River Wandle from Jan. 1988 to Dec. 1988. ---------- **53**

**Figure 3.4** Concentrations of copper in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **56**

**Figure 3.5** Concentrations of cadmium in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **56**

**Figure 3.6** Concentrations of chromium in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **57**

**Figure 3.7** Concentrations of nickel in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **57**

**Figure 3.8** Concentrations of lead in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **58**

**Figure 3.9** Concentrations of zinc in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. ---------- **58**

**Figure 3.10** Concentrations of total copper in samples of water taken from the River Wandle at various sites on 12th Dec. 1988 (left), 8th Jun. (middle), and 6th Jul. (right), 1989. ---------- **59**

**Figure 3.11** Concentrations of dissolved copper in samples of water taken from the River Wandle at various sites on 6th Jul. 1989.--- **59**

**Figure 3.12** Concentrations of copper in samples of 3-spined stickleback taken from the River Wandle at various sites (marked B-I) and from the River Thames (TH). ---------- **60**

**Figure 3.13** Concentrations of chromium in samples of 3-spined stickleback taken from the River Wandle at various sites. ----- **60**

**Figure 3.14** Concentrations of lead in samples of 3-spined stickleback taken from the River Wandle at various sites. ----- **61**

**Figure 3.15** Concentrations of zinc in samples of 3-spined stickleback taken from the River Wandle at various sites. ----- **61**

**Figure 4.1** An example of a Cassie curve using length-frequency data from sticklebacks at site E in 21st July 1989. ---------- **71**

**Figure 4.2** The relationship between the dry weight of parasites and the dry weight, or the total length of the host fish at the three sampling sites (A, B, & E) in the River Wandle. ---------- **75**

**Figure 4.3** The relationship between the parasite index and the dry weight, or the total length of the host fish at the three sampling sites (A, B, & E) in the River Wandle. ---------- **76**

**Figure 4.4** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site A in the River Wandle. ---------- **79**

**Figure 4.5** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site B in the River Wandle. ---------- **80**

**Figure 4.6** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site E in the River Wandle. ---------- **81**

**Figure 4.7** The relative percentage of different age groups in non-parasitised (NP) and parasitised (P) sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **82**

**Figure 4.8** The relative percentage of different age groups in four seasonal samples (Jul., Oct., Jan., & Apr.) at the three sites (A, B, & E) in the River Wandle. ---------- **83**

**Figure 4.9** The relationship of dry weight (DW) and total length (TL) in non-parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **89**

**Figure 4.10** The relationship of dry weight (DW) and total length (TL) in parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **92**

**Figure 4.11** A comparison of growth rates by length and dry-weight of non-parasitised (NP) and parasitised (P) at tri-monthly intervals for the $0^+$ age group (left-hand side) and 12 month intervals for older fish (right-hand site) ate the three sites (A, B, & E) in the River Wandle. ---------- **93**

**Figure 4.12** Comparisons of body burdens of Cu and Cd, and concentrations of Cu and Cd of non-parasitised, parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle. Histograms present the means, and + indicate the standard deviations. ---------- **95**

**Figure 4.13** The relationships between log total copper burdens and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. -- **99**

**Figure 4.14** The relationships between log total cadmium burdens and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **100**

**Figure 4.15** The relationships between log total copper concentrations and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **103**

**Figure 4.16** The relationships between log total cadmium concentrations and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **104**

**Figure 4.17** The relationships between log total burdens of Cu or Cd, and log total lengths, or log dry weights in cestode parasites in the parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **107**

**Figure 4.18** The relationships between log concentrations of Cu or Cd, and log total lengths, or log dry weights in cestode parasites in parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. ---------- **108**

**Figure 5.1** Comparison of the percentage relative abundances of individual food items in the stomachs of 3-spined sticklebacks at the three sites (A, B and E) in the River Wandle. ---------- **149**

**Figure 5.2** Seasonal changes in the percentage relative abundances of major food groups in the stomachs of 3-spined sticklebacks at the three site (A, B and E) in the River Wandle. ---------- **152**

**Figure 5.3** Comparison of the percentage relative abundance of major food items in the stomachs of 3-spined sticklebacks according to different size classes and sites (A, B and E). ---------- **154**

**Figure 5.4** Comparison of the percentage relative abundances of major food items in the stomachs of female and male sticklebacks at the three sites (A, B and E) in the River Wandle. ---------- **156**

**Figure 5.5** Comparison of the percentage relative abundances of major food items in the stomachs of non-parasitised (N-PAR) and parasitised (PAR) sticklebacks at the three sites (A, B and E) in the River Wandle. ---------- **156**

**Figure 6.1** Concentrations of Cu and Cd in samples of water, organic detritus (floc) and sediment at the three sites in the River Wandle. ---------- **166**

**Figure 6.2** Copper and cadmium concentrations in *Spirogyra* sp., *Lymnaea peregra* and *Asellus aquaticus* at the three sites in the River Wandle. ---------- **173**

**Figure 6.3** Copper and cadmium concentrations at the different trophic levels in the River Wandle. ---------- **175**

**Figure 6.4** The distributions of copper and cadmium in biota, water and sediment phases at the three sites (A, B and E) in the River Wandle. ---------- **176**

**Figure 7.1** Distribution of copper concentrations in the tissues of three groups of sticklebacks; (1) left bar: low-metal non-parasitised fish, (2) middle bar: high-metal non-parasitised fish, (3) right bar: high-metal parasitised fish. ---------- **193**

**Figure 7.2** Distribution of cadmium concentrations in the tissues of three groups of sticklebacks; (1) left bar: low-metal non-parasitised fish, (2) middle bar: high-metal non-parasitised fish, (3) right bar: high-metal parasitised fish. ---------- **193**

**Figure 7.3** Percentage of the total amount of copper in the tissues of three groups of sticklebacks; low-metal non-parasitised fish, high-metal non-parasitised fish, and high-metal parasitised fish. ---- **198**

**Figure 7.4** Percentage of the total amount of cadmium in the tissues of three groups of sticklebacks; low-metal non-parasitised fish, high-metal non-parasitised fish, and high-metal parasitised fish. ---- **199**

**Figure 7.5** Gel filtration elution profiles (Sephadex G-75) of buffer extracts of the liver of sticklebacks from the metal contaminated sites. (Vo: void volume is 125 ml, Vt: bed volume is 440 ml). ---------- **200**

**Figure 7.6** Calibration curve of gel filtration column (Sephadex G-75), Kav = (Ve-Vo)/(Vt-Vo). ---------- **200**

**Figure 8.1** Copper distribution in a urban river system. Values represent in sequence order as sites A, B, and E in the River Wandle. ---------- **214**

**Figure 8.2** Cadmium distribution in a urban river system. Values represent in sequence order as sites A, B, and E in the River Wandle. ---------- **215**

# LIST OF PLATES

**Plate 3.1** A view of sampling site A, Waddon pond, in the River Wandle. ---------- **44**

**Plate 3.2** A view of sampling site $A^+$, at Croydon Arm just below Waddon pond at Aldwich Rd., in the River Wandle. ---------- **44**

**Plate 3.3** A view of sampling site B, at Croydon Arm beside Church Rd. at Beddington Park, in the River Wandle. ---------- **45**

**Plate 3.4** A view of sampling site C, at Croydon Arm beside London Rd. outside Manor Garden, in the River Wandle. ---------- **45**

**Plate 3.5** A view of sampling site D, at Carshalton Arm below Wandle Rail Bridge at Mill lane, in the River Wandle. ---------- **46**

**Plate 3.6** A view of sampling site E, at the junction of two Arms beside Hack Bridge, in the River Wandle. ---------- **46**

**Plate 3.7** A view of sampling site F, Beddington sewage effluent channel at Goat Road. ---------- **47**

**Plate 3.8** A view of sampling site G, below the junction of Beddington sewage effluent channel to the River Wandle. ---------- **47**

**Plate 3.9** A view of sampling site H, a section of the River Wandle beside Morden Cottage at Morden Hall Park. ---------- **48**

**Plate 3.10** A view of sampling site I, a section of the River Wandle beside Steerforth street at Summertown. ---------- **48**

**Plate 3.11** A view of sampling site J, a section of the River Wandle above the mouth of the river to The Thames at Wandsworth. -- **49**

**Plate 3.12** Showing two types of basket samplers (Plate A and B) used in the study, and the settled community after more than 3 weeks planting (Plate C). ---------- **64**

# CHAPTER ONE

# GENERAL INTRODUCTION

## 1.1 Heavy Metals in Aquatic Environment

The discharge of materials into rivers, streams and lakes, is often used as a way of disposing of by-products and wastes. Ideally, the dilution effect and the self-purification ability of water reduce concentrations so that wastes do not harm the environment. The toxic effects of many contaminants are reduced through the biological degradation processes. However, there is a limit to the capacity of rivers to absorb wastes. Moreover, among the pollutants there are two factors which make the deleterious effects of heavy metals as environmental pollutants significant: heavy metals cannot be destroyed through biological degradation; and heavy metals tend to accumulate in the environment.

Realistically, in the industrialised world it is inevitable that there will be a continuous discharge of industrial, domestic and agricultural wastes into rivers, lakes, and estuaries. Accordingly, a number of questions can be raised. How much waste can a water body absorb ? What sort of water quality must be maintained if the water body is needed for purposes such as potable water, irrigation, or conservation of aquatic life ? To what extent must toxic contaminants in the wastes be removed in treatment processes ?, and in this connection how do toxic substances behave in a aquatic ecosystem ? These questions have exhausted hydroengineers, biologists, toxicologists, and environmental policy administrators over the past 3 decades.

The detrimental effects of heavy metals in the aquatic environment have attracted much scientific attention (Hynes 1960, Hartung 1973, Phillips 1977, Forstner & Prosi 1979, Coombs 1980, Sheehan et al. 1984, Leland & Kuwabara 1985, Hellawell 1986, Mance 1987, Clarkson et al. 1988, Evans 1988). Numerous experiments have been done on the toxic effects of heavy metals to aquatic organisms in many laboratories, in order to build up a data base enabling the establishment of water quality criteria. These experiments have included acute toxicity tests, chronic toxicity tests, and safety

concentration tests with a single metal or a combination of metals (Alabaster & Lloyd 1982).

There are two ways that heavy metals may affect biota in the aquatic environment; one is through severe and acute exposure whereby fish and aquatic invertebrates are eliminated; the other is through chronic exposure in which organisms receive lower doses over a prolonged period. In this case the transport of the toxicant or its biogeochemical cycle is important. Also of concern is metal accumulation of fish which are important sources of food for human.

Historically, there is a predominance of acute lethal toxicity studies based on laboratory experiments. Nowadays, although the mortality of aquatic organisms is no longer the only evaluated parameter, the results deduced from laboratory experiments often criticised as being of limited relevance to real situations (Abel 1989). This is because data obtained from laboratory studies most were limited to a few particular factors, such as a single pollutant in a series of fixed chemical and physical situations. Results have then been used to predict the behaviour of a heavy metal in field situations. Such extrapolations from laboratory data to field situations are risky. The coexistence of metals in natural environments may change the toxicity, through antagonistic, additive, or synergistic effects. Moreover, other environmental factors such as temperature (Edgren & Notter 1980), dissolved oxygen (Voyer et al. 1975), organic matter (Rashie & Leonard 1973, Gamble & Schnitzer 1974, Gardiner 1974b, Giesy et al. 1977, Winner 1984), suspended solids (Olsen et al. 1982), humic acids, and bicarbonates (Stiff 1971 a&b, Kinkade & Erdman 1975, Calamari et al. 1979, Carroll et al. 1979, Buckley et al. 1985) in nature also affect toxicity (Nishikawa & Tabata 1969, Morris & Russle 1973, Pagekopf et al. 1974, Elder 1975, Howarth & Sprague 1978, Florence 1982, Ramamoorthy & Blumhagen 1984, John et al. 1987).

Since the fauna of polluted waters is more commonly exposed to relatively low concentrations of poison for a long periods, rather than to high levels of pollution which cause rapid mortality, it is probably more important to study the effects on aquatic organisms of exposure to sublethal levels over a period representing at least a substantial proportion of their life cycles.

The complexity of the natural environment, therefore makes it impossible to reproduce the exact conditions in a laboratory for any substantial period of time, such as a year. Moreover, the maintenance of constant experimental conditions for periods up to or exceeding a year is expensive in human and physical resources, and the longer an experiment continues the greater is the chance of failure due to accident or equipment malfunction. In addition it should be noted that relatively few species can be satisfactorily maintained in the laboratory for long periods and fewer still can complete their life cycle under such conditions since their environmental requirements are complex, unknown, or both.

Consequently, Taylor (1983) stated that 'results of laboratory experiments can only be taken as a guide, usually a pessimistic one, to the likely effects in natural waters'. Biological field verification of the results of laboratory studies for water quality management is necessary before sound conclusions can be reached regarding the behaviour of heavy metals and other contaminants in natural systems. Therefore, a well designed field survey could contribute more information of value.

Several field surveys of metal pollution have been reported notably on the effects of a mixture of industrial and sewage wastes on the freshwater ecosystem (Mathis & Cummings 1973, Eustace 1974, Wiener & Giesy 1979, Dallinger & Kautzky 1985). Researches focused only on the metals in domestic wastes are limited (Mason & Barak 1990, Mclean et al. 1991). Nevertheless, in the modern world, an elevated heavy metal content in sewage wastes is common.

Since the chemical forms of heavy metals in wastewater containing a high proportion of organic matter and suspended solid are likely to be complicated. Metal ions tend to bind to the suspended particles and organic substances, and thereby associate with river sediment with the possibility of transport via food-chain relationships into the biota. The concept of field verification is, therefore, useful in the difficult task of evaluating and comparing the relative importance of water and sediment sources of heavy metal uptake which in a relatively 'short-term' laboratory experiment may impossible.

Among the heavy metals, zinc, copper, lead, mercury, nickel, chromium and cadmium are of principal interest as pollutants (Winder et al. 1973, Eustace 1974, Abo-Rady 1979, Vinikoar et al. 1980, Lowe et al. 1985, Ajmal et al. 1988, Singh et al. 1990). Historically, Cu, Pb and Zn were regarded as the most significant but more recently Hg and Cd have been prominent (Janben & Brune 1984, Abel 1989, Barak & Mason 1989, 1990 a&b, Ozretic et al. 1990). Among the list of environmentally hazardous metals, both copper and cadmium are common contaminants (Forstner & Wittmann 1983). However one of them Cu, is an essential element to the aquatic animal, and the other Cd is obligatorily unessential to the life of organisms.

Copper has been studied as the principle toxicant in a sewage effluent (Clark & Fraser 1983). It has been a subject in many toxicity tests (i.e. Pickering & Henderson 1966, McKim & Benoit 1974, Pagenkopf et al. 1974, Howarth & Sprague 1978, Brown & Rattigan 1979, Chernoff & Dooley 1979, Steele et al. 1990, Dave & Xiu 1991) and a very common element in numerous field surveys of freshwater ecosystems (Lucas et al. 1970, Uthe & Bligh 1971, Mathis & Cummings 1973, Kelso & Frank 1974, Abo-Rady 1979, Wiener & Giesy 1979, Roch et al 1985, Dallinger & Kautzky 1985, Lowe et al. 1985, Weber 1985, Bradley & Morris 1986, Gokhale & Patil 1989, Harrison & Klaverkamp 1990).

Cadmium present is generally found within suspended particulate matter and the sediments (Preston 1973, Olsen et al. 1982). It is the subject of considerable research because of its toxicity (Pickering & Gast 1972, Kumada et al 1973, Eaton 1974, Kinkade & Erdman 1975, Larsson 1975, Giesy et al 1977, McCarty et al 1978, Reichert et al 1979, Sastry & Gupta 1979, Hawkins et al. 1980, Buckley et al 1985, Joensen & Korsgarrd 1986, Pascoe et al 1986, Ghosh & Chakrabarti 1990) and its potential to accumulate in food chains (Badsha & Goldspink 1982, Janben & Brune 1984, Dallinger & Kautzky 1985, Roch et al. 1985, Barak & Mason 1990 a,b). Most research on Cd in the environment focuses upon its toxicity to plants (Mclean & Jones 1975) and animals (Clubb et al 1975, Mayes et al. 1977), especially those of agricultural importances, and upon public health aspects of Cd exposure.

From some field surveys a general tendency for metals to rank highest in sediment is elucidated (Mathis & Cummings 1973, Mathis & Kevern 1975, Enk & Mathis 1977, Lewis 1980). The relative contributions of dietary intake vs. direct absorption from the water and/or sediments to the metal-loading of aquatic animals is poorly understood. Hardisty et al (1974) demonstrated a high correlation between the percentage of crustacean in the diet, and the cadmium and lead levels in various estuarine fishes from Severn estuary and Bristol channel. Apart from this, there is very little information available on the movement of Cu and Cd in aquatic food webs (Dallinger & Kautzky 1985, Dallinger et al. 1987). Food as a source of Cd may be relatively more important in nature than can be implied from laboratory studies due to significantly higher concentrations of Cd in food items than in the surrounding water.

Through the study of these two common metals which have extremely different physiological characteristics, a general comparison of the pathways of the metals in the aquatic environment can be made.

## 1.2 The Effects of Pollution to Three-spined Stickleback

The 3-spined stickleback, *Gasterosteus aculeatus* L., is a cosmopolitan fish species. It is found abundantly in the coastal region between 35$^{o}$N and 70$^{o}$N of Europe, northern Asia, and much of North America, but is absent from Africa (Wootton 1976, Wheeler 1979). It is present in fresh, brackish and salt water, but with two restrictions to its distribution. One is that it is restricted to the coastal waters of seas and oceans, and another restriction is that it is not found in steep, fast-flowing streams, hence it is rare or absent in mountainous areas. The fish is most common in the slow flowing backwaters and tributaries of rivers and in ditches, dykes, sheltered bays and harbours. It is also common in lakes and ponds where there are areas of emergent or submerged, rooted vegetation (Wootton 1976). It is widely spread more or less wherever one looks for it (Wheeler 1979). It seems that their existence in the aquatic environment is linked with human settlements in river floodplains.

It could be suggested that modern civilization bringing domestic wastes, industrial discharges, and sewage effluents affect its

distribution. Like most of aquatic organisms, populations may diminish due to overfishing by mankind, lost habitats, or by the whole environment being rendered unsuitable because of pollution. Fortunately, due to its adaptability, it is one of the first fish to appear in the recovery of heavily polluted rivers. For example, in Willow Brook, Northamptonshire, U.K., the 3-spined stickleback was the most widely distributed fish and the only species found in the section below discharge points. It appeared to be the most tolerant fish species of pollution in the system (Solbe 1973).

Reviewing the natural history of fishes in the restoration of the River Thames and its tributaries, even in the critical period from 1900 to 1950 when the distribution and abundance of fishes in the tidal Thames was very limited, the stickleback was the most frequently seen fish in the period. In the 1920s, fishes were evidently common in the river at Chiswick, where stickleback abounded on the surface of marginal water at high tide in summer (Polunin, N. in Wheeler 1969). At the same time the river itself was in exceedingly bad condition. Also at this time The London Docks contained freshwater fishes including 3-spined stickleback in large numbers (Horn, P.W. in Wheeler 1979). During the Second World War it was reported that the stickleback inhabited the tidal river in the vicinity of Chelsea by Dr. Hentschel, C.C. (in Wheeler 1979) and, therefore, it must have been living in the freshwater river.(Dr. Sutton, M., in Wheeler 1979).

In the 1957 survey of the Thames fish fauna it was concluded that there were no fish between Richmond and Tilbury in the tidal Thames (cf. Harrison and Grant 1976, in Wheeler 1979). Nevertheless, the stickleback was abundant upstream in the Richmond area (Wheeler 1958 in Wheeler 1979) even when pollution of the metropolitan reaches was severe. Then, during the 1967-73 power-station survey, its occurrence was demonstrated from the first years of collection. Overall it was a common inhabitant of the Thames during 1967-1973, being most abundant upstream and least abundant at the mouth of the river, and probably increasing in number as the pollution of the river lessened. It was also extremely abundant in tributaries of the Thames and in still waters adjacent to it (Wheeler 1979). The information suggests that the stickleback never completely

disappeared from this river system, and where it had disappeared it was the first fish to return to the river.

Why do the stickleback populations recover more quickly than those of other fishes in a polluted aquatic ecosystem ? Are the sticklebacks more tolerant to the contaminants than the others species ? To what extent is their life-cycle affected by their stressed environment ?

In freshwater ecosystems the stickleback is essentially a secondary consumer. In a polluted freshwater environment does the contaminant act directly killing the fish, or could it lead to shortage of food, thereby indirectly restricting the distribution, growth and reproduction of the sticklebacks ?

Examples of studies of the tolerance of 3-spined stickleback to different types of pollutants in the literature are limited. One of the earliest examples that of Jones (1935, 1938, 1939), who conducted a series of studies about the toxic action of heavy metal salts on the fish in the 1930s, followed in the 1940s by studies on the responses of the stickleback to toxic solution (Jones 1947 a&b, 1948, 1952). Later, Matthiessen and Brafield carried out a series of studies on zinc toxicity to 3-spined stickleback (Matthiessen & Brafield 1973, 1977, Brafield & Matthiessen 1976) and Legore and Desvoigne (1973), studied the effect on the fish of exposure to resuspended contaminated harbour sediment. Pascoe and Cram (1977a), and Pascoe and Mattey (1977) studied the toxicity and the effects of parasitism on the toxicity of cadmium to the fish, and Oronsaye and Brafield (1984), examined the effects of dissolved cadmium on gill structure. More recently Holm et al. (1991) studied the effects of long-term exposure to bis(tributyltin)oxide (TBTO) on reproduction and histopathology of the fish.

The responses of 3-spined stickleback to a long-term, sustained, chronic exposure to pollutants in a natural aquatic environment has been little studied. Of particular interest is the route and supply of persistent, sublethal contaminants, such as metals, to sticklebacks in a natural freshwater ecosystem.

In addition, the use of measurement of toxic metal concentrations in the fish body as a biological monitor of metal loading in the natural

aquatic environment (Mason 1987) may be applied using this widely distributed fish.

## 1.3 Aims and Objectives

As stated previously a considerable amount is known about the relationship between metal exposure and uptake by aquatic organisms in laboratory systems. Most of these studies have been based on the uptake of metals directly from the water media and very few have attempted to evaluate the importance of intake of metal via food. One school of thought assumes that the food route is unimportant whereas another has found evidence which indicates that some of the metal burden in aquatic organisms is derived from food (Dalinger & Kautzky 1985). One aspect that tends to be overlooked is that most trace metals such as cadmium, zinc, copper, lead and mercury in aquatic systems are partitioned between the aqueous and solid phases and even within the aqueous phase these metals often exist as complexes with inorganic radicals e.g. chlorides, carbonates, phosphates, nitrates and sulphates and soluble organic compounds. Consequently the concentration of the free metal ion in water, which as a rule is the predominant, if not the only, bioavailable form can be very low. The partitioning and complexing of metals is most pronounced in hardwaters and seawater. Therefore in such environments exposure to metals can be mainly from food, whereas in laboratory experiments which have considered the efficiency of uptake from food and water, the comparison is based on equivalent exposures.

It was known from the outset of the project that the river Wandle is a hard water body which receives much of its flow of water from sewage effluents. A pilot study quickly established that elevated metal concentrations could be found in sediments at a number of locations along the river. It was also known that the most abundant fish in the river was the stickleback. As stated above, this is a resilient territorial fish which feeds on a wide range of organisms. Finally it was known that the concentrations of certain metals e.g. Cu, Cd, Zn,... etc. were elevated in fish and the pattern corresponded closely with the metal contamination found in the sediments.

The aims and objectives of the main part of the study were formulated from these considerations and the results of the pilot study. The stickleback populations in the Wandle provided an ideal opportunity to examine metal accumulation in freshwater fish in a hard water body and to evaluate the route and rate of supply of metal to the fish. This was tackled by setting up a regular programme of sampling at selected locations along the river. At the design stage it was realised that only three sites could be maintained and that there would be only time for analysing two metals on a routine basis. Consequently a reference or background site and two metal contaminated sites were chosen. The decision to concentrate on Cu and Cd was very much a compromise based on levels found in the preliminary analysis, analytical sensitivity, physicochemical properties in aquatic systems and the fact that Cu is an essential element, and Cd is a well known pollutant.

It was decided to carry out four major surveys at the three selected sites. These were undertaken during each season of the year from July 1989 to April 1990. During each survey samples were taken of water, sediment, organic detritus, sticklebacks, and the most abundant plants and invertebrates that occurred at each site. All these samples were analysed for Cu and Cd. The composition of the diet of sticklebacks was assessed from the stomach contents of fish at different sizes. From this it was envisaged that it would be possible to; determine the metal distribution in the habitat of the fish; and from a knowledge of the diet it would be possible to assess the relative importance of metal exposure from food and water. A further objective was to examine the distribution of the metals in the tissues of the stickleback in order that the major tissues of metal accumulation could identified.

An further and important aspect of the study was developed during the early stages of the project. It was realised that a significant number of the sticklebacks at the sites in the Wandle were infected with the cestode parasite, *Schistocephalus solidus*. It was therefore decided to treat the infected sticklebacks as separate group and examine growth and metal relationships in the same way as for the non-infected fish.

# CHAPTER TWO

# DEVELOPMENT OF METHODS TO DETERMINE COPPER AND CADMIUM IN SAMPLES OF FISH, INVERTEBRATES, PLANTS, SEDIMENTS AND WATERS

## 2.1 INTRODUCTION

There are three techniques which have been widely used in laboratories for the analysis of trace elements in biological or environmental samples. They are atomic absorption spectrophotometry (AAS), plasma emission spectrometry (ICP), and anodic stripping voltammetry (ASV) (Gilber and Kakareka 1985). Also, there are alternative methods, such as neutron activation analysis, and spark source mass spectrometry (SSMS). However, the latter two methods require special facilities and are beyond the means of most toxicology laboratories. AAS, ICP and ASV have formed the basis of standard methods published by the U.S. Environmental Protection Agency (EPA) and the American Society of Testing Material (ASTM).

The sensitivity of ICP is higher than that of flame AAS for determination of elements, such as antimony, arsenic, lead, nickel, silver, thallium, and zinc. In some cases, e.g. chromium, beryllium, and copper, it is either equivalent or even better than that of flameless AAS (Gilber and Kakareka 1985). However, ICP is more susceptible to spectral interferences than AAS. This is caused by the stray light which results in a matrix-induced shift in background emission intensity, and overlapping of emission lines. The main advantage of ICP is that it is a multi-element facility for measuring a number of elements at one time (up to 48 elements). Because of the problem of interference it may be a time-consuming technique to develop and in the wide variety of samples of fish, invertebrates, plants, sediments and waters which were used in this study together with limited volume of sample available in each case, the ICP method has not been chosen.

The major drawback of the method of ASV which precludes its use for routine analysis of a large number of samples is the lengthy procedure involved (on average 30-60 min per sample). This is partly due to the

fact that the precise quantification can only be achieved by employing time-consuming standard addition methodology. Moreover, the technique is only recognised as a standard method by the ASTM for the measurement of cadmium and lead in water.

Atomic absorption spectrophotometry (AAS) has become the most commonly used technique for measuring trace elements in a wide variety of materials. Its popularity is based on several factors: low cost, ease and speed of operation, relative freedom from interference, and good degree of sensitivity. Although the sensitivity of flame AAS for number of elements cannot compete with ICP, the development of graphite furnace technology in AAS since 1970 has provided the main analytical method for routine determination of many elements at trace and ultratrace levels with sufficiently high sample throughput. In general, the sensitivity achieved by flameless AAS is 100 times superior to that of flame AAS. The limit of detection for copper is 1 ug/l, and 0.1 ug/l for cadmium.

In view of the nature of the project and the sample throughput required, both flame and flameless AAS offer the best choice of methods. There are two reasons for this decision, firstly, the limit of detection is a piority of the choice of analytical method. Comparing the limit of detection of copper and cadmium by flameless AAS and ICP, while ICP is more sensitive for copper, it is much less sensitive for cadmium (Gilber and Kakareka 1985). Since cadmium concentrations in biological samples are generally very low, it was decided that flamless AAS offered the best compromise.

Secondly, the amount of sample available, both in terms of weight of individuals and total number of individuals, restricted the volume of sample that could be prepared for sample determination. The sample volume required for the ICP and flame AAS is 1 to 3 ml per analysis. However, flameless AAS usually requires less than 100 ul of sample. Therefore, 5 ml of sample would be sufficient for the determination of Cu and Cd in the study by using a combination of flame and flameless AAS.

## 2.2 METAL ANALYSIS TECHNIQUES

Flame and flameless (electrothermal atomisation) AAS were used throughout the study for the determination of copper and cadmium in a wide range of organisms, sediments and waters. At the outset the aim was to develop suitable methods for preparation of the materials. Where possible the flame method of AAS was used but for a number of samples, where metal levels were very low, the graphite furnace AAS was used.

The following sections set out the procedures adopted for the analysis of copper and cadmium. Particular emphasis has been placed on: (1) Procedures to minimise metal contamination and loss during handling and preparation of samples. (2) Methods for decomposition of samples. (3) Optimisation of AAS instruments.

### 2.2.1 Procedures to Minimise Metal Contamination and Loss During Handling and Preparation of Samples.

Many of the problems associated with trace metal analysis are due to contamination and/or loss during preparation. There are a number of possible external sources of contamination, these include airborne particulates from the laboratory environment, impurities in reagents, and direct contact with metal surfaces. Losses of metals during preparation stages may be due to absorption to container surfaces, spillages and volatilisation. Therefore, the following precautions were undertaken in this study.

**(A)** All glass and plastic utensils were meticulously cleaned according to the following procedure:

1. soaking in 2-5% of Decon-90 in single distilled water (SDW) for 24 hours.

2. rinsing with single distilled water (SDW) to completely remove the detergent, followed by rinsing with DDW.

3. soaking with 10% nitric acid ('AnalaR' grade, BDH) in DDW for at least 24 hours.

4. rinsing three times with DDW and then drying in a stainless steel oven.

5. the glassware and plasticware were stored in clean, and sealed polythene bags.

**(B)** For glassware and plasticware that were used to prepare samples for flameless AAS extra care as taken and an additional soaking in 10% $HNO_3$ was undertaken to ensure complete removal of metals from surfaces.

**(C)** In all instances only the natural coloured polythene or polypropylene plastics were used. For example, yellow-coloured disposable pipette tips were not used, even after acid washing. Moreover, all containers which had been previously used for storing either samples or working standards were disposed off, rather than re-cycled.

### 2.2.2 Methods for Decomposition of Samples

All solids and many liquid samples require some pretreatment to decompose organic matter and the solid phases in order that metals are released into solution. In the literature there are many methods for the destruction of organic matter for the analysis of trace elements, among which the two most common methods are dry-ashing and wet-ashing.

Dry-ashing is a way of breaking down solid samples by direct application of heat at 500-550$^{o}$C is sufficient to completely destruct organic matter. Although it is a convenient and widely used method, there is a risk of loss of volatile elements, such as cadmium, at elevated temperatures of 400-450$^{o}$C. Also during ashing, metals such as copper, may be strongly absorbed and retained on surfaces such as silica crucibles (Cowley 1978 in Blake 1980).

For metals extracted from biological samples wet-ashing has many advantages, especially for the purpose of multiple element analyses (Gilber and Kakareka 1985). It is a fairly rapid pre-treatment method, it is applicable to a wide variety of samples and it is less prone to volatilisation losses (Blake 1980, Schachter & Boyer 1980). Also

decomposition using strong inorganic acids prevents the formation of metal oxides which would otherwise be retained on container walls (Bull 1975).

Wet-ashing can be achieved by using a single acid or a combination of acids. The acid(s) used for wet-ashing also needs to be considered carefully, since chlorine-containing mineral acids such as hydrochloric and perchloric acids are known to cause serious chemical interference and analyst loss in the ashing stage of flameless AAS determination of cadmium (Culver et al. 1975, Rothery 1982). Sulphuric acid boils at 338$^{o}$C and when it is used in digestions the excess acid is very difficult to remove, also it may form insoluble compounds with alkaline earth elements which are not easily re-extracted (Blake 1980). Wet-ashing with nitric acid has been used with good recoveries in variety of food samples (Schachter & Boyer 1980). Although it might not break down the organic matrix completely, it has several advantages, for example, the extraction solution is amenable for multi-element analysis, minimizing the possibilities of chemical interferences, especially in flameless AAS. It is a relatively safe and quick acting acid for most organic matrices, and with its relatively low boiling point, excess acid can be removed more easily than perchloric or sulphuric acid (Blake 1980). It was highly recommended by Gilber and Kakareka (1985) for the destruction of organic tissues for multiple trace element analyses.

When considering all the factors mentioned above and other practical considerations, such as availability of apparatus for analysis of large batches, safety, and cost of analysis, wet-ashing with nitric acid offered the best compromise. Although for sediment which is a mixture of organic and inorganic substances, a combination of $HNO_3$ and HCl (2:1) was used.

Digestion procedure:

(1) Predigestion stage:

Previously dried samples were weighed in either conical flasks or boiling tubes (Pyrex) and concentrated $HNO_3$ (or $HCl/HNO_3$ for sediment) was added in a volume to weight ratio of 15:1.

'Spectrosol' and even 'AristaR' grade acid (BDH) was used throughout the analysis. The mouth of containers were covered with PTFE tape (Gallenkamp) and left at room temperature for overnight. In order to check for contamination, a minimum of two blanks (glassware and acid only) were prepared per batch of 20 digestions.

(2) Digestion stage:

The PTFE covers were replaced with Pyrex refluxing droplets and the digestions were placed on a hot plate or hot block, set at 90 to 110$^{o}$C and refluxed for 24 hours.

(3) Evaporation stage:

The temperature was raised to 120$^{o}$C for at least two hours or until brown fumes were no longer evolved (Bull 1975). The glass pear drops were then removed, excess acid was evaporated and the digestion was reduced almost to dryness (usually about 0.5 to 1 ml was left in the vessel).

(4) Final volume:

i) For fish, and invertebrates samples, 'AnalaR' water (BDH) was used to make up to a final volume of 5 ml in graduated conical centrifuged tubes and these were then centrifuged at 4000 rpm for 15 mins.

ii) The plant and sediment digestions were filtered with Whatman no. 541 filter papers to remove the particulates and 1 M 'Spectrolsol' $HNO_3$ was used to make up to a volume of 10 ml and 25 ml respectively in volumetric flasks.

iii) The clear supernatant and filtered samples were finally transferred to acid-washed Pyrex tubes, sealed with PTFE tape and stored at 4$^{o}$C awaiting determination of Cu and Cd.

### 2.2.3 Optimisation of AAS Instruments

**(A)** Preparation of standard solution:

In the preparation of standard solutions it is important that, (1) the matrix of the samples and standards are closely matched, (2) stock solutions are maintained in acid solution to prevent the degradation by time, and (3) all the dilution procedures and pipettes are accurately calibrated.

Copper and cadmium stock standard solutions were prepared from commercial 1000 ug/ml cadmium nitrate and copper nitrate standard solutions (BDH 'Spectrosol' grade) with 1 molar nitric acid (BDH 'Aristar' grade) in double distilled water (for flame AAS), or in 'AnalaR' water (BDH) (for flameless AAS). All the working standards were prepared by serial dilution from stock solutions freshly on the day of analysis using acid-washed grade 'A' volumetric glassware.

**(B)** Flame Atomic Absorption Spectrometry (Flame AAS):

All determinations were carried out using a Varian AA-1475 Atomic Absorption Spectrophotometer, with the following settings:

| Parameter | Copper | Cadmium |
|---|---|---|
| Manufacturer's lamp | Juniper/Cathodeon | Cathodeon |
| Lamp current, mA | 5-6/3-4 | 3-4 |
| Wavelength, nm | 324.8 | 228.6 |
| Spectral bandpass, nm | 0.5 | 0.5 |
| Flame stoichiometry | fuel lean | fuel lean |
| Ratio of air:acetylene | 4.8:1.4 | 4.5:1.2 |
| Double beam | on | on |
| Deuterium background corrector | off | on |
| Integrated time second, sec | 3 | 3-5 |
| Working range, ug/l | 0.5-3.0 | 0.2-0.8 |
| Detection limit, ug/l | 0.2 | 0.04 |

Flame AAS was set up according to the Varian Instrument Operation Manual and to the comments of Bull (1975). Moreover, Brodie and Rowland (1981) published " A simple fault-finding guide for flame atomic absorption spectrometry" which was consulted regularly.

Essential maintainence procedures were followed for all determinations, these included; maintaining a clean burner and optical path, achieving optimum alignment of optics and operating temperatures.

Normal calibration methods were used to determine the metal concentration of samples and only the linear part of calibration curve was used in these determinations. Figures 2.1 and 2.2 show typical calibration curves of standards against absorbance for Cu and Cd. The absorbance-concentration linear regression line was calculated using computer worksheet software, Lotus` 1.2.3, (the regression coefficents were normally greater than 0.99) and the values of absorbances were back-calculated into concentrations. Standard solutions were aspirated at regular intervals to monitor for a shift in baseline and deterioation of signals.

**(C)** Flameless Atomic Absorption Spectrometry (Flameless AAS):

A Varian AA-1475 Atomic Absorption Spectrophotometer fitted with a Varian GTA-95 Graphite Tube Atomiser and a automatic sample dispenser was used throughout with the following settings:

| Parameter | Copper | Cadmium |
|---|---|---|
| Manufacturer's lamp | Cathodeon | Cathodeon |
| Lamp current, mA | 3-4 | 3-4 |
| Wavelength, nm | 324.8 | 228.6 |
| Spectral bandpass, nm | 0.5 | 0.5 |
| Double beam | on | on |
| Deuterium background corrector | off | on |
| Working range, ng | 0.10-0.40 | 0.01-0.04 |
| Detective limit, ng | 0.06 | 0.004 |
| Injection volume, ul | 20 | 28 |
| Purge gas* | nitrogen | nitrogen |
| Cooling water, l/min | 1.5-2 | 1.5-2 |
| Reading mode | peak height | peak height |

* oxygen-free nitrogen.

"The Analytical Methods for Graphite Tube Atomizers" edited by Rothery, E. (1982) was found to be very useful guide to flameless AAS. The initial setting up of this instrument is similar to that for Flame AAS analysis. In addition very careful attention was directed to optimising the light path through the graphite furnace since the

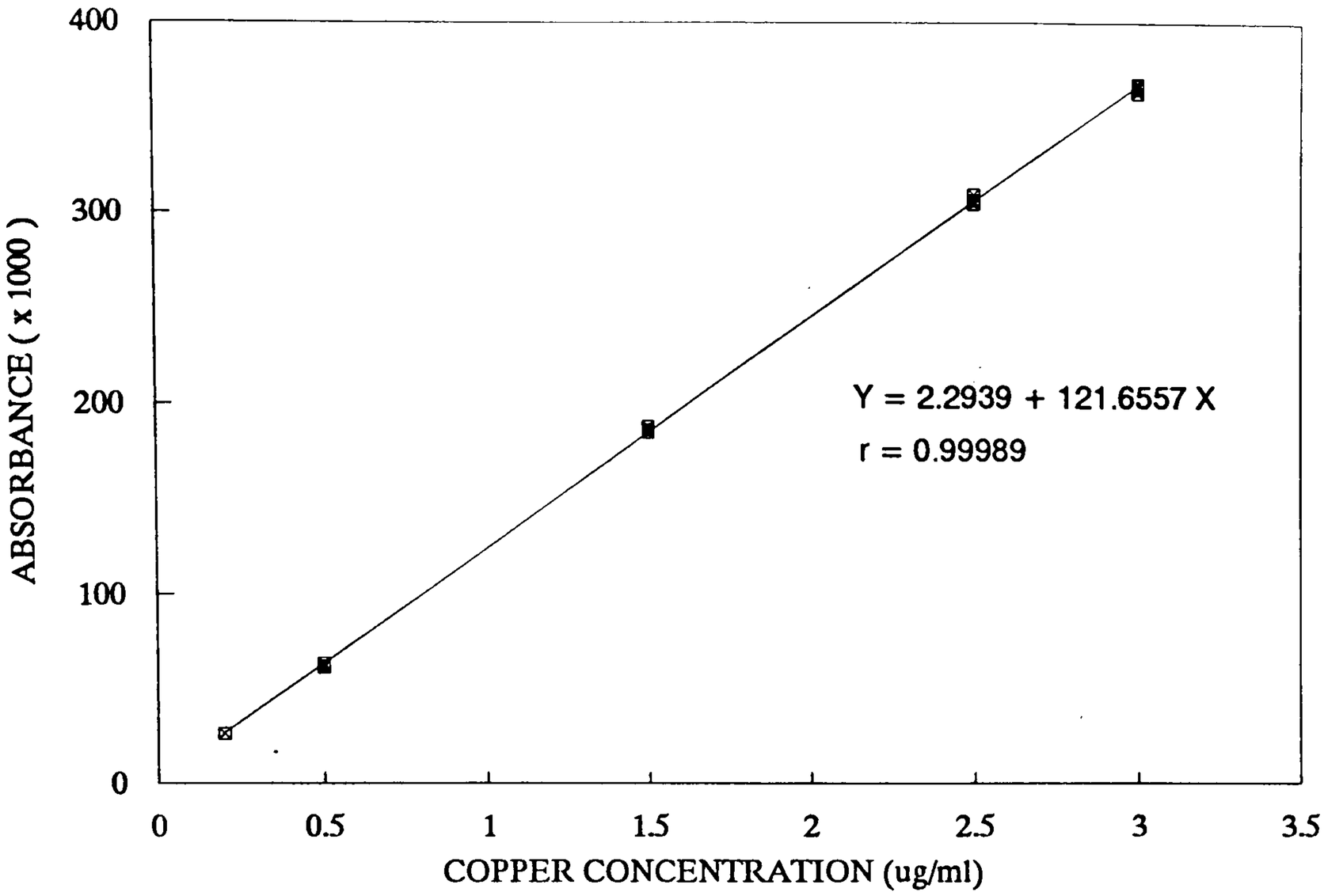



**Figure 2.1** Copper calibration curve by flame atomic absorption spectrophotometry.

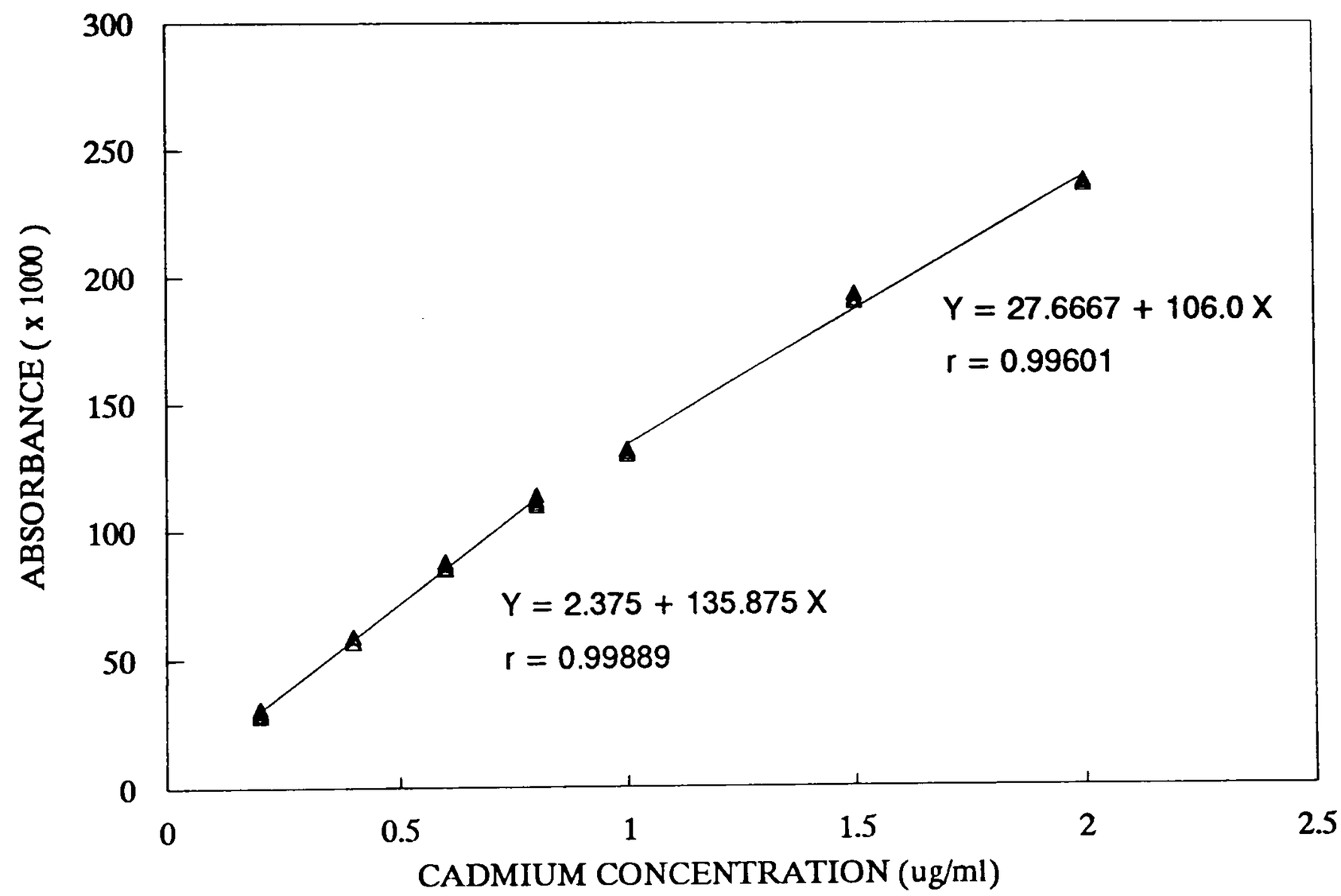



**Figure 2.2** Cadmium calibration curve by flame atomic absorption spectrophotometry.

diameter of the furnace is only 0.5 cm, the alignment of the light path and adjustment of the atomiser position need more care. The quartz windows which are located at the two ends of atomizer were cleaned regularly. Installation of graphite tubes into the atomising chamber was done carefully so that the nozzle of the capillary injection tube was precisely at the centre of the opening of the atomising chamber.

The Varian GTA was equipped with an automatic dispenser. The position of the injection arm was adjusted as that the height of the tip on the injection arm delivered a droplet just touched the inner surface of the graphite tube when fully dispensed. All the capillary tubing of the dispenser was kept very clean and metal-free, and any gas bobbles in the tubing and the dispensing syringe were removed. The whole system was rinsed in 0.01% $HNO_3$ for Cu analysis. However, it was found that an addition of 0.005% of Triton X-100 in the system was improved the precision of Cd determinations.

The reproducibility of the analytical technique was expressed as the relative standard deviation (RSD %), which is defined by the equation:

$$RSD\% = (s/x) \times 100\%$$

Where $s$ is the standard deviation of a series of measurements; $x$ is the mean value of the series of measurements. Each sample were determined three times. Where the RSD % was greater than 5 %, the analysis was repeated.

## 2.3 DEVELOPMENT OF GRAPHITE FURNACE PROGRAMMES

### 2.3.1 Design of the Graphite Furnace Programmes

There are three basic stages to a graphite furnace programme, drying, ashing and atomisation. Each one is specific to the element to be determined and the matrix in which it is dispersed. To achieve the maximum precision and reproducibility, the aim is to develop a programme with a set of temperatures and time settings which successively complete in each stage without affecting the others. This

is particularly difficult to achieve for the more volatile elements and with samples containing refractory organic matter (Issaq 1979).

The furnace programmes for cadmium and copper were carefully designed in order to optimise sensitivity, precision, and accuracy of the analysis.

(1) Drying stage:

Smooth and complete drying of the injected aliquot is an important criterion for achieve satisfactory precision. The drying stage is a series steps (1 to 4) in the furnace programmes for removing water from the sample and standard. The time selected was based on the guideline that is 1 to 3 seconds are required for each microlitre injected (Rothery 1982). Selection of temperatures was based on the nature and components in a sample. For example, with a sample containing $HNO_3$, the temperature was first set at $90^oC$ to remove the water and then increase to $125^oC$ to remove the $HNO_3$.

(2) Ashing stage:

Ashing normally takes place in a series of steps of increasing temperature to remove organic components to leave behind an inorganic matrix containing the element of interest. It is highly sample matrix dependent and the temperature and time setting required for the ashing stage are influenced by the nature and amount of organic matter in the sample. For a new type of sample, the customary approach is to plot absorbance readings for an analyst against different ashing temperatures (at this point the atomisation temperature is set recommended value by the manufacturer's manual) and from these ash plots the optimum conditions for a particular element in a particular type of sample can be ascertained. Ashing usually consists of three to four stages.

(3) Atomisation stage:

The aim of atomisation stage is to achieve a temperature in which the element of interest is completely vaporised. To achieve the best

atomisation temperature according to different matrices, the atomisation temperature plots should be determined. This is done by keeping the ashing temperature constant but successively increasing the atomisation temperature. It is taken as the lowest value which does not reduce the signal or introduce a memory effect. Operating with an atomisation temperature at the lower end of the range also has the advantage of increasing the life expectancy of a graphite tube. The ramp rate (i.e. the rate of temperature increase) between the ashing and the atomisation stages needs to be carefully selected in order to achieve a maximum precision without causing too much reduction in sensitivity and minimising the non-atomic signals. It is usual to repeat the atomisation stage to ensure complete vaporisation and finally with the carrier gas flushing the system to clean the furnace.

(4) Standard Additions:

In the development of a method, a series standard additions were carried out to check for presence of chemical interference in the sample matrices and to ensure that the normal calibration curves could be used routinely.

### 2.3.2 Development of Graphite Furnace Programme for Copper

Figure 2.3 and 2.4 are examples of calibration curves in peak height mode covering the range from 0.08 to 5 ng of copper. It was found that double distilled water produced a significant copper signal (see Figure 2.3), and so in all subsequent determinations "AnalaR" $H_2O$ purchased from BDH was used. It was found that a drying time of 40-60 seconds was needed for a 20 ul sample injection (Figure 2.5). Figure 2.6 shows the effect of different ashing temperature for a typical standard solution and a fish digest. A temperature profile including a stage at 700$^{o}$C for 10 to 15 second and at 900$^{o}$C for 5 second was found to give the most reproducible results. An ashing temperature at 900$^{o}$C ashing temperature up to 30 second did not reveal significant losses of Cu for a wide range of different types of samples, i.e. fish, chironomid, mollusc, *Gammarus*, leech and

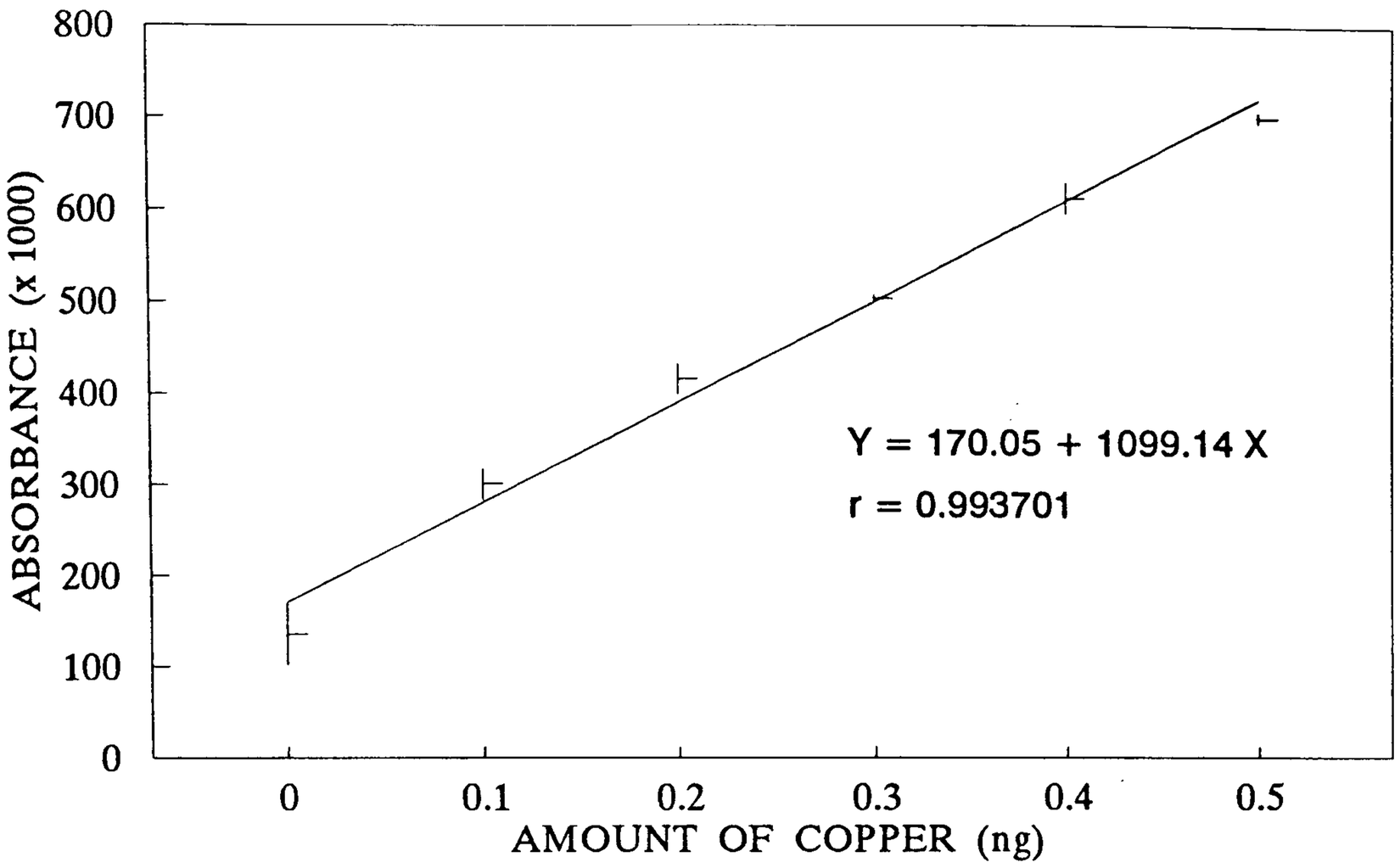



**Figure 2.3** A standard curve for copper by electrothermal atomisation. The vertical lines indicate the standard deviations; the horizontal dashes indicate the mean values.

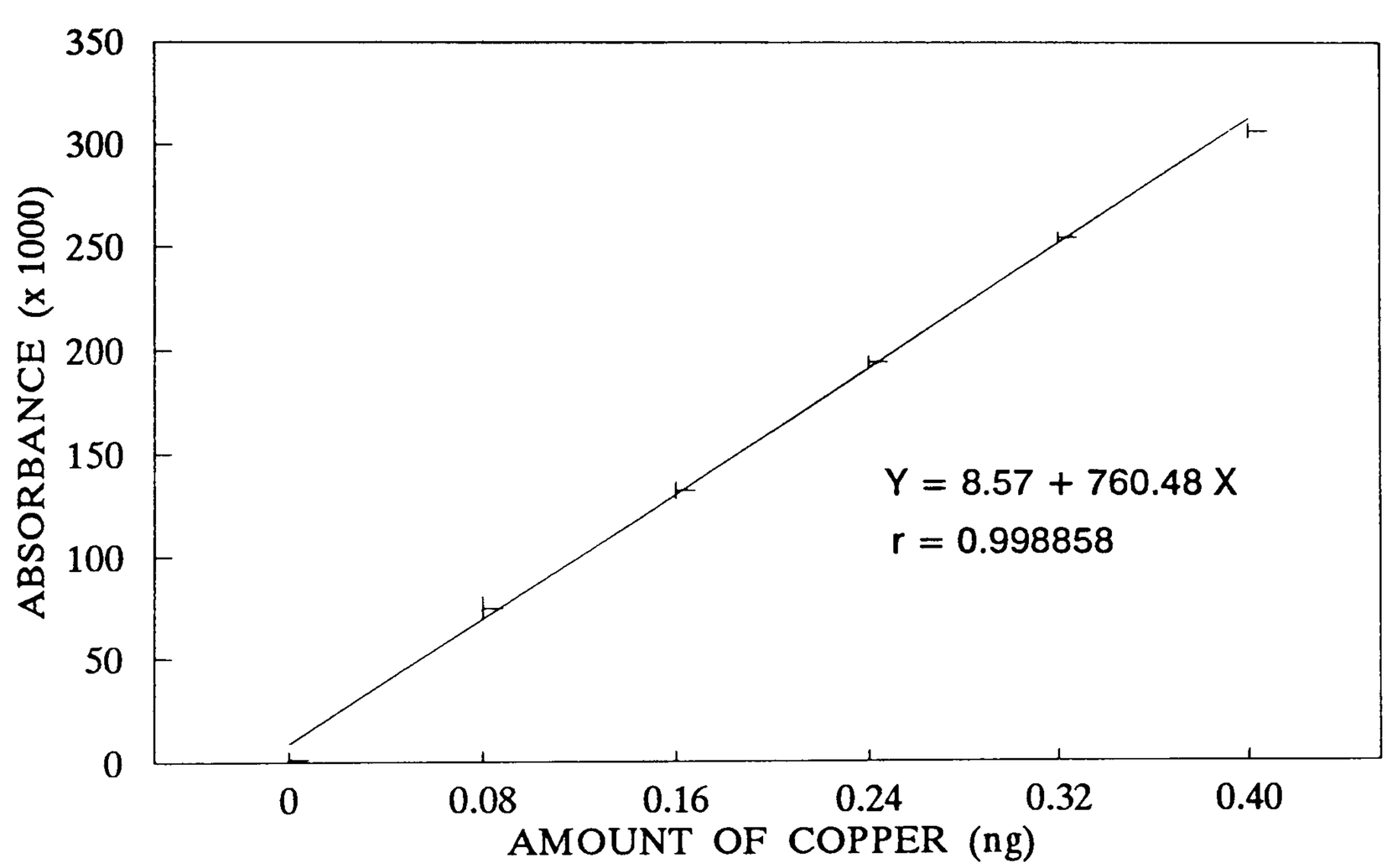



**Figure 2.4** Typical standard curve for copper by electrothermal atomisation. The vertical lines indicate the standard deviations; the horizontal dashes indicate the mean values.

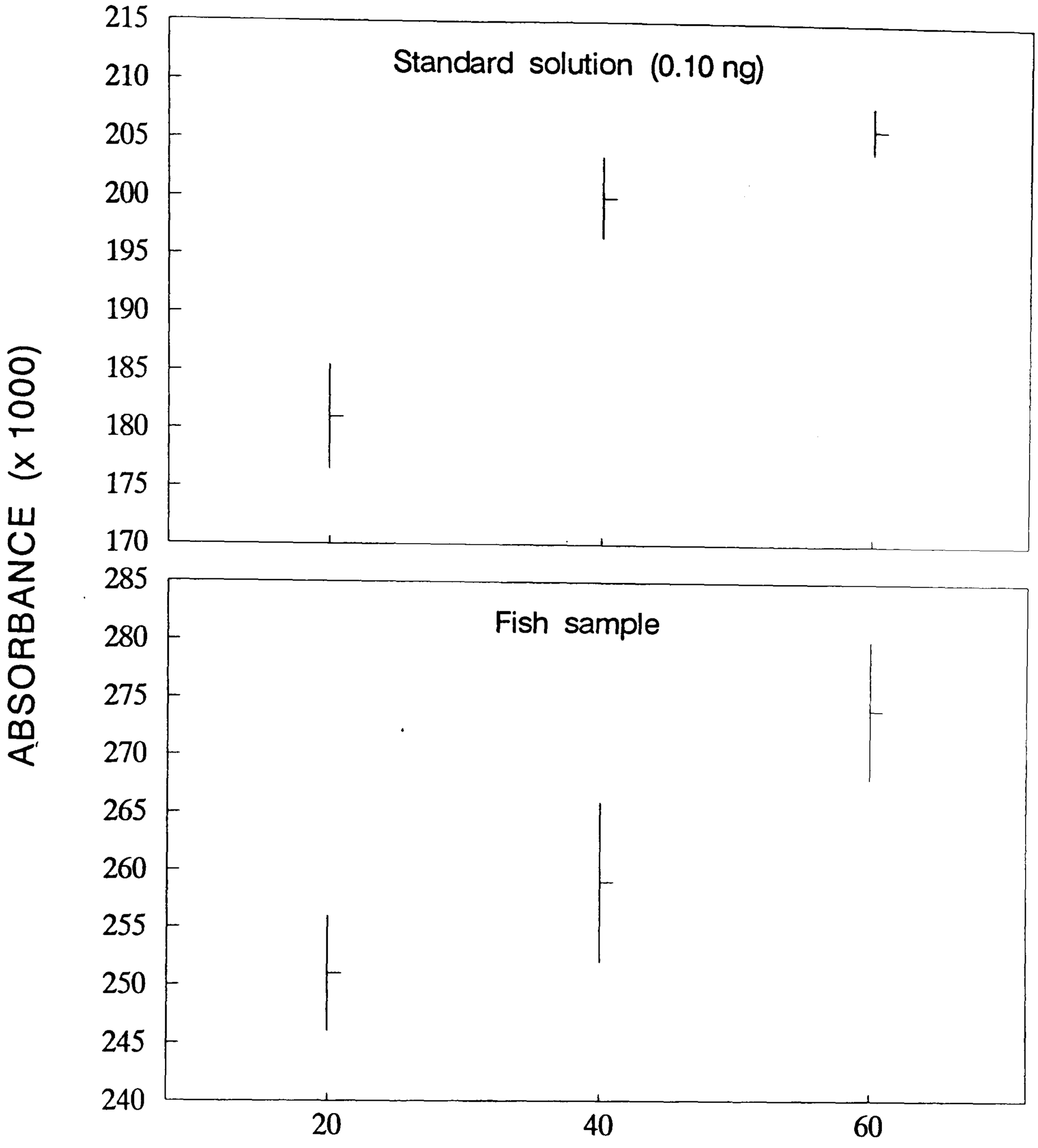



**Figure 2.5** Comparison of Cu absorbance values with different drying times. The vertical lines indicate the standard deviations; the horizontal dashes indicate the mean values.

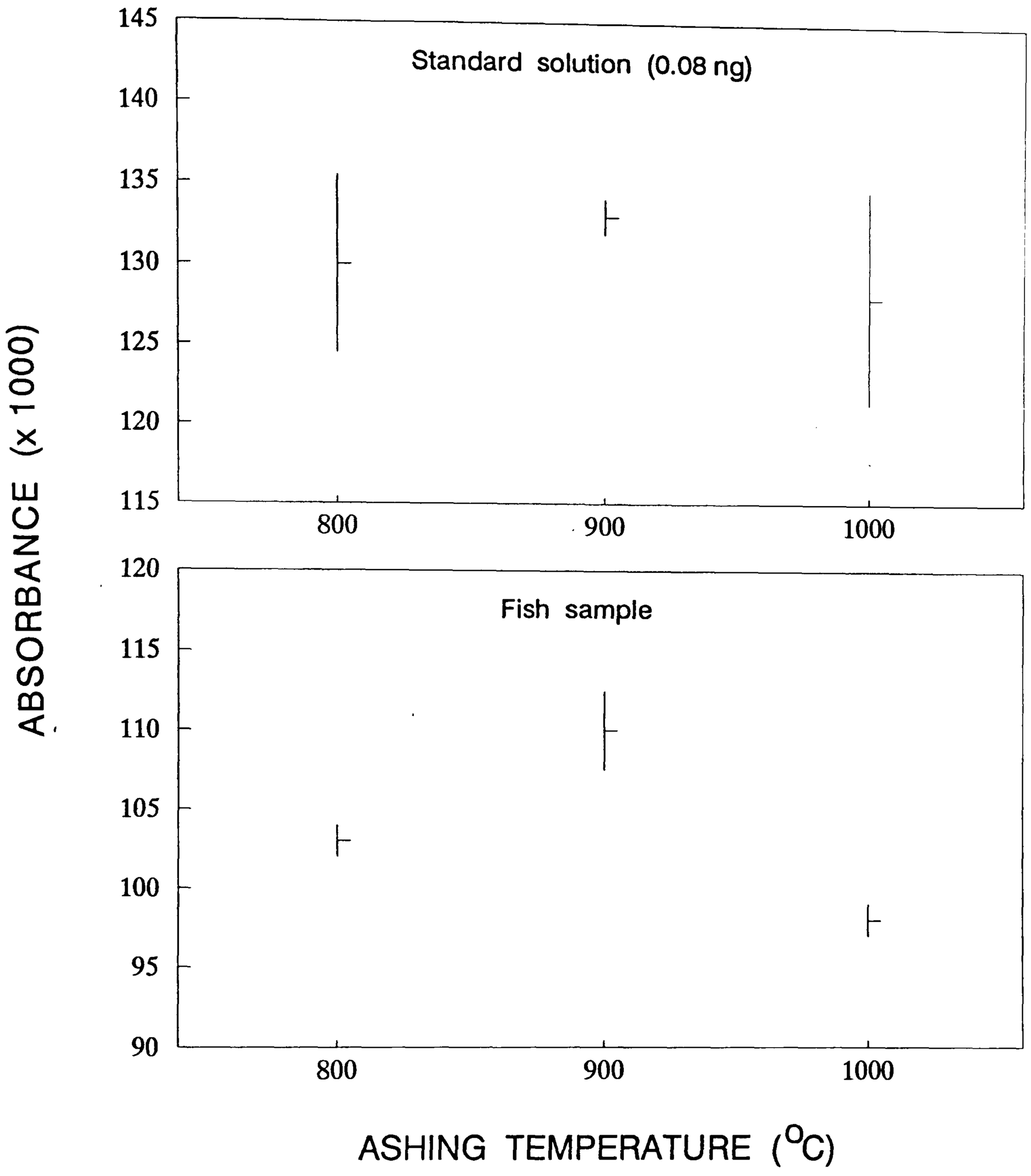



**Figure 2.6** Comparison of Cu absorbance values with different ashing temperatures. The vertical lines indicate the standard deviations; the horizontal dashes indicate the mean values.

standard solution (Figure 2.7). Atomisation temperatures above 2200$^{o}$C gave constant absorbance values for all tissues analysed (Figure 2.8). The graphite furnace programme for copper that was developed from the above procedures is given below:

GRAPHITE FURNACE PROGRAMME FOR COPPER DETERMINATION:

| Step | Temp($^{o}$C) | Time(sec) | Gas | Flow Rate(l/min) | Read |
|---|---|---|---|---|---|
| 1 | 75 | 5 | $N^2$ | 3 | |
| 2 | 90 | 50 | $N^2$ | 3 | |
| 3 | 120 | 10 | $N^2$ | 3 | |
| 4 | 120 | 5 | $N^2$ | 3 | |
| 5 | 700 | 10 | $N^2$ | 3 | |
| 6 | 900 | 5 | $N^2$ | 3 | |
| 7 | 900 | 1 | $N^2$ | 0 | |
| 8 | 2300 | 0.6 | $N^2$ | 0 | * |
| 9 | 2300 | 2 | $N^2$ | 0 | * |
| 10 | 2300 | 2 | $N^2$ | 3 | |
| 11 | 40 | 20 | $N^2$ | 3 | |

* indicates setting for absorbance reading.

The above programme was used for a series of standard additions of Cu to the different types of samples used in the study, i.e. Stickleback, *Schistocephalus*, chironomid, mollusc, *Asellus*, *Gammarus*, leech and suspended solid. It can seen from Figure 2.9 that all the standard addition calibration lines were parallel with the normal calibration line. It was therefore concluded that there was no significant chemical interference in the samples and the above programme was used in all subsequent Cu determinations.

### 2.3.3 Development of Graphite Furnace Programme for Cadmium

The working range for cadmium (Cd) is shown in Figure 2.10 and the range of 0.01 to 0.04 ng Cd was found to be linear. The recommended maximum ashing temperature for Cd in $HNO_3$ is 300$^{o}$C (Rothery 1982). Figure 2.11 shows that there was no loss of Cd up to 60 seconds. As Figure 2.12 shows, ashing temperatures of 600$^{o}$C resulted in marked differences in Cd absorbances between standard solution and the digested samples, and the trends suggest that Cd was present in the samples in more stable complexes, although the

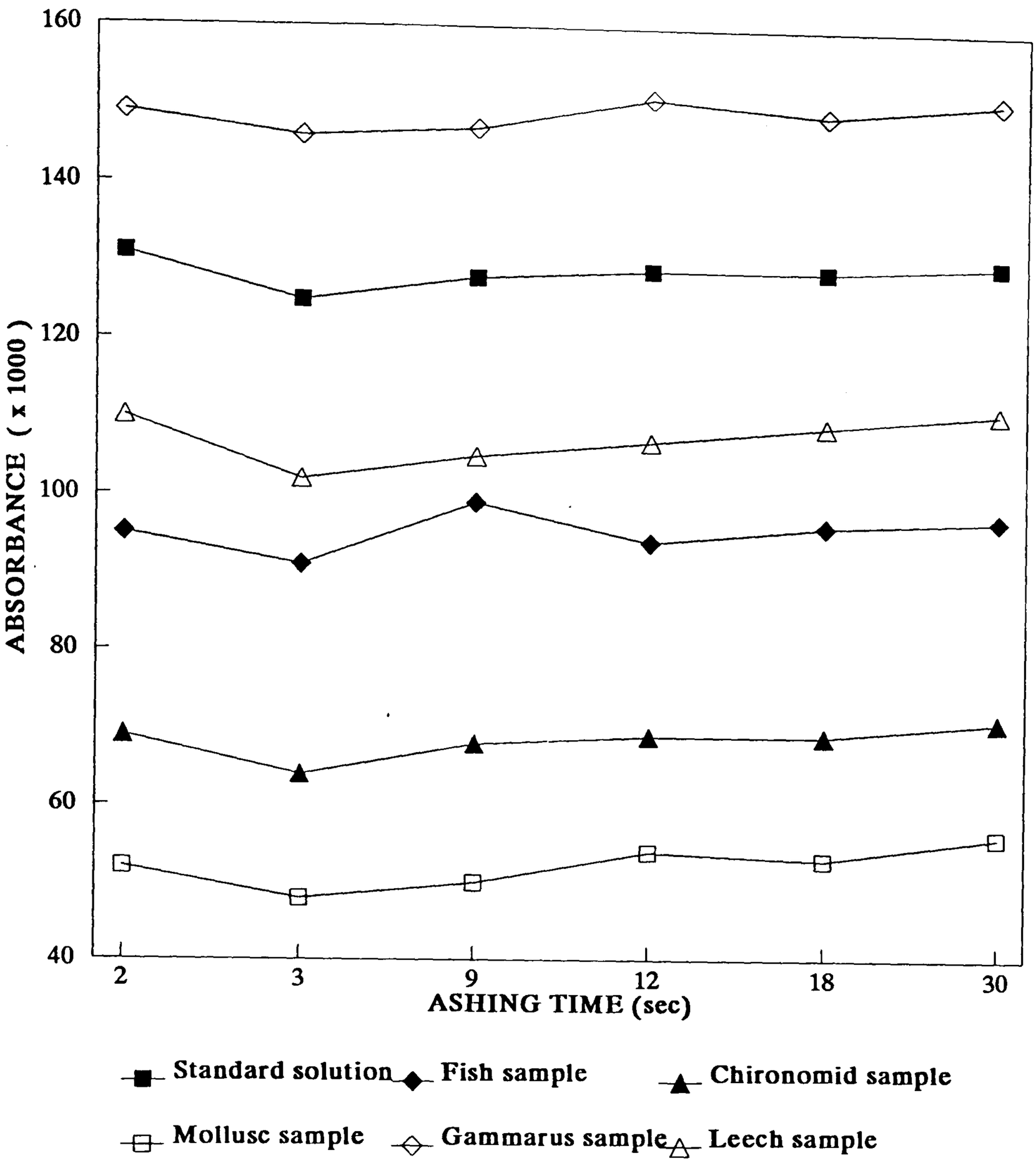



**Figure 2.7** Comparison of Cu absorbance values for various types of samples with different ashing times at 900$^{o}$C of ashing temperature . (The original data are listed in Appendix A.1).

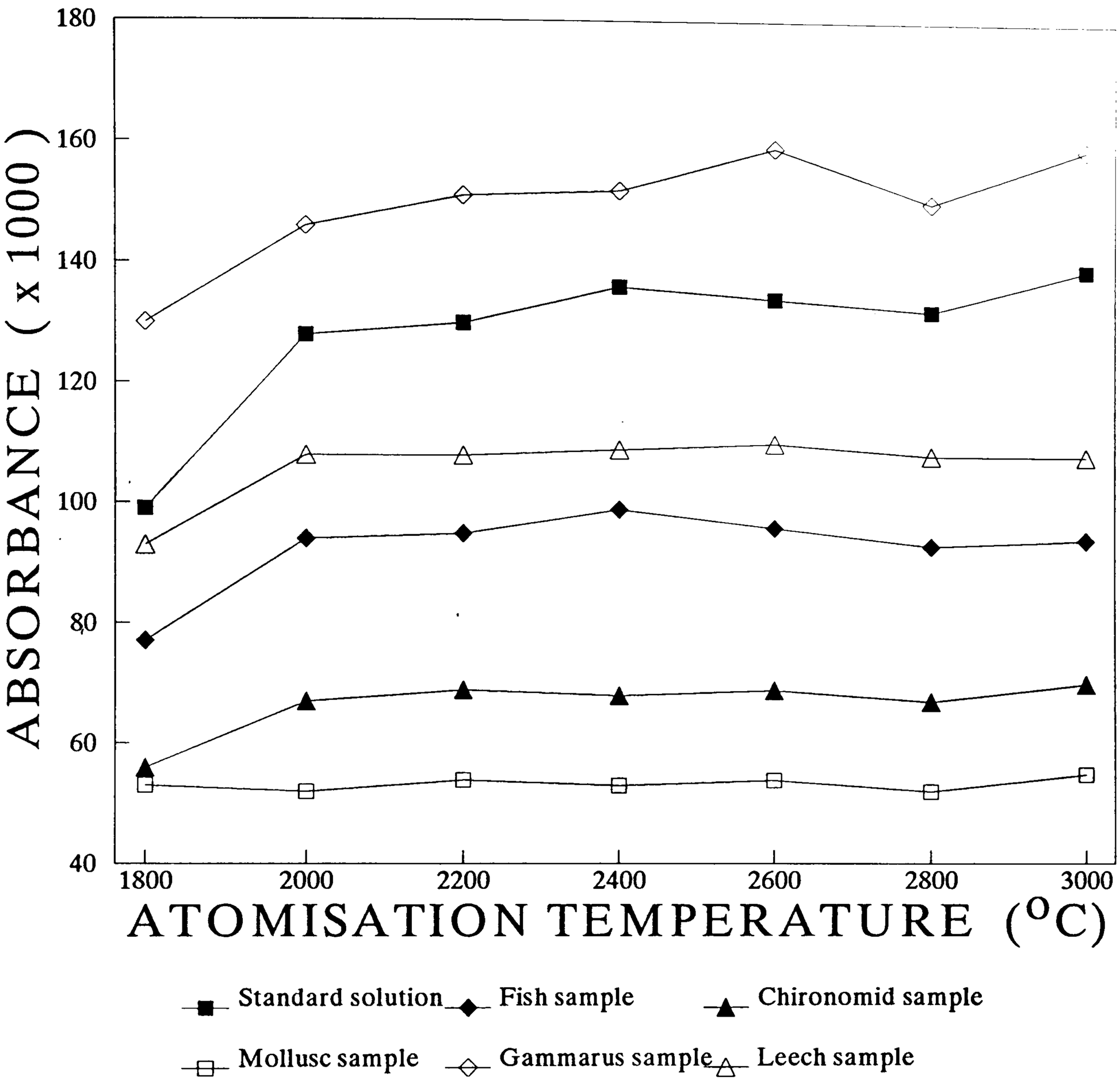



**Figure 2.8** Comparison of Cu absorbance values for various types of samples with different atomisation temperatures. (The original data are listed in Appendix A.2).

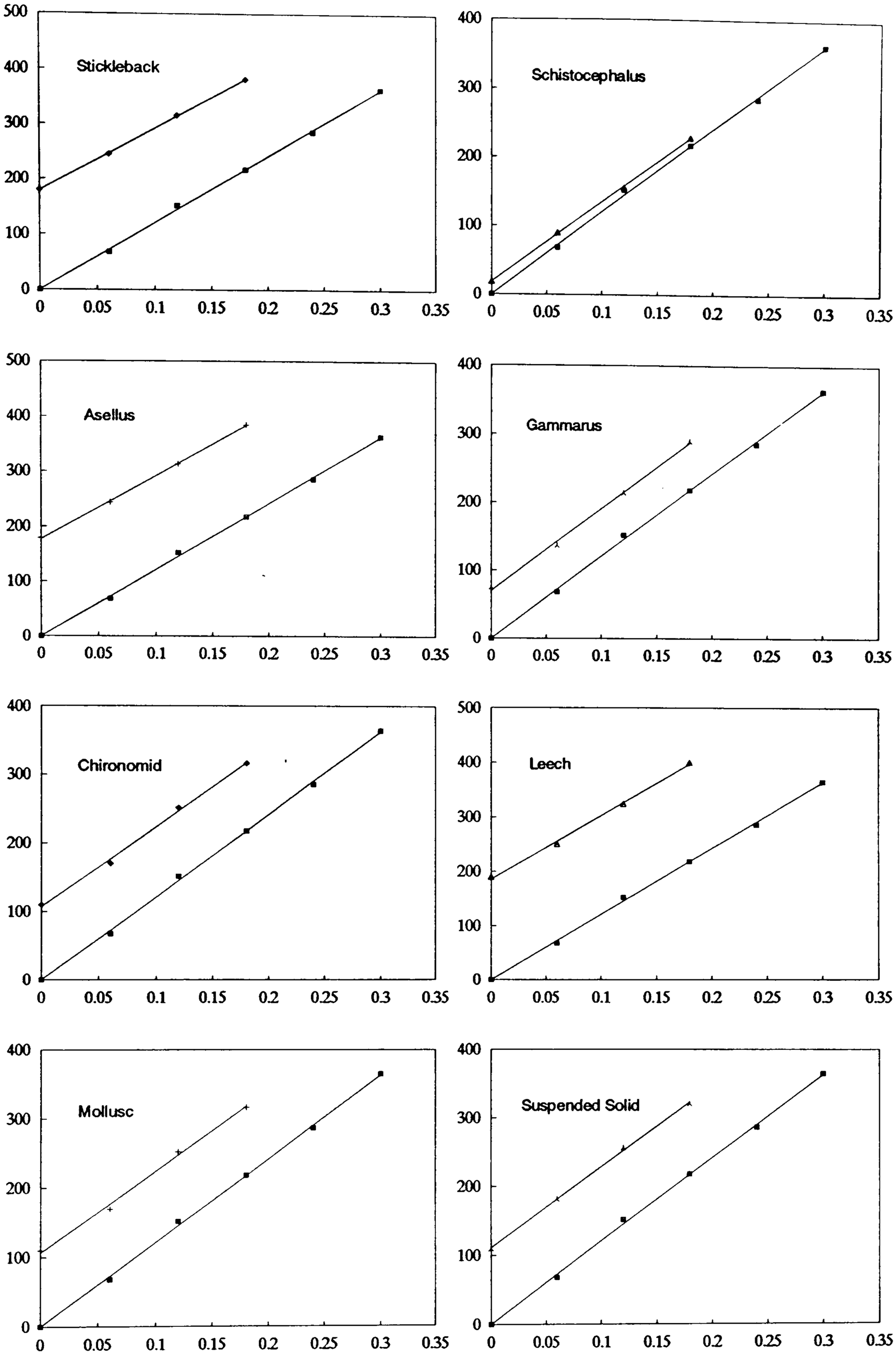



**Figure 2.9** Standard addition curves of various types of samples in the copper graphite furnace programme.

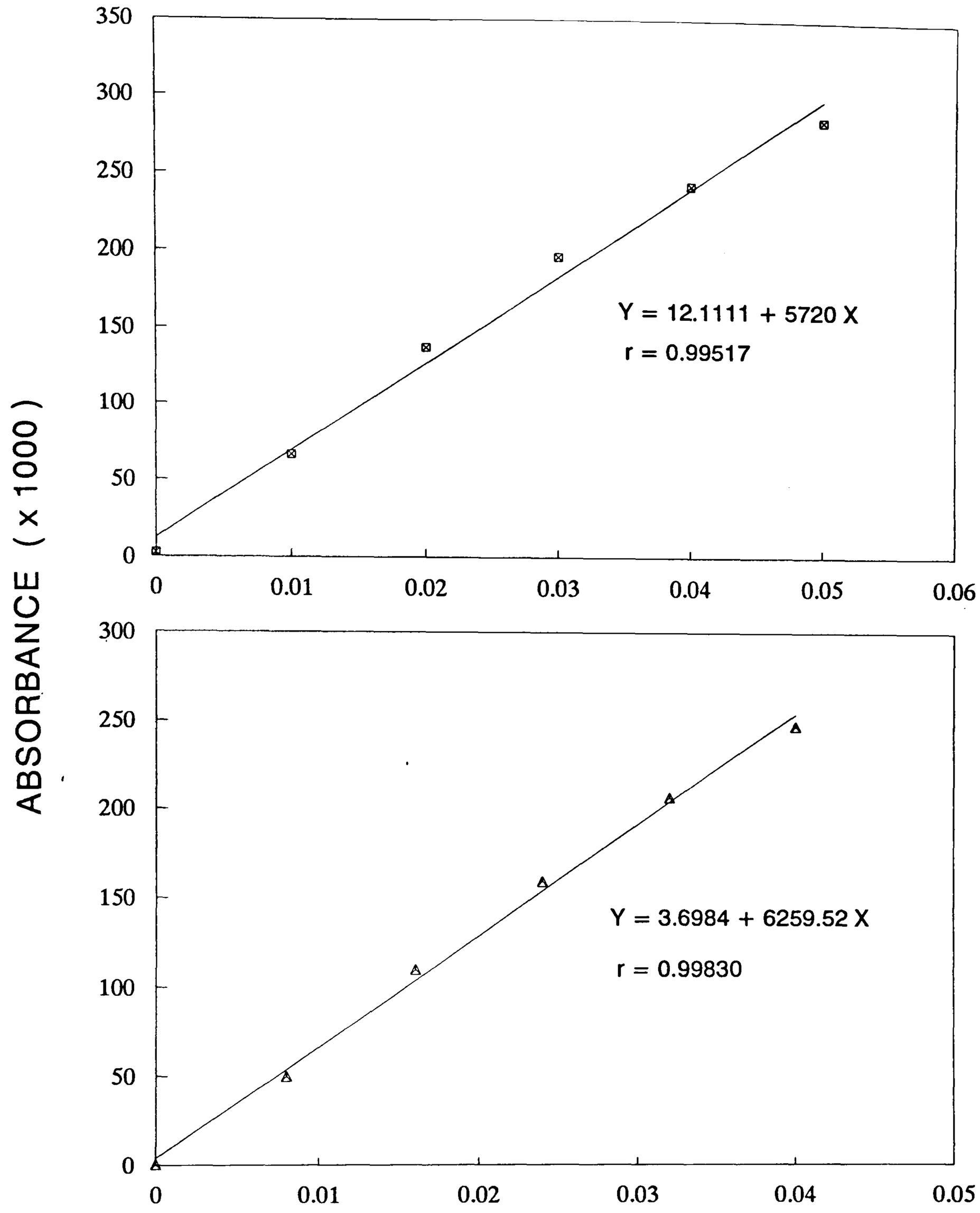



**Figure 2.10** Typical standard curves for cadmium by electrothermal atomisation. (The original data are listed in Appendix A.3).

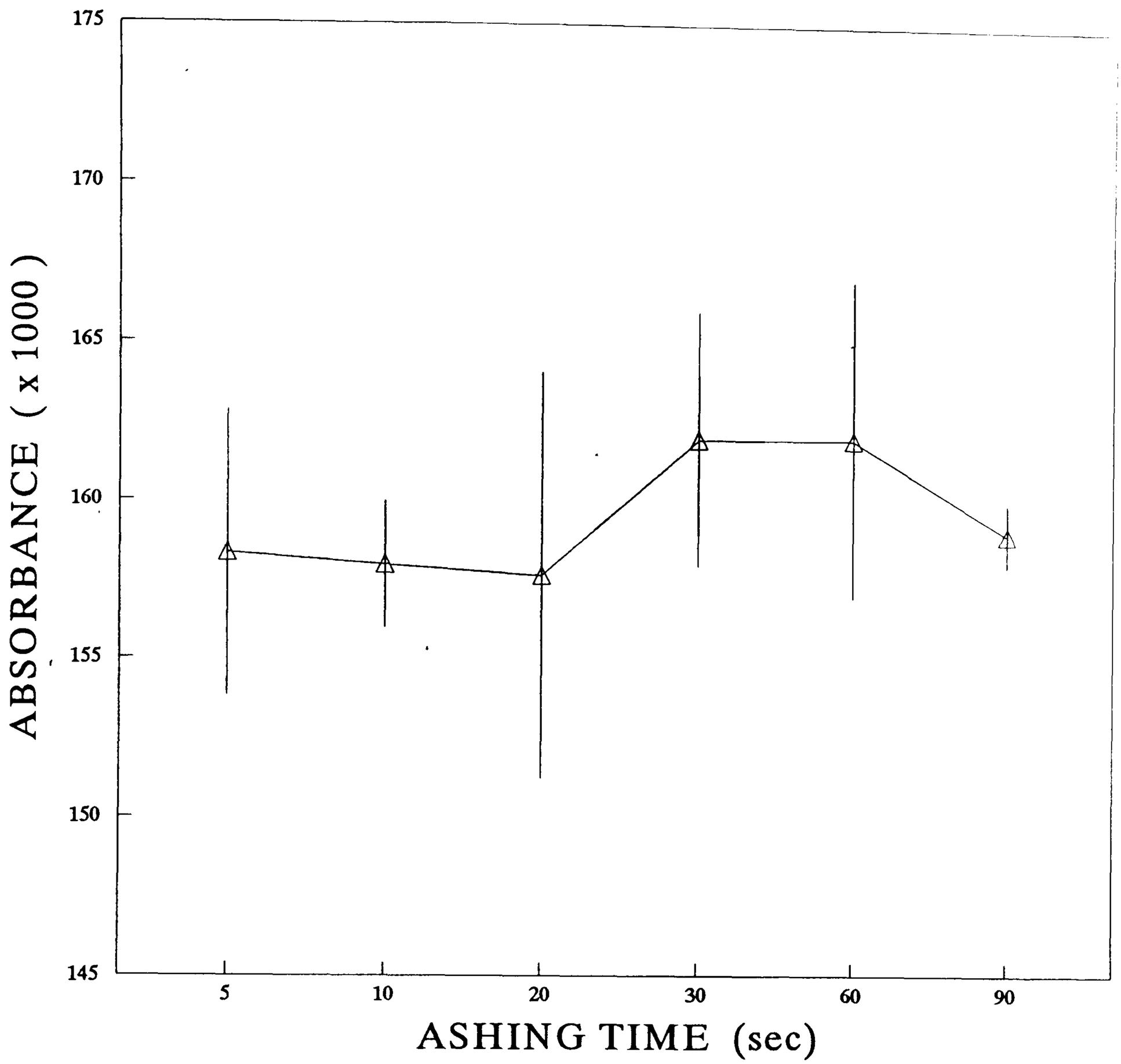



**Figure 2.11** Comparison of Cd absorbance values with different ashing times at 300$^{o}$C of ashing temperature. The vertical lines indicate the standard deviation; the triangles indicate the mean values.

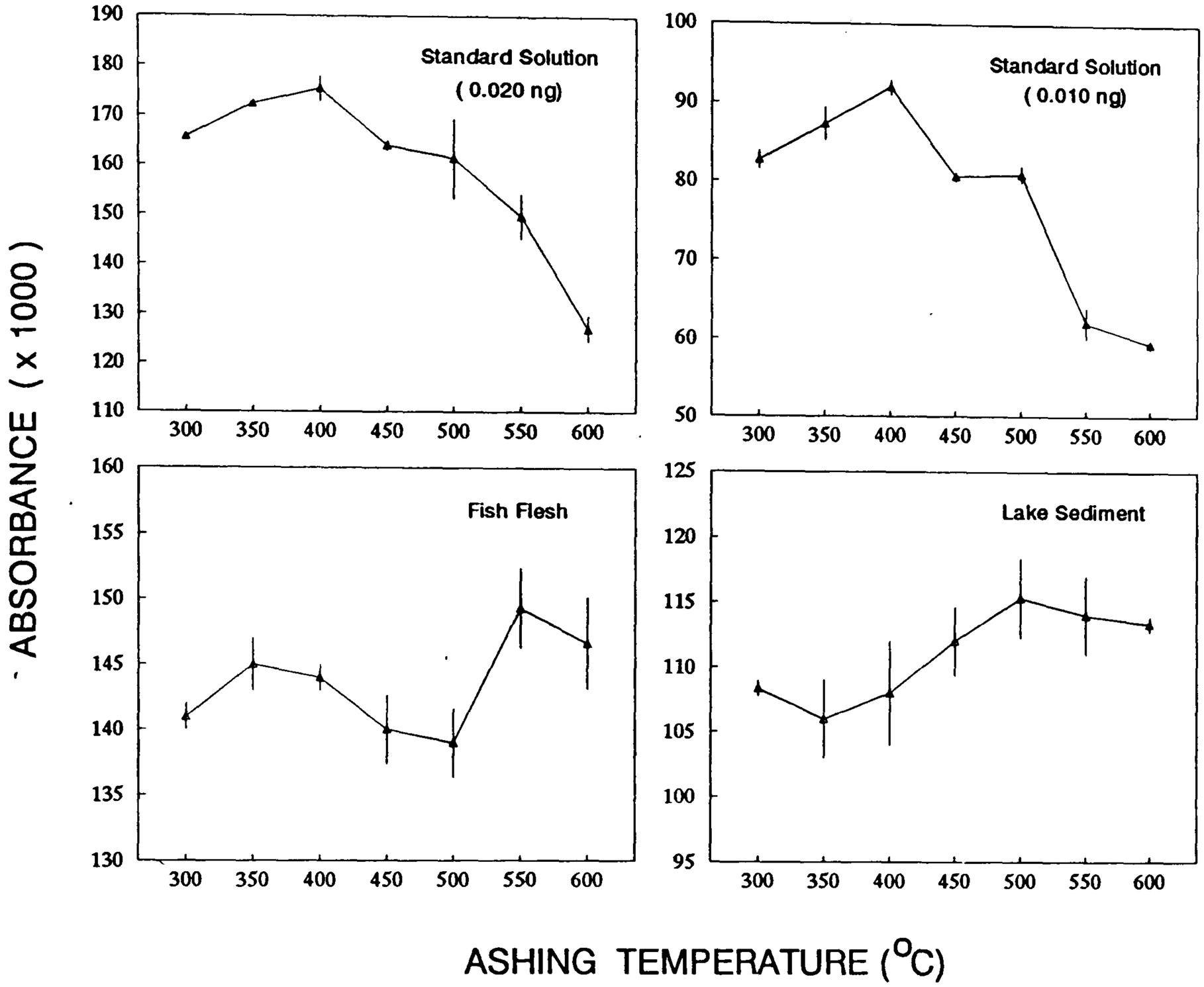



**Figure 2.12** Comparison of Cd absorbance values for various types of samples with different ashing temperatures. The vertical lines indicate the standard deviations; the triangles indicate the mean values.

analysis of Cd in $HNO_3$ at 600°C of ashing temperature was reported by Issaq (1979). Figure 2.13 shows that the maximum ashing time at 400°C was 5 to 10 seconds. The best atomisation temperature for Cd was found to be in the range of 1700°C to 2000°C (Figure 2.14). The graphite furnace programme for Cd in the nitric acid dissolved samples was as below:

GRAPHITE FURNACE PROGRAMME FOR CADMIUM DETERMINATION:

| Step | Temp(°C) | Time(sec) | Gas | Flow Rate(l/min) | Read |
|---|---|---|---|---|---|
| 1 | 75 | 5 | $N^2$ | 3 | |
| 2 | 90 | 50 | $N^2$ | 3 | |
| 3 | 120 | 10 | $N^2$ | 3 | |
| 4 | 120 | 5 | $N^2$ | 3 | |
| 5 | 300 | 10 | $N^2$ | 3 | |
| 6 | 400 | 5 | $N^2$ | 3 | |
| 7 | 400 | 1 | $N^2$ | 0 | |
| 8 | 1900 | 0.6 | $N^2$ | 0 | * |
| 9 | 1900 | 2 | $N^2$ | 0 | * |
| 10 | 1900 | 2 | $N^2$ | 3 | |
| 11 | 40 | 20 | $N^2$ | 3 | |

* indicated setting for absorbance reading.

Using the above programme for a series of standard additions with different types of samples, it was found that several samples such as *Gammarus*, stickleback, *Schistocephalus*, and lake sediment gave significantly non-parallel line when they were compared to standard curves (Figure 2.15). It was significant that during the atomisation stage of these samples smoke fumes were emitted, indicating incomplete breakdown of organic matter. Nevertheless, plants samples, i.e. *Spirogyra*, *Callitriche*, showed a good degree of parallelarity.

Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) has been used as chemical modifier in determination of Cd in order to form a more stable complex of Cd that would withstand higher ashing temperature (Rothery 1982, Andersson and Borg 1988). Addition of 1%, 0.5% or 0.25% of $H_3PO_4$ equal to or half the volume of the sample was tested in the GTA-Cd programme. With the exception of the least usage of 3 ul of 0.25% of $H_3PO_4$, the addition of modifier caused significant smoking at the stage of ashing and atomisation.

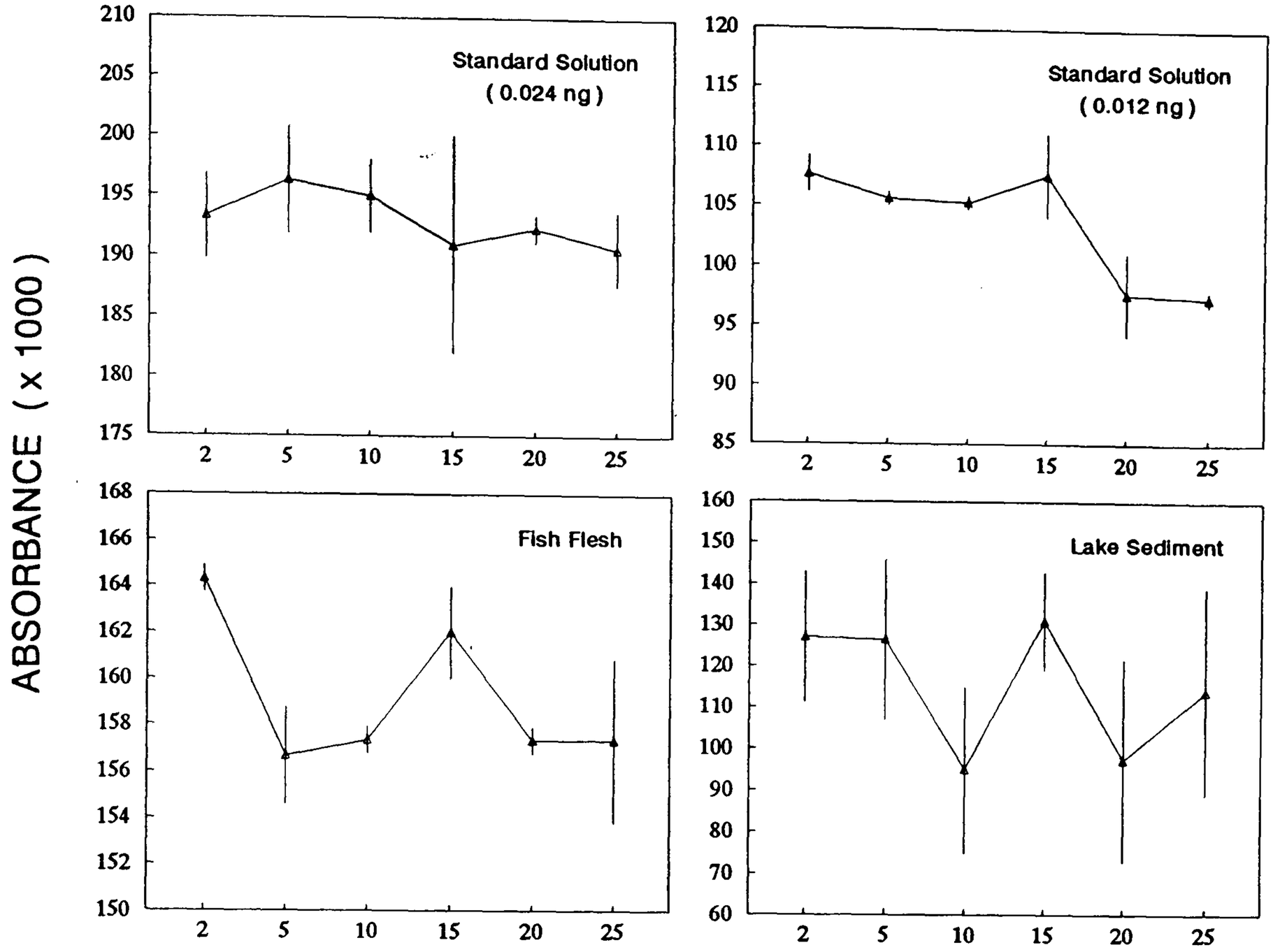



**Figure 2.13** Comparison of Cd absorbance values for various types of samples with different ashing times at 400$^{o}$C of ashing temperature. The vertical lines indicate the standard deviations; the triangles indicate the mean values.

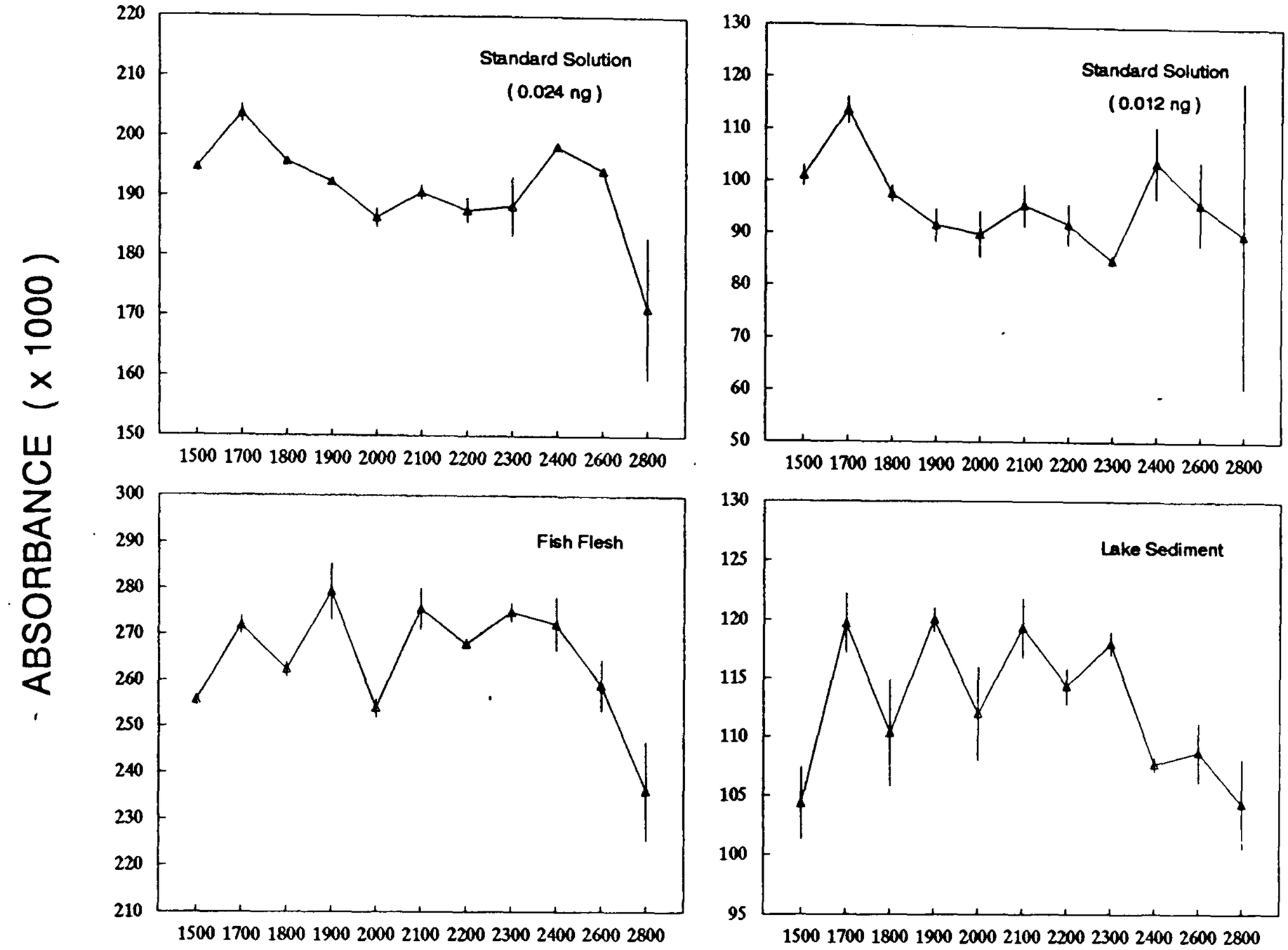



**Figure 2.14** Comparison of Cd absorbance values for various types of samples with different atomisation temperatures. The vertical lines indicate the standard deviations; the triangles indicate the mean values.

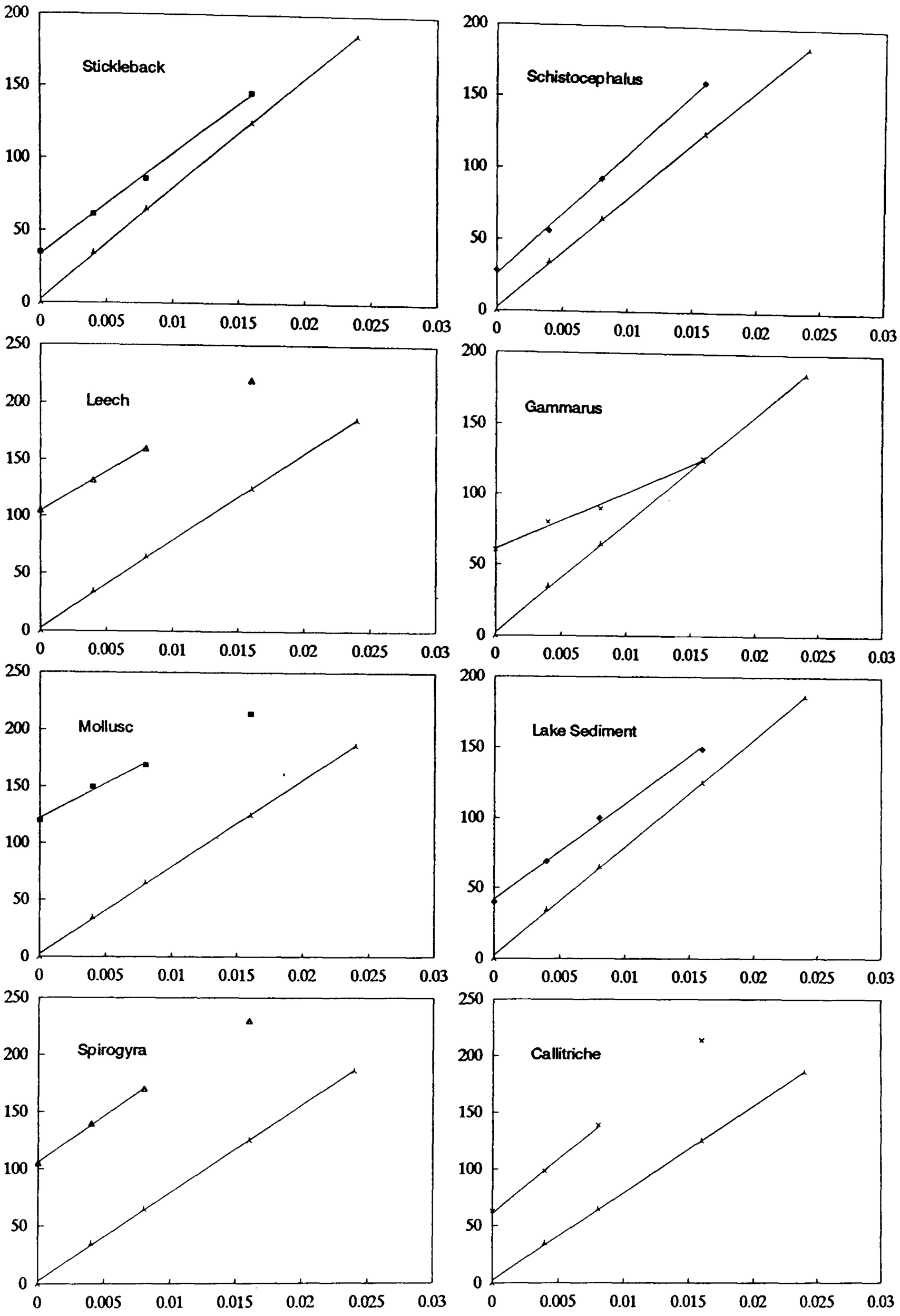



**Figure 2.15** Standard addition curves of various types of samples in the cadmium graphite furnace programme without chemical modifier.

The ashing temperatures were re-examined with the addition of 0.25 % of $H_3PO_4$ and the results revealed that the ashing temperature could be raised to 700°C without any losses of Cd (Figure 2.16). The best atomisation temperature was found to be 1900°C (Figure 2.17). The final cadmium graphite furnace programme using 3 ul of 0.25% $H_3PO_4$ is shown below:

GRAPHITE FURNACE PROGRAMME FOR CADMIUM DETERMINATION (WITH 0.25% $H_3PO_4$ AS CHEMICAL MODIFIER):

| Step | Temp(°C) | Time(sec) | Gas | Flow Rate(l/min) | Read |
|---|---|---|---|---|---|
| 1 | 75 | 5 | $N^2$ | 3 | |
| 2 | 90 | 50 | $N^2$ | 3 | |
| 3 | 120 | 10 | $N^2$ | 3 | |
| 4 | 120 | 5 | $N^2$ | 3 | |
| 5 | 600 | 10 | $N^2$ | 3 | |
| 6 | 700 | 5 | $N^2$ | 3 | |
| 7 | 700 | 1 | $N^2$ | 0 | |
| 8 | 1900 | 0.6 | $N^2$ | 0 | * |
| 9 | 1900 | 2 | $N^2$ | 0 | * |
| 10 | 1900 | 2 | $N^2$ | 3 | |
| 11 | 40 | 20 | $N^2$ | 3 | |

* indicated setting for absorbance reading.

Using the above programme a variety of different types of samples were determined by standard additions (Figure 2.18). All the samples tested gave parallel calibrations between the sample standard addition curves and standard curves, except for the lake sediment.

## 2.4 DISCUSSION

The techniques developed in this study were checked by using five standard reference materials: Lake Sediment (SL-1), Fish Flesh (MA-A-2/TM), and Dried Copepoda (MA-A-1/TM) from IAEA (International Atomic Energy Agency, Analytical Quality Control Services, Austria); and Pond Sediment (NIES No.2) and Tea Leaves (NIES No.7) from NIES (National Institute for Environmental Studies, Japan), in order to

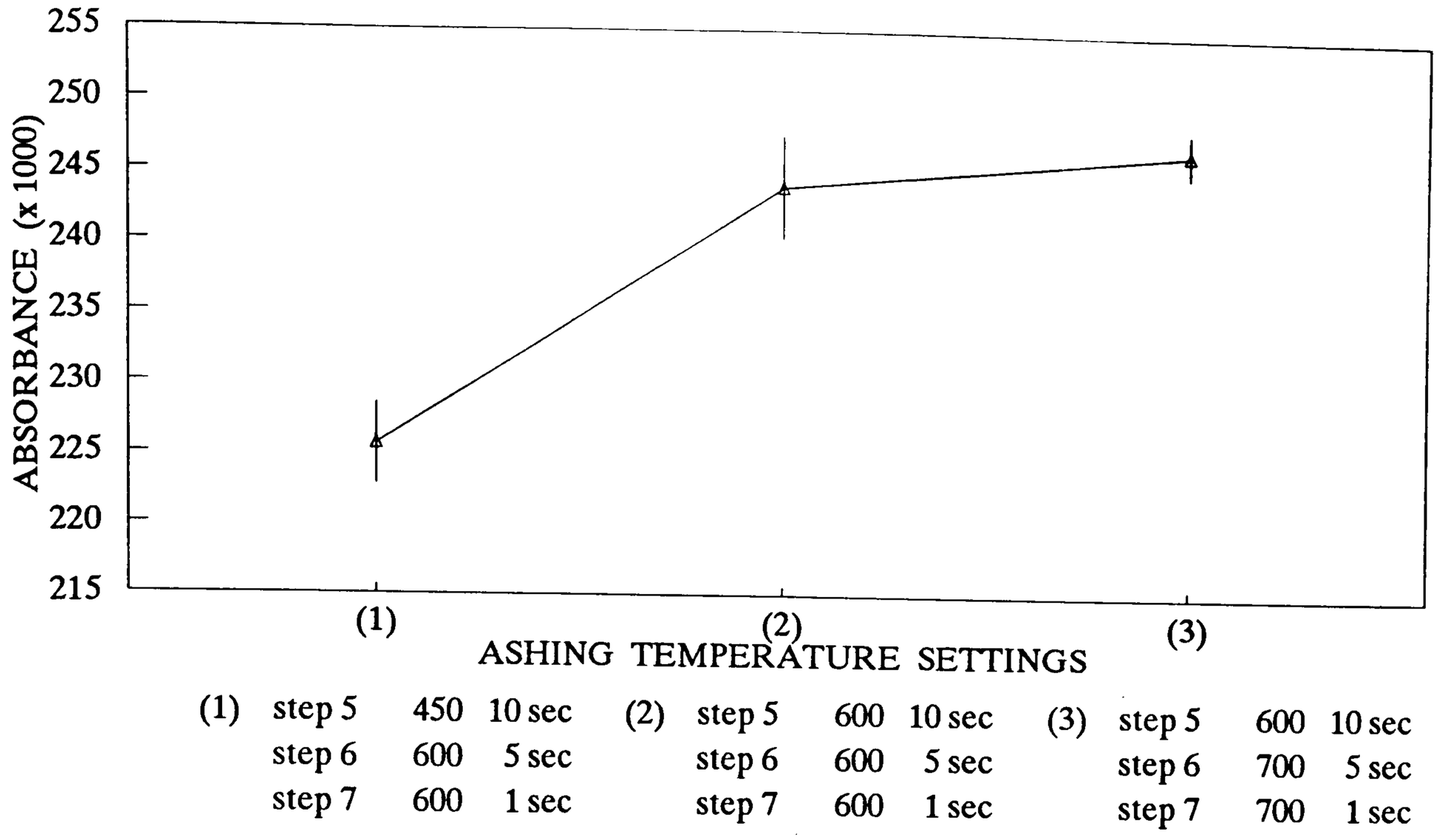



**Figure 2.16** Comparison of Cd absorbance values with 0.25 % $H_3PO_4$ as chemical modifier at different ashing temperature settings. (The original data are listed in Appendix A.4).

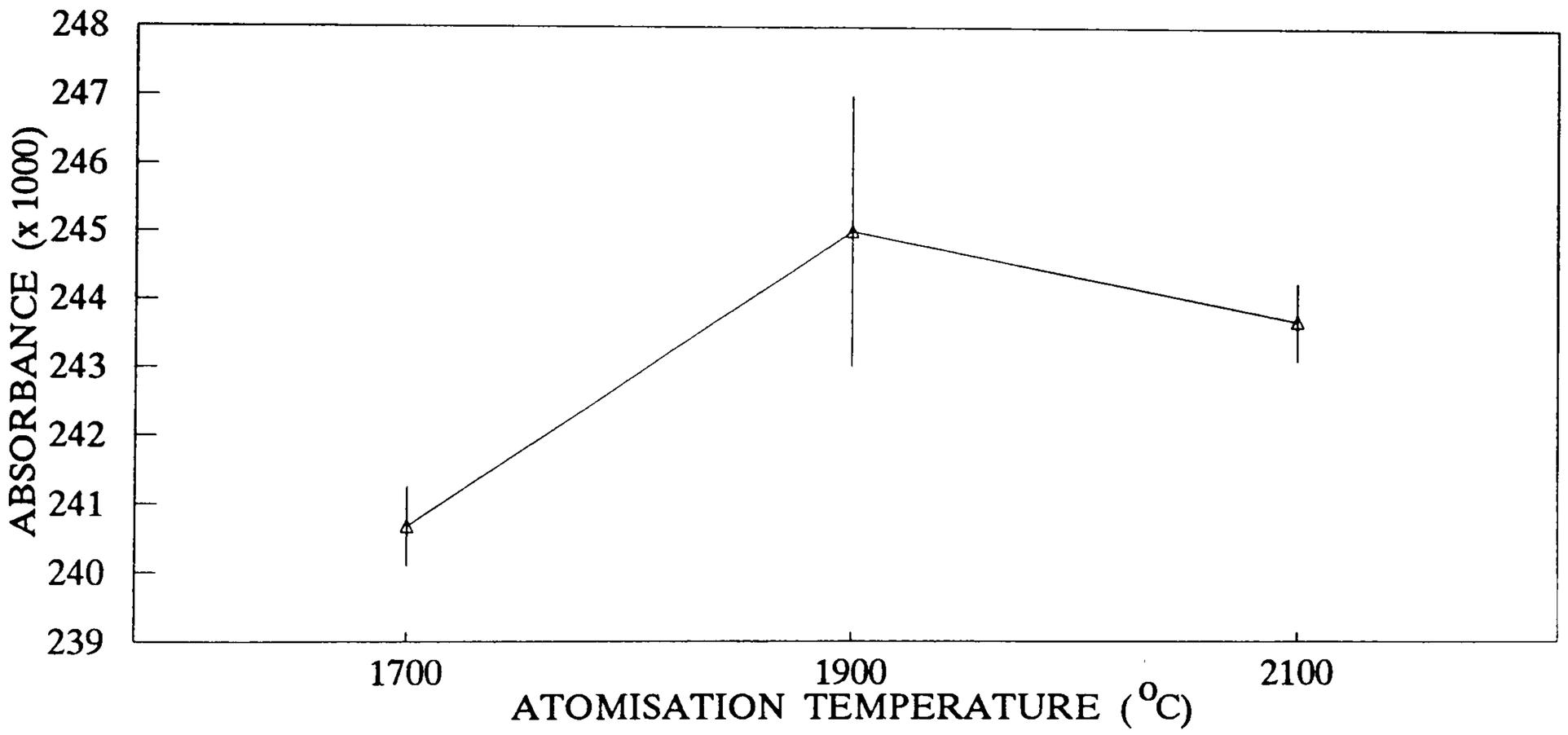



**Figure 2.17** Comparison of Cd absorbance values with 0.25 % $H_3PO_4$ as chemical modifier at different atomisation temperatures. (The original data are listed in Appendix A.4).

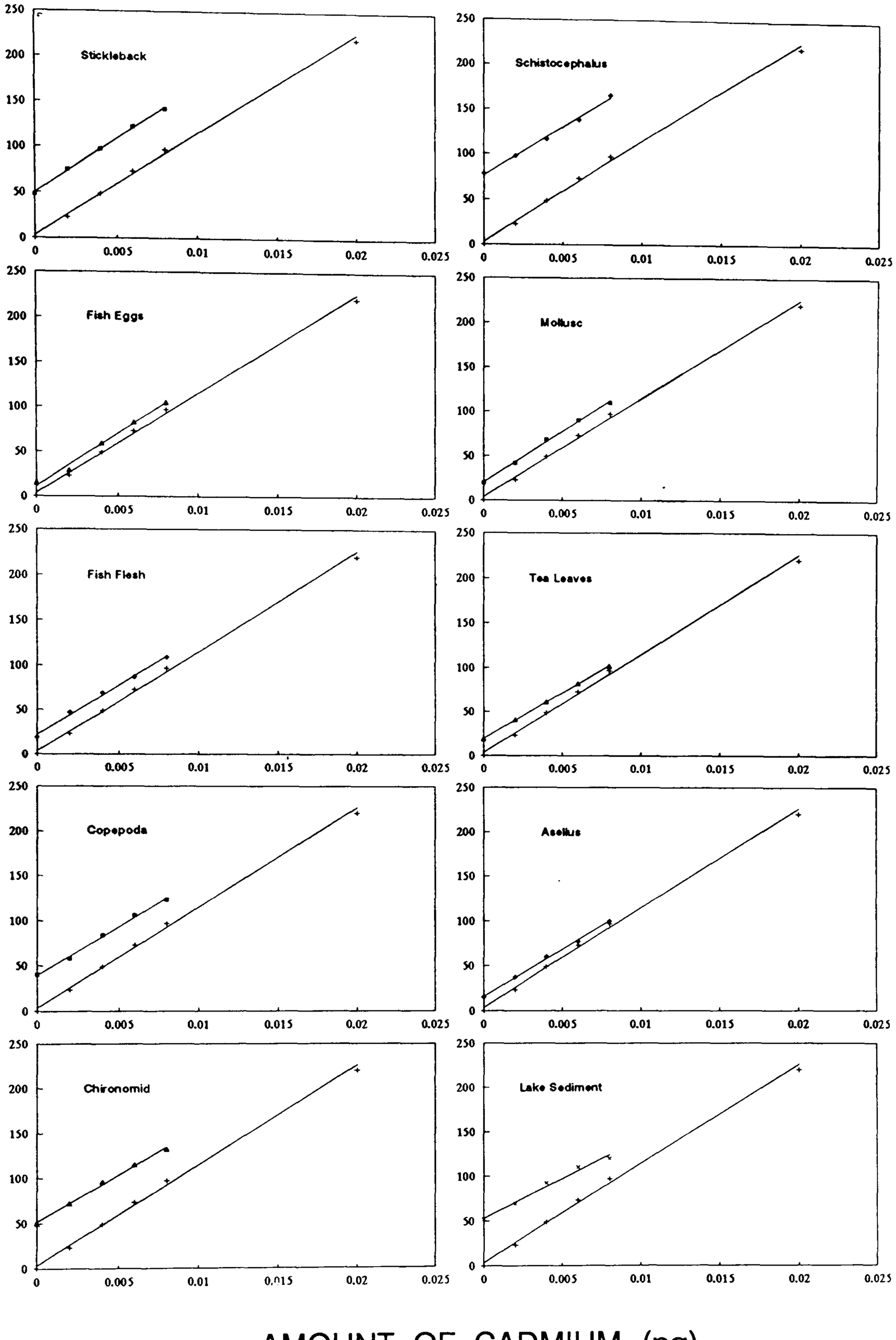



**Figure 2.18** Standard addition curves of various types of samples in the cadmium graphite furnace programme with 0.25 % $H_3PO_4$ as chemical modifier.

check the degree of analytical accuracy (i.e. the closeness of the measured value to the certificated or accepted value).

Table 2.1 shows the comparisons of the measured values and the certificated values of the standard materials. With one exception, the copper values in the different samples are within 5% of the confidence limits of the certificated values. Pond sediment, NIES No.2, is within 10% of the confidence limit. The cadmium values of Tea Leaves, NIES No.7 and Pond Sediment, NIES No.2, are within 5 % confident limit of the certificated values, but for lake sediment and fish flesh the values were on average 16% greater than the certified values, but for copepods it was greater than 45 %.

In conclusion there was very little difficulty in developing a suitable graphite furnace programme for Cu. However, Cd proved more difficult. Essentially this is because it is a more volatile element and the ashing temperature that can be used without losing any Cd from the samples are restricted. Using a modifier of $H_3PO_4$ improved the programme to considerable extent and it is concluded that only Copepod data should viewed with uncertainty. However because the actual study was comparative, it was decided to proceed with the above developed methods. It was later found that addition of the surfactant Triton X-100 together with the chemical modifier further improved the precision of the analysis.

**Table 2.1** The results of validation by various standard materials. (The original data are listed in Appendix A.5).

| | | | COPPER (ug/g dw) | | | | | CADMIUM (ug/g dw) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard Material | Values | Method | No. | Mean | S.D. | R% | Method | No. | Mean | S.D. | R% |
| Fish Flesh | Certificated | | | 4.00 | 0.10 | | | | 0.066 | 0.004 | |
| IAEA MA-A-2/TM | This study | Flame | 8 | 4.02 | 0.22 | 100.38 | | | – | – | |
| | This study | GTA | 8 | 3.92 | 0.13 | 98.05 | GTA | 8 | 0.077 | 0.008 | 116.36 |
| Copepoda | Certificated | | | 7.60 | 0.20 | | | | 0.75 | 0.03 | |
| IAEA MA-A-1/TM | This study | Flame | 8 | 7.30 | 0.34 | 95.99 | GTA | 8 | 1.09 | 0.04 | 145.47 |
| Tea Leaves | Certificated | | | 7.00 | 0.30 | | | | 0.030 | 0.003 | |
| NIES No.7 | This study | Flame | 8 | 6.69 | 0.12 | 95.57 | GTA | 8 | 0.032 | 0.005 | 105.67 |
| Lake Sediment | Certificated | | | 30.00 | 5.00 | | | | 0.26 | 0.05 | |
| IAEA SL-1 | This study | Flame | 16 | 28.55 | 1.49 | 95.17 | Flame | 3 | 0.30 | 0.06 | 116.92 |
| Pond Sediment | Certificated | | | 210.00 | 12.00 | | | | 0.82 | 0.06 | |
| NIES No.2 | This study | Flame | 6 | 192.71 | 1.52 | 91.77 | Flame | 6 | 0.86 | 0.13 | 104.88 |

Flame means flame AAS. GTA means graphite furnace AAS

# CHAPTER THREE

# PILOT STUDY AND SAMPLING SCHEME

## 3.1 THE RIVER WANDLE AND ITS FISHES

The River Wandle is the largest of London's Metropolitan water courses draining into the Thames. It arises from springs, the largest of these being found at Carshalton and Croydon, and then flows northwards for about 10 miles through a densely populated area with high industrial development and joins the Thames at Wandsworth. In the early urban development of London, the Wandle attracted many factories which were built along its banks for deriving power from the river. Up to 90 water mills were once found along its length.

In the 18th century with its abundant clean chalk water it was a trout river, containing *Salmo trutta* (L.), together with gudgeon, *Gobio gobio*, and minnow, *Phoxinus phoxinus* (Wheeler 1957 in Wheeler 1979). Accompanying the southward development of London's population, however housing and industry progressively polluted the river. In the 1930s the water quality had greatly deteriorated. The increased population made heavy demands on potable supplies from the ground water which reduced the natural springs supplying the River. At the same time increasing quantities of sewage effluent were discharged from works at Beddington, Wandle Valley and Wimbledon. All these works allowed partially treated sewage to enter the river which at times could account for 90% at the average river flow. Finally industrial discharges contributed to the general decay of river quality, and for much of its length the Wandle became an "open metropolitan sewer". The bullhead, *Cottus gobio* (L.) survived in headstreams and the stickleback, *Gasterosteus aculeatus* remained abundant throughout the area into the 1960s. The survival of the stickleback was undoubtedly due to its ability to withstand a great deal of pollution (Marlborough 1963). Between 1962 to 1968 there was no record of fish in the River Wandle (Marlborough 1965, 1969).

Since the early 1960s, considerable progress has been made in cleaning up the Wandle. Several small old sewage works were closed and their flow diverted to Crossness works. Industrial discharges now

go to the sewage system rather than directly to the river. Beddington sewage works, which serves Croydon, has been rebuilt and extended and is now the main sewage effluent discharge. Currently, its flow is only about 25 million gallons per day (mgd ).

Given its historical background of pollution and ongoing urban and light industrial nature with one major input of sewage effluent and many miscellaneous sources from seepages, spills and storm drains of organic and industrial materials, the River Wandle provides an ideal river system in which to investigate the effects of a constant input of pollutants on the aquatic ecosystem in general and on fish populations. Since stickleback were reported to be widely distributed in the Wandle in recent years it was considered that a detailed study of these fish would provide an insight into current water quality impacts.

## 3.2 A PILOT STUDY

### 3.2.1 Introduction

Good sampling design is a key to success in a ecological research. A PILOT study of over one year was carried out, to discover if and where fish lived in the river and which sites had value for research purposes.

Along the river, 11 sampling sites, marked A to J, from upstream to downstream (Figure 3.1) were identified. These were selected to be representative of the river along its course, but in part the selection was governed by ease of access. Views of each site are shown in Plate 3.1 to Plate 3.11. The site F is located close to the Beddington sewage effluent channel. Site A+ is a site just downstream of a factory.

The initial survey, from December 1987 to June 1989, included attempts to catch fishes, together with the measurements of water temperature, conductivity, pH and total hardness. Where stickleback populations were found, they were sampled regularly. Samples of sticklebacks were placed into their correct morphs by counting lateral scutes along the flanks (Wootton 1976), and total lengths were measured and sexes determined. Sediment samples from the river bed, water samples and sticklebacks were taken for metal analysis.

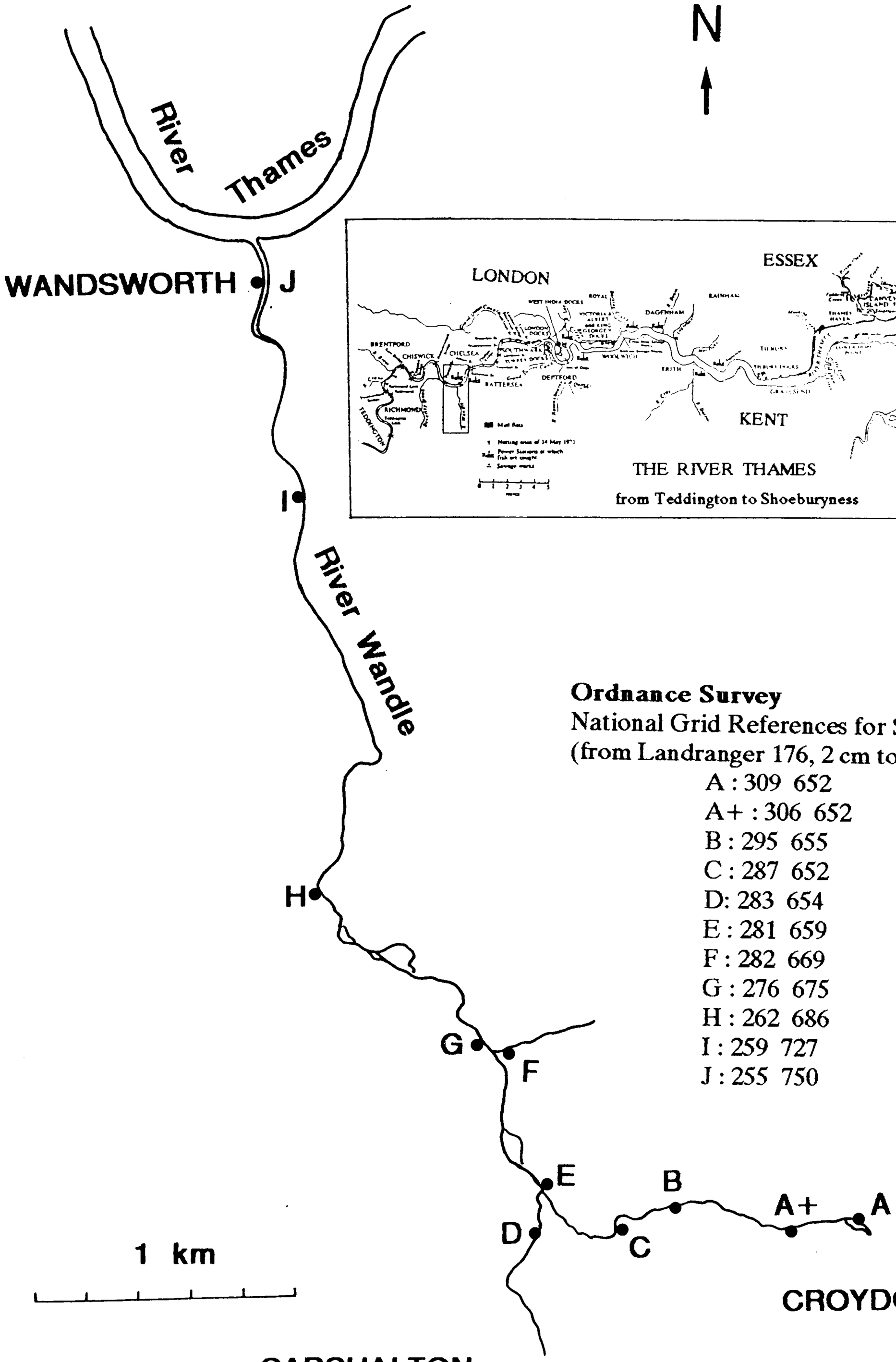



**Figure 3.1** A map shows the 11 sampling sites in the River Wandle.



Plate 3.1 A view of sampling site A, Waddon pond, in the River Wandle.



Plate 3.2 A view of sampling site A+, at Croydon Arm just below Waddon pond at Aldwich Rd., in the River Wandle.




Plate 3.3 A view of sampling site B, at Croydon Arm beside Church Rd. at Beddington Park, in the River Wandle.




Plate 3.4 A view of sampling site C, at Croydon Arm beside London Rd. outside Manor Garden, in the River Wandle.




Plate 3.5 A view of sampling site D, at Carchalton Arm below Wandle Rail Bridge at Mill lane, in the River Wandle.




Plate 3.6 A view of sampling site E, at the junction of two Arms beside Hack Bridge, in the River Wandle.




Plate 3.7 A view of sampling site F, Beddington sewage effluent channel at Goat Road.




Plate 3.8 A view of sampling site G, below the junction of Beddington sewage effluent channel to the River Wandle.

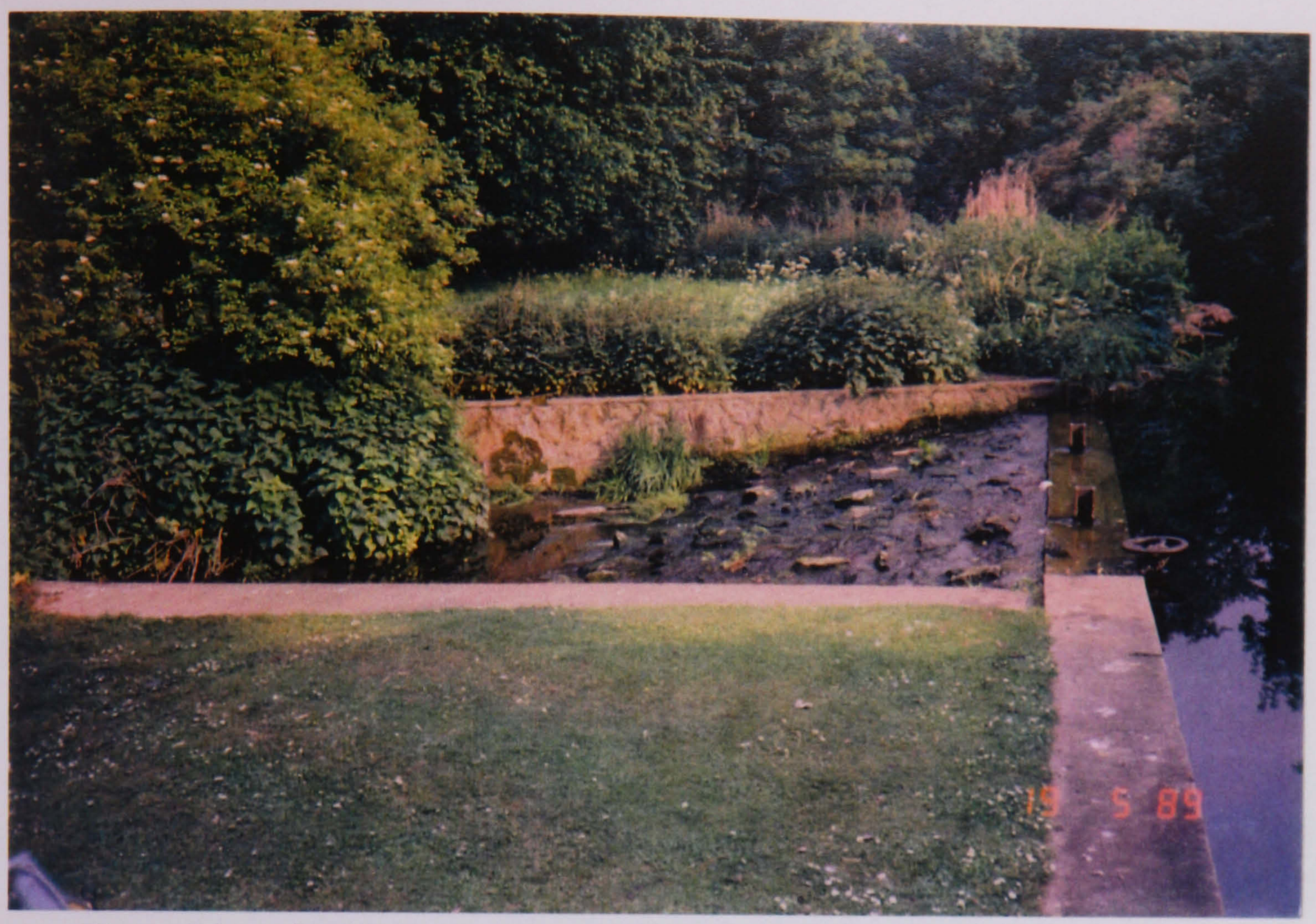



Plate 3.9 A view of sampling site H, a section of the River Wandle beside Morden Cottage at Morden Hall Park.




Plate 3.10 A view of sampling site I, a section of the River Wandle beside Steerforth street at Summertown.



Plate 3.11 A view of sampling site J, a section of the River Wandle above the mouth of the river to The Thames at Wandsworth.

The methods for determination of metals were as described in Chapter 2.

### 3.2.2 The Results

(A) Resistant fish species

Three-spined stickleback, *Gasterosteus aculeatus* L., was the most abundant fish species in the River Wandle, accompanying with 9-spined stickleback, *Pygosteus pungitius* L., and bullhead, *Cottus gobio* in the middle section of sampling sites, C, D, and E, where the *G. aculeatus* and *P. pungitius* always coexist. Stone loach, *Noemacheilus barbatulus* (L.), and eel, *Anguilla anguilla* (L.) were occasionally caught at various sites (Table 3.1).



BIBL. LONDIN. UNIV.

**Table 3.1** Major fish species observed in the River Wandle between Jan. 1988 and Jul. 1989. The numerical basis of classification: +++++ = fish were abundant, ++++ = fish were frequent, +++ = fish were common, ++ = fish were occasional, + = fish were rare, and - = no fish were found.

| Site | *Gasterostus aculeatus* | *Pygosteus pungitius* | *Cottus gobio* | Other fish spieces which were occasionally found |
|---|---|---|---|---|
| A+ | – | – | – | |
| B | ++++ | – | – | |
| C | +++++ | ++ | ++ | |
| D | +++++ | + | ++ | *Noemacheilus barbatulus* |
| E | +++++ | +++ | – | |
| F | – | – | – | |
| G | ++ | ++ | – | |
| H | +++++ | – | – | |
| I | ++ | – | – | *Anguilla anguilla* |
| J | – | – | – | |

(B) <u>The water quality</u>

The day time water temperature ranged from 8 to 19.2$^{o}$C and the seasonal variation is shown in Figure 3.2. The pH value ranged from 6.6 to 8.4, the conductivity from 270 to 2390 $\mu$s/cm, with site F the sewage works effluent always showing the highest values. The total water hardness ranged from 140 to 310 $CaCO_3$ mg/l, and the percentage of dissolved oxygen from 48 to 120 %, with site F having the lowest measurement (see Appendix B.1).

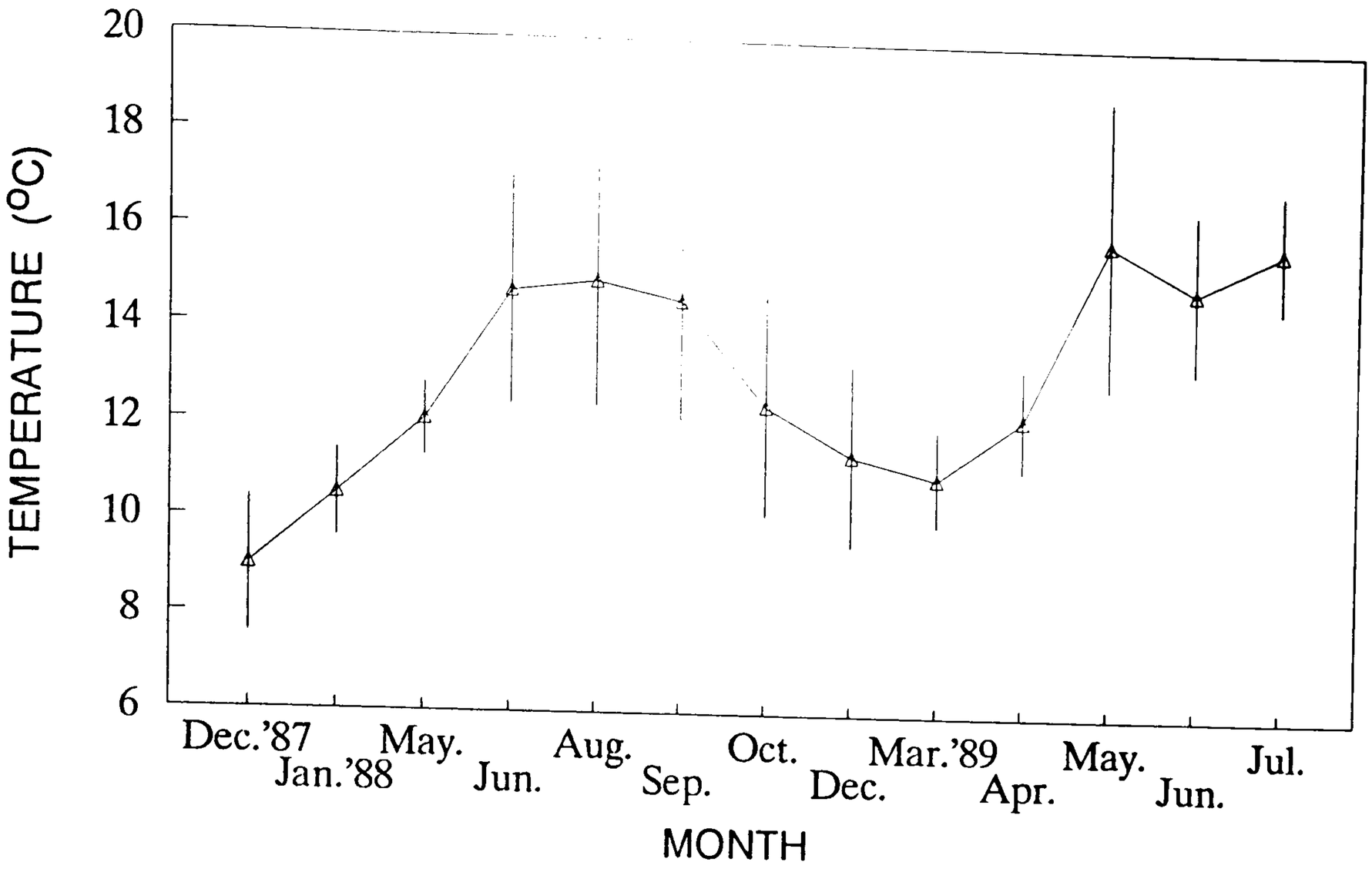



**Figure 3.2** Day-time water temperature, sampled on a monthly basis from December 1987 to July 1989.
The vertical lines indicate the standard deviations.

(C) <u>The biology of Wandle's 3-spined stickleback</u>

The examination of the lateral scutes along the flanks of the 3-spined stickleback followed the conservative criteria recommended by Wootton (1976). The morphs are distinguished by the number of the lateral scutes and the presence of a caudal keel. The 'trachurus' morph has a caudal keel and a complete row of lateral scutes, stretching from just in front of the pectoral fin to the tail, which normally consists of 30 to 35 plates. The 'leiurus' morph has only a few lateral plates in the anterior region of the body and lacks a keel in the caudal peduncle. Its lateral plates number 1 to 9 in a row or occasionally they are absent. The morphological characters of the 'semiarmatus' are fall between those of the previous two morphs. It presents a caudal keel and 8 or 9 to 30 plates along the flanks.

The frequency distribution of the different morphs of stickleback in the River Wandle is shown in Table 3.2. Since the different morphs

**Table 3.2** The frequency of the different morphs at each sampling sites in the River Wandle from Jan. 1988 to Oct. 1988.

| Site | Leiurus | Semiarmatus | Trachurus | Sum | Leiurus's percentage |
|---|---|---|---|---|---|
| B | 179 | 4 | 0 | 183 | 97.81 |
| C | 326 | 17 | 3 | 346 | 94.22 |
| D | 567 | 30 | 0 | 597 | 94.97 |
| E | 272 | 4 | 1 | 277 | 98.19 |
| G | 225 | 9 | 0 | 234 | 96.15 |
| H | 214 | 7 | 0 | 221 | 96.83 |
| I | 100 | 7 | 0 | 107 | 93.46 |

reflect the different ecological habits, both the 'trachurus' and 'semiarmatus' could be a landlocked or anadromous populations. However the 'leiurus' morph characterises a solely landlocked population. The stickleback populations in the River Wandle were ascertained to be predominantly a 'leiurus' population, mixed with a very small proportion of 'semiarmatus' morphs. This strongly suggests that the Wandle sticklebacks are resident populations which live in the freshwater river throughout the year.

The length frequency distribution of 3-spined sticklebacks on each sampling occasion at various sites is given in Figure 3.3. The results indicate the life cycle and growth pattern of the populations at the different sites. Typically an overwintering $0^+$ age groups (modal lengths 30-34 mm TL) is apparent in the histograms for January and March. The modal right hand skew of the length-frequency distribution is indicative of considerable variation in the $0^+$ growth achieved and overlap with the surviving $1^+$ and older age groups fish. New generations recruit in late June samples so that spawning occurs in May and early June. The new recruits can be followed through their summer growth with increasing modal lengths at sites B, E, G and H. At site C this generation is accompanied by strong survival of the parental (new $1^+$ age group) stock. No satisfactory interpretation for June to October at site D can be made. A more detailed examination of length-frequency distribution at three selected sites is made using Cassie curve analysis in Chapter 4.

Sticklebacks were examined for infection by the plerocercoid stage of *Schistocephalus solidus* L.. The incidence of infestation varied between 3.37% to 15.96% according to the site (Table 3.3).

**Table 3.3** The incidence of infestation by the plerocercoid stage of *Schistocephaus solidus* in the 3-spined stickleback from the River Wandle from Jan. 1988 to Oct. 1988.

| Site | Sample size | No. of Infested stickleback | Incidence of Infestation (%) |
|---|---|---|---|
| B | 183 | 29 | 15.96 |
| C | 346 | 22 | 6.49 |
| D | 597 | 94 | 15.79 |
| E | 277 | 42 | 15.31 |
| G | 234 | 34 | 14.48 |
| H | 221 | 7 | 3.37 |
| I | 107 | 13 | 12.14 |

The sex ratio of fish was also examined. The results reveal that the males were between 36.5% to 48.8% at each sampling site (Table 3.4).

**Table 3.4** Sex ratio and male percentage at each sampling sites in the River Wandle from Jan. 1988 to Oct. 1988. Only the fish that total length larger than 25 mm were examined.

| Site | Male | Female | Sum | Male Percentage |
|---|---|---|---|---|
| B | 44 | 81 | 125 | 35.2 |
| C | 138 | 157 | 295 | 46.8 |
| D | 211 | 367 | 578 | 36.5 |
| E | 79 | 137 | 216 | 36.6 |
| G | 82 | 86 | 268 | 48.8 |
| H | 80 | 89 | 269 | 47.3 |
| I | 38 | 61 | 99 | 38.4 |

(D) Metal levels in the sediment and the water

The different metals examined were copper, cadmium, chromium, nickel, lead, and zinc. The distributions in the sediment samples at each sampling site along the River Wandle are shown in Figure 3.4 - 3.9. Among the upstream sampling sites, where fish were relatively abundant, sites B and E had comparatively elevated metal loadings.

A similar result was found with regard to copper concentrations in the water samples (Figures 3.10 and 3.11).

(E) Metal levels in the 3-spined stickleback

Levels of the different metals were also examined in the stickleback from the river Wandle and Thames. The copper concentration found in the fish from the Wandle were higher than those for the fish from the Thames (Figures 3.12, 3.13, 3.14, and 3.15).

As the dominant fish species in the Wandle, *G. aculeatus* was chosen to be the target organism in the project. It lives in the River Wandle throughout life without migration, superficially at any rate, unaffected by the current pollution levels. However, the copper concentration of fish from the sites containing elevated copper in the sediment were higher than those in fish from the main River Thames. Interesting questions were raised. How do these metals enter the fish and to what extent does the sewage effluent affect to the fish population?

In addition, cadmium which is a non-essential element to organisms by contrast to copper, was chosen to compare pathways of trace elements in an aquatic system.

## 3.3 SAMPLING SCHEME AND PROTOCOL

From the results of the pilot study, three sites were chosen, these were site A: Waddon pond, site B: Church street, and site E: Junction of Croydon Arm and Carshalton Arm, along the River Wandle (see Figure 3.1). These sites were selected as the concentration of copper and cadmium in the water and the sediment were different between sites. Site B and E were chosen as suitable contaminated sites. Sticklebacks were invariably present and these upriver sites were

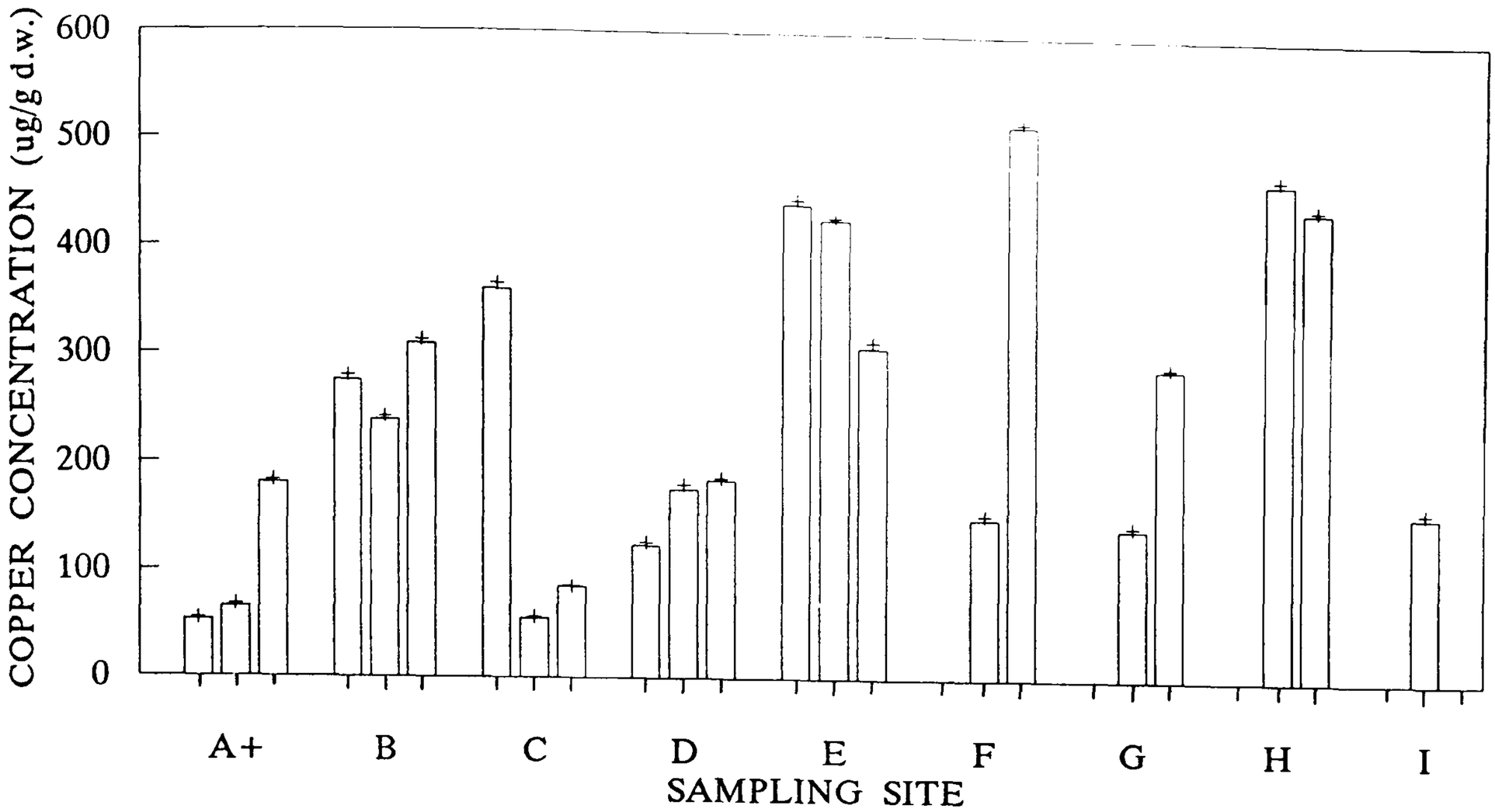



**Figure 3.4** Concentrations of copper in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

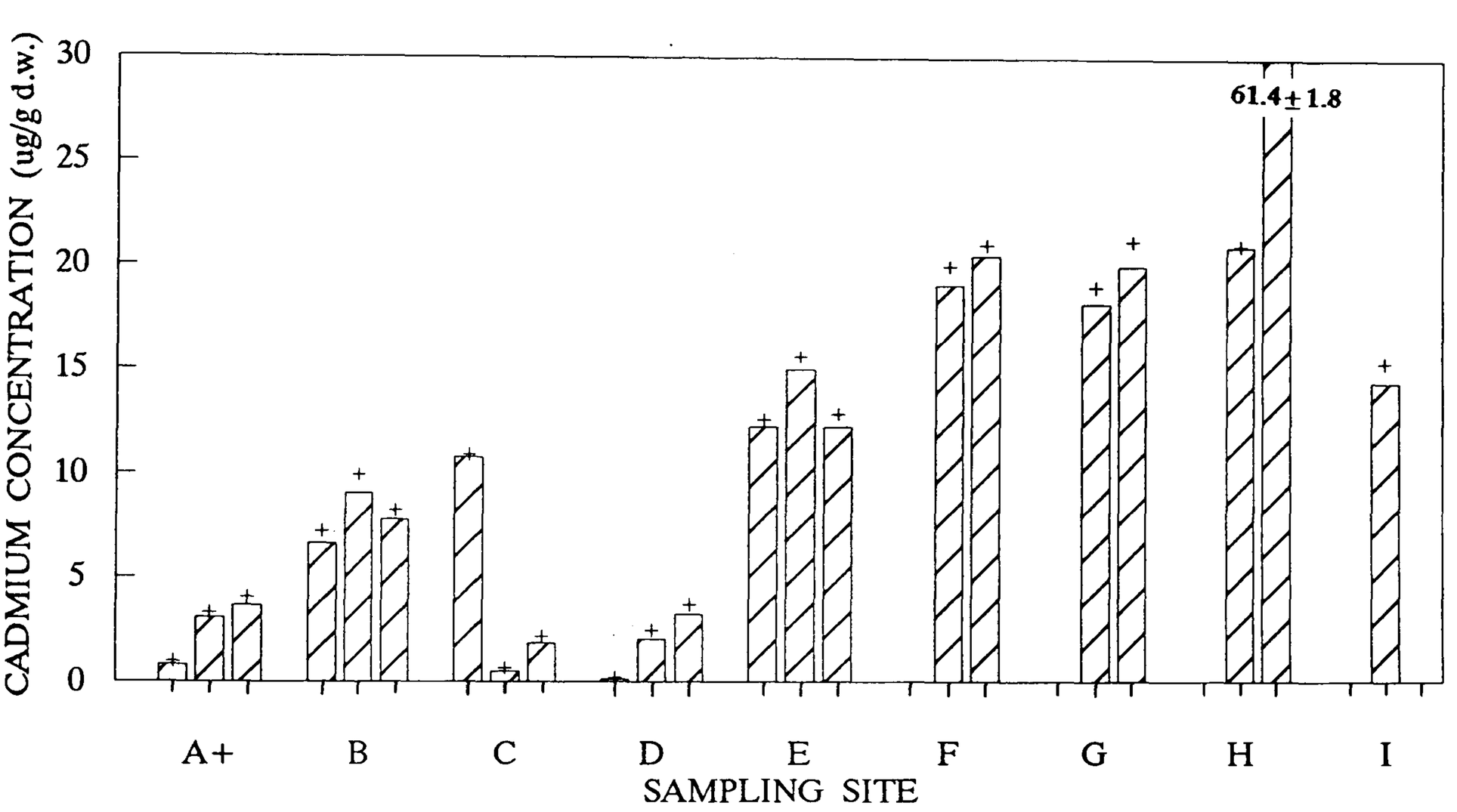



**Figure 3.5** Concentrations of cadmium in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

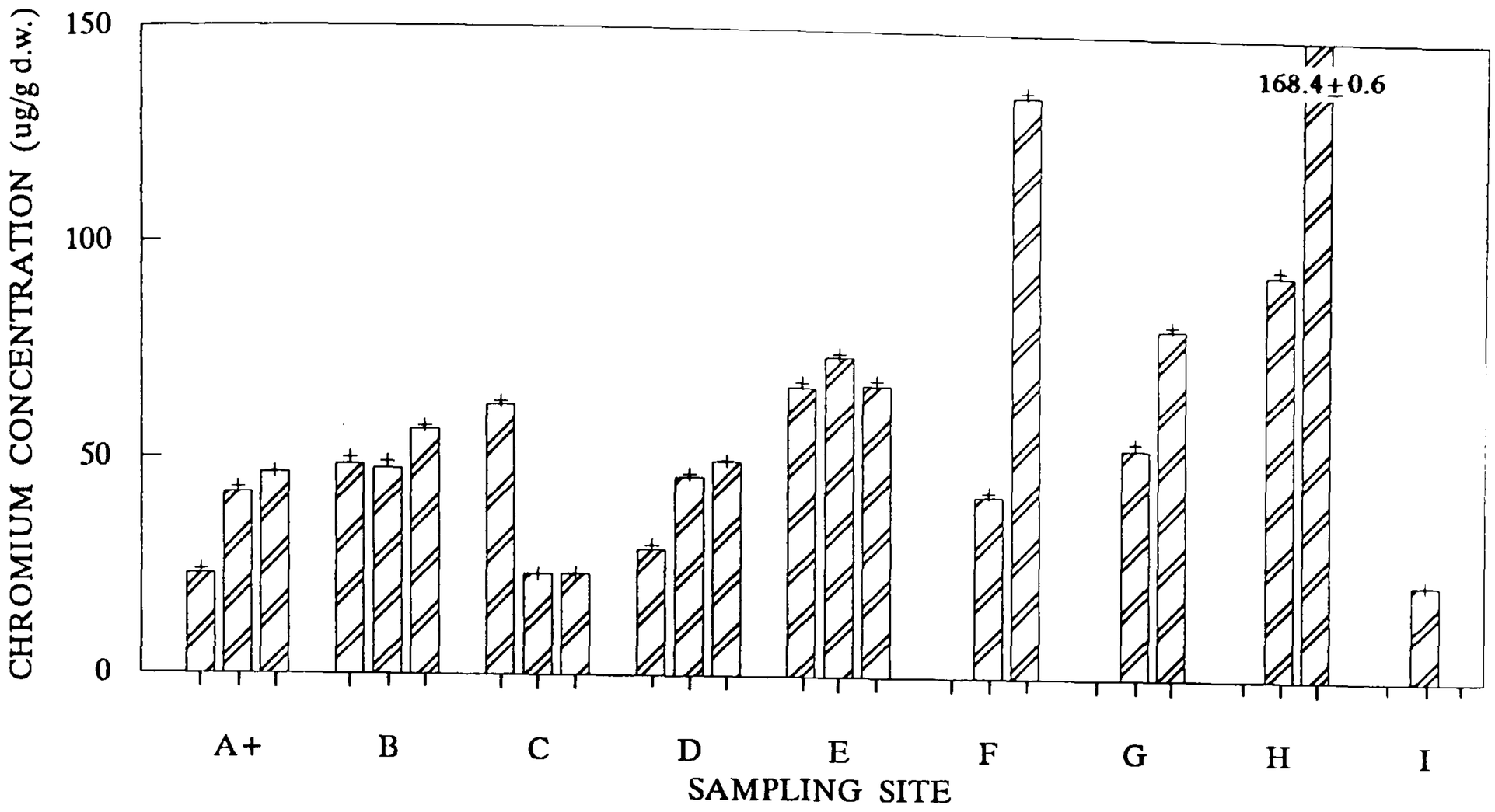



**Figure 3.6** Concentrations of chromium in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

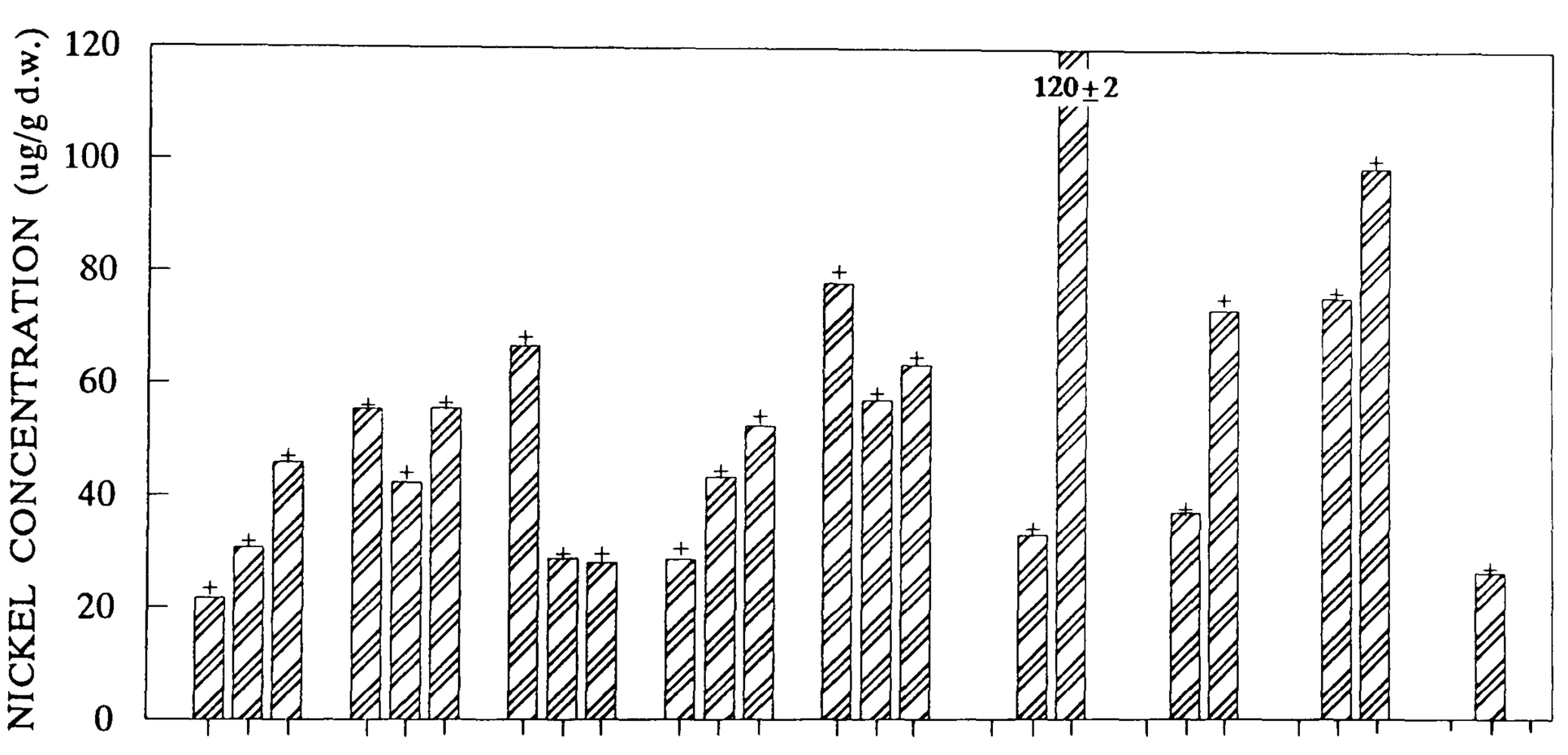



**Figure 3.7** Concentrations of nickel in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

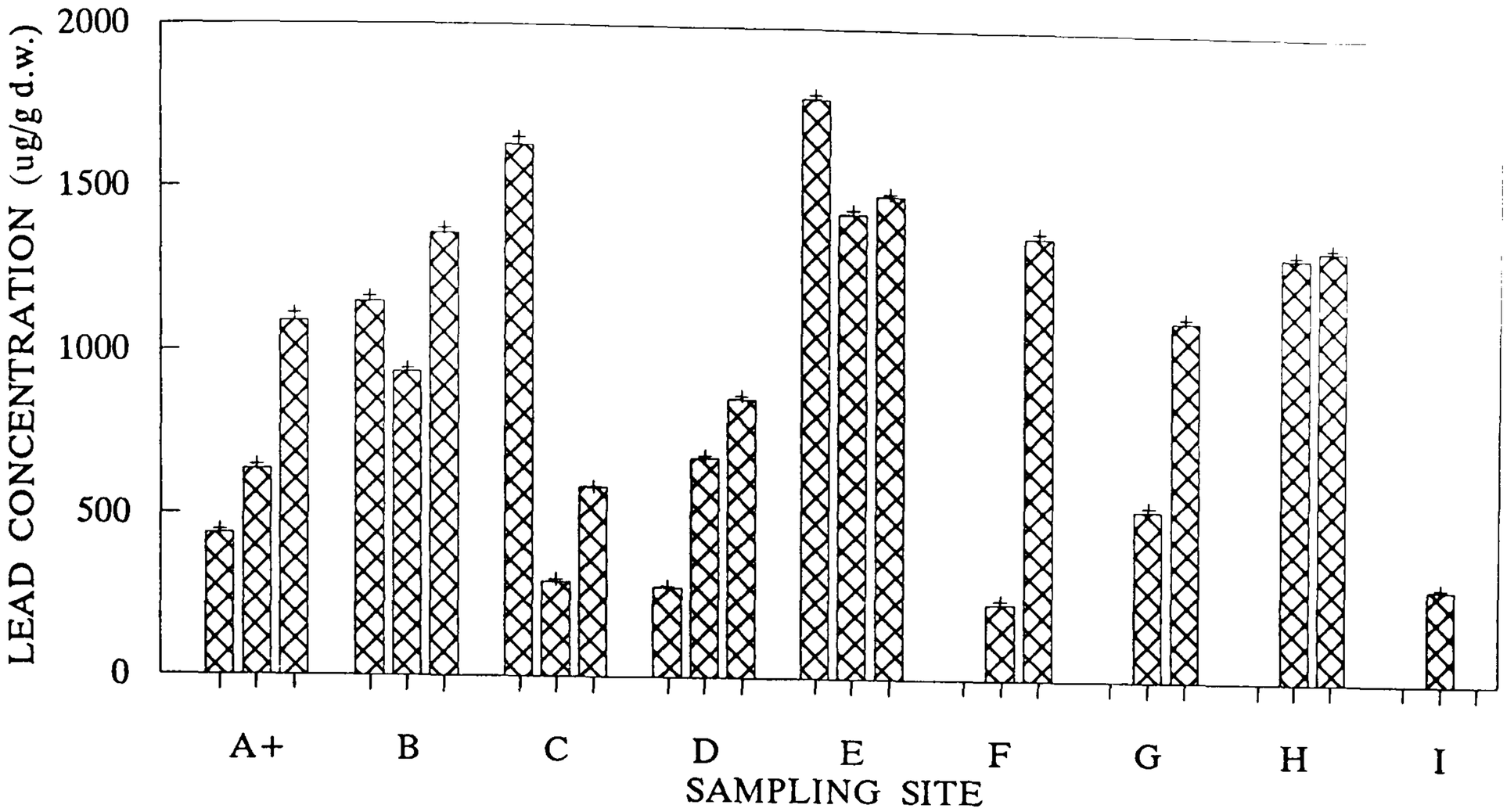



**Figure 3.8** Concentrations of lead in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

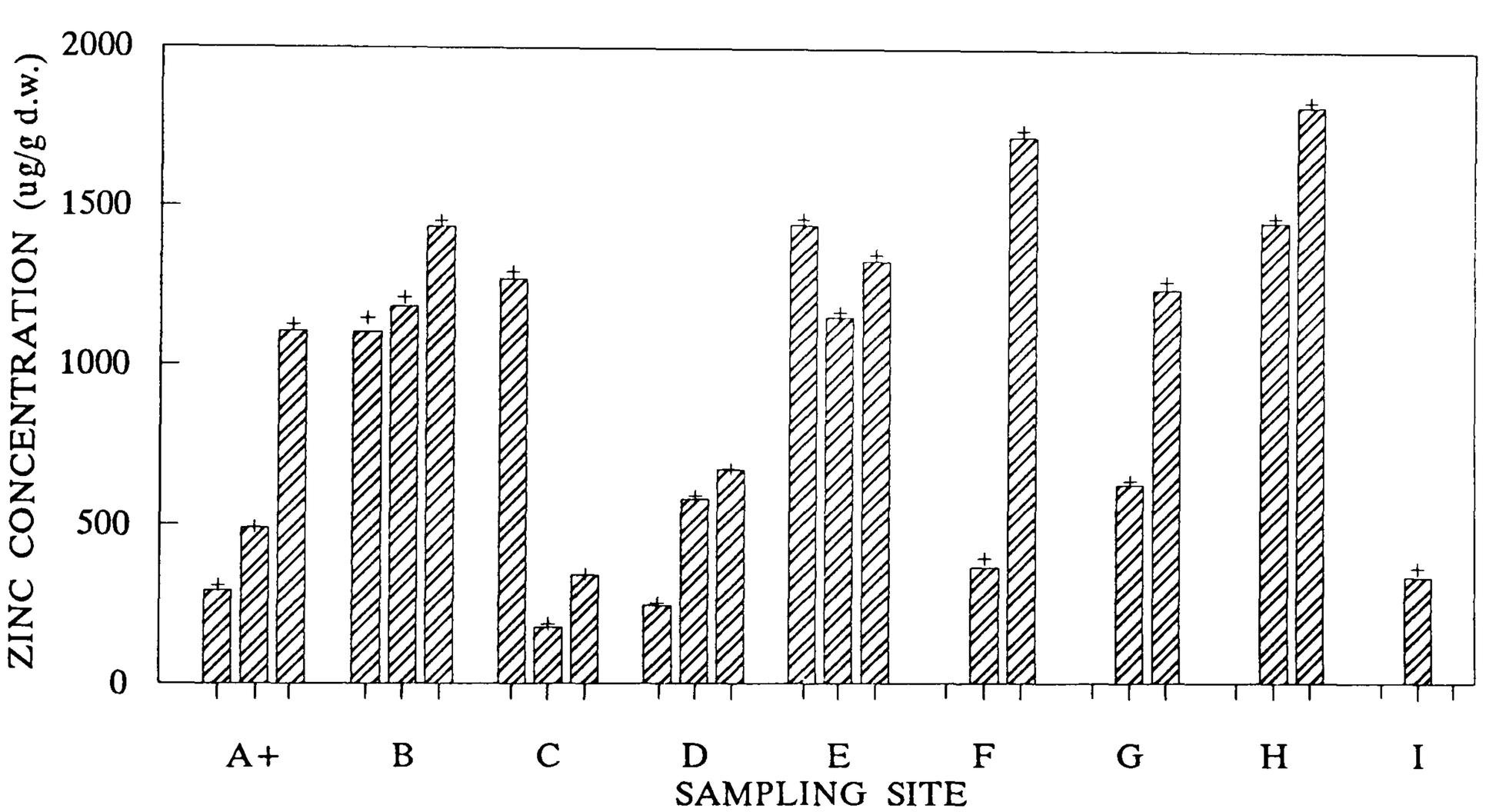



**Figure 3.9** Concentrations of zinc in samples of sediment taken from the River Wandle at various sites on 4th May (left), 29th Jun. (middle), and 3rd Aug. (right), 1988. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.2).

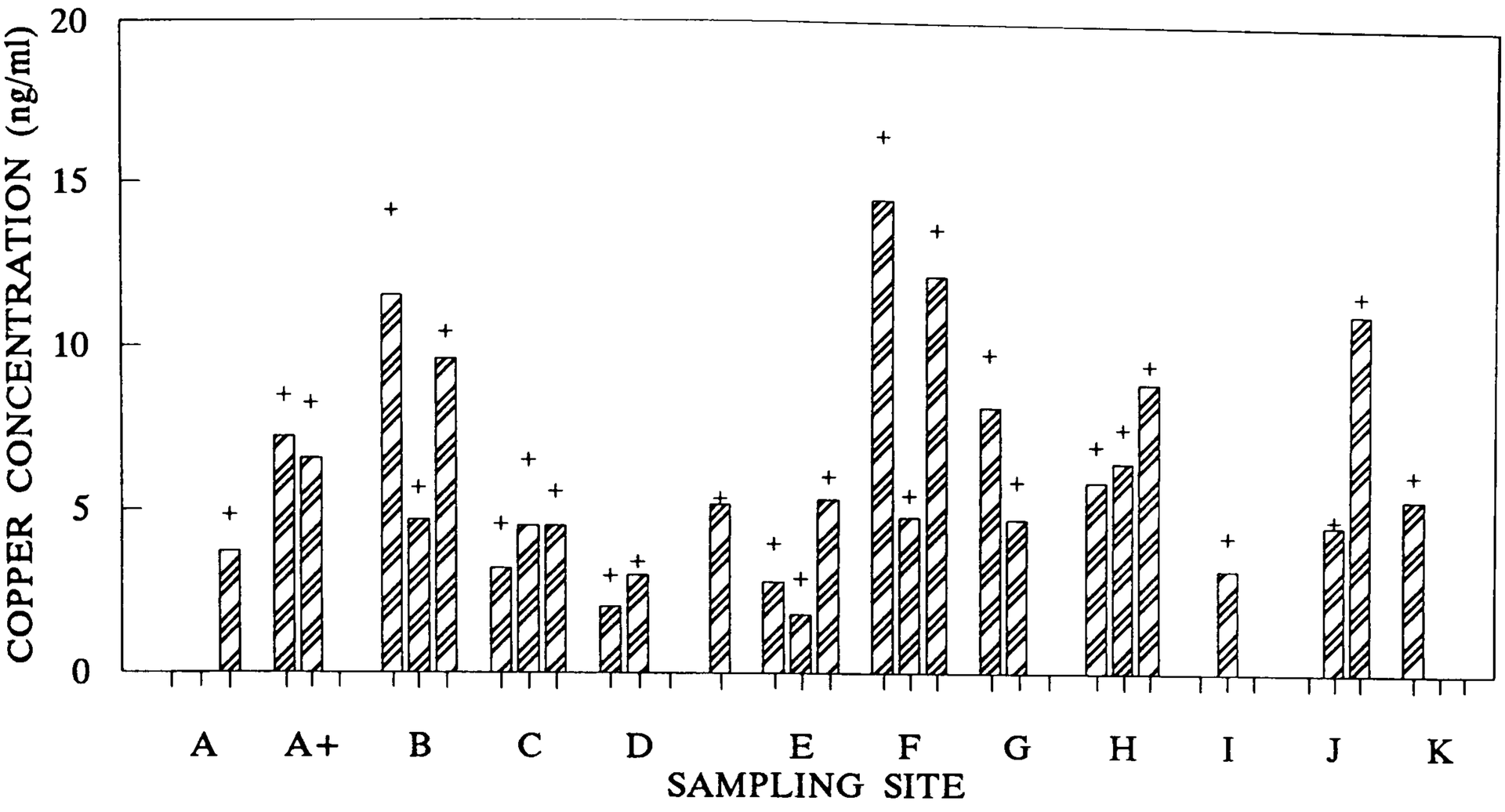



**Figure 3.10** Concentrations of total copper in samples of water taken from the River Wandle at various sites on 12th Dec. 1988 (left), 8th Jun. (middle), and 6th Jul. (right), 1989. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.3).

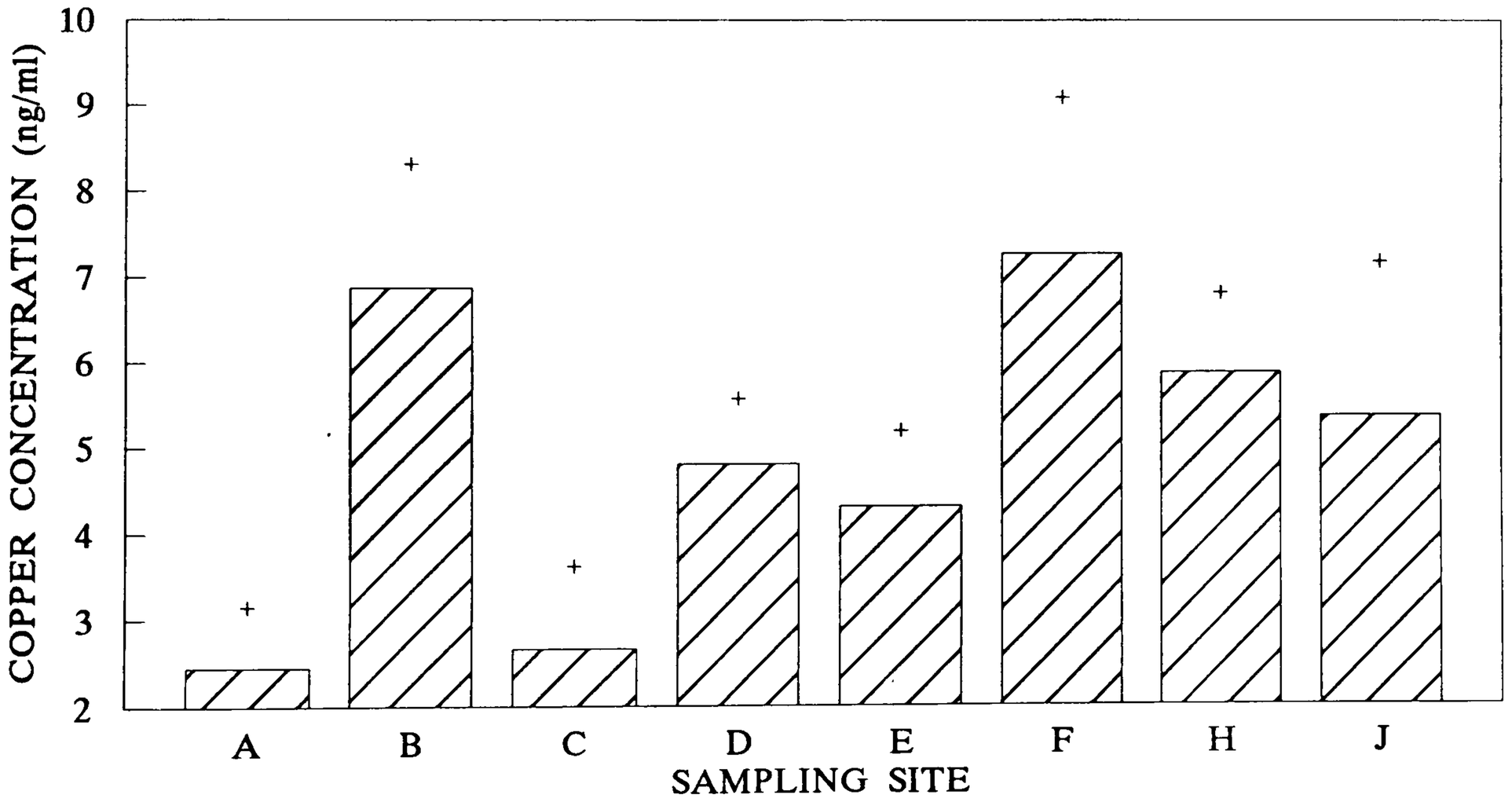



**Figure 3.11** Concentrations of dissolved copper in samples of water taken from the River Wandle at various sites on 6th Jul. 1989. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.3).

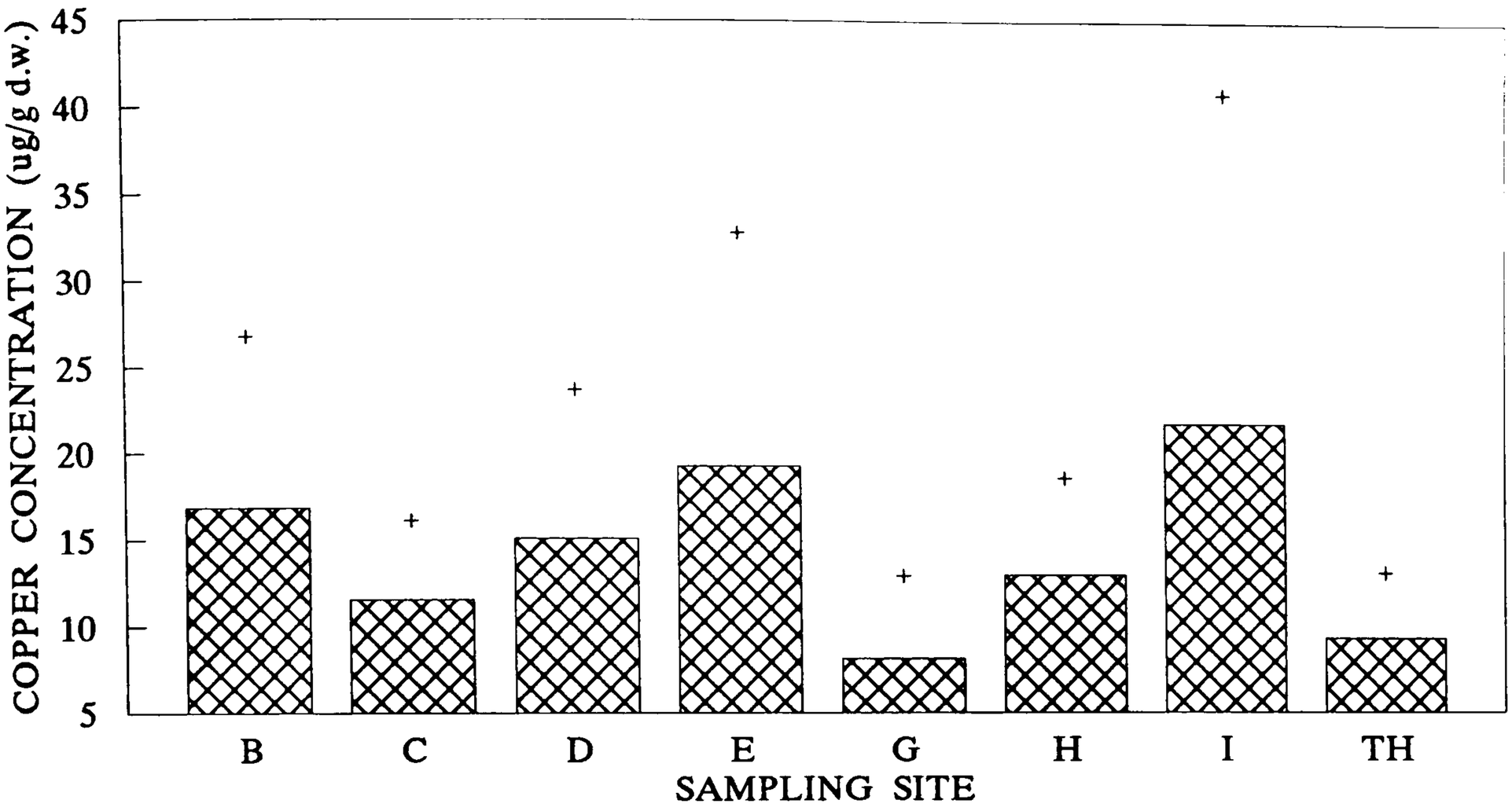



**Figure 3.12** Concentrations of copper in samples of 3-spined stickleback taken from the River Wandle at various sites (marked B-I) and from the River Thames (TH). + indicates the value of mean plus S.D. (The original data are listed in Appendix B.4 and B.5).

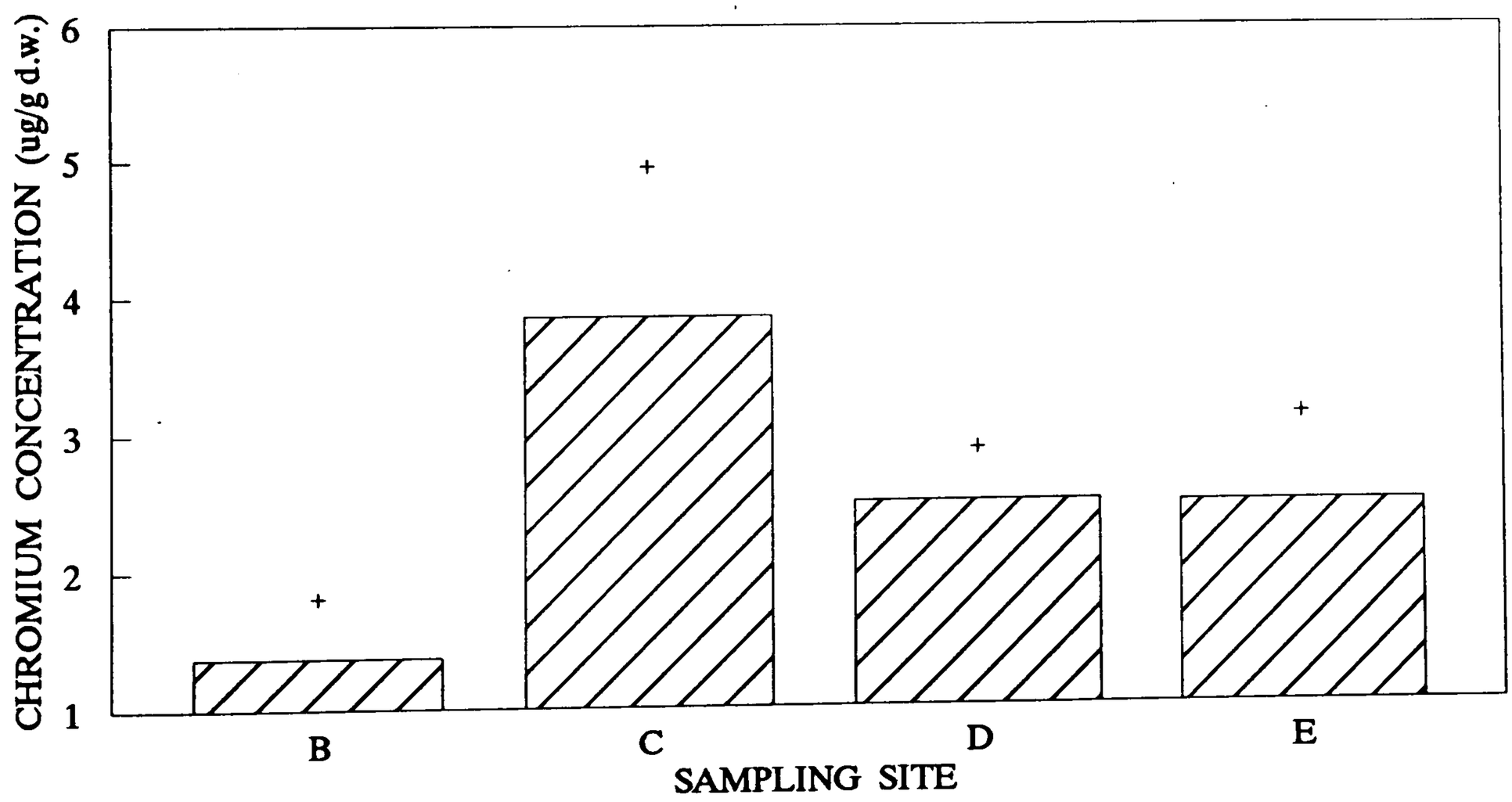



**Figure 3.13** Concentrations of chromium in samples of 3-spined stickleback taken from the River Wandle at various sites. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.4).

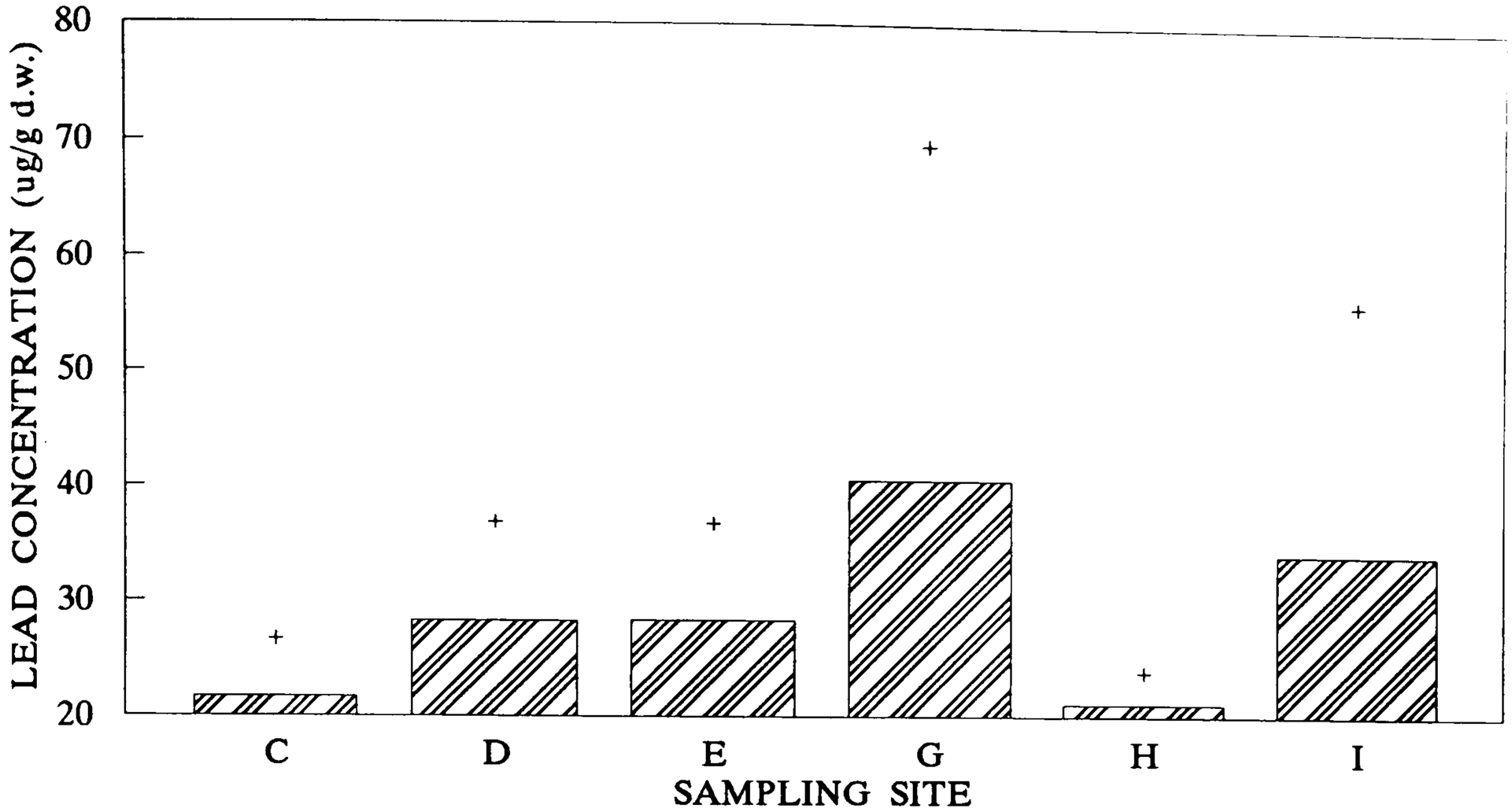



**Figure 3.14** Concentrations of lead in samples of 3-spined stickleback taken from the River Wandle at various sites. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.4).

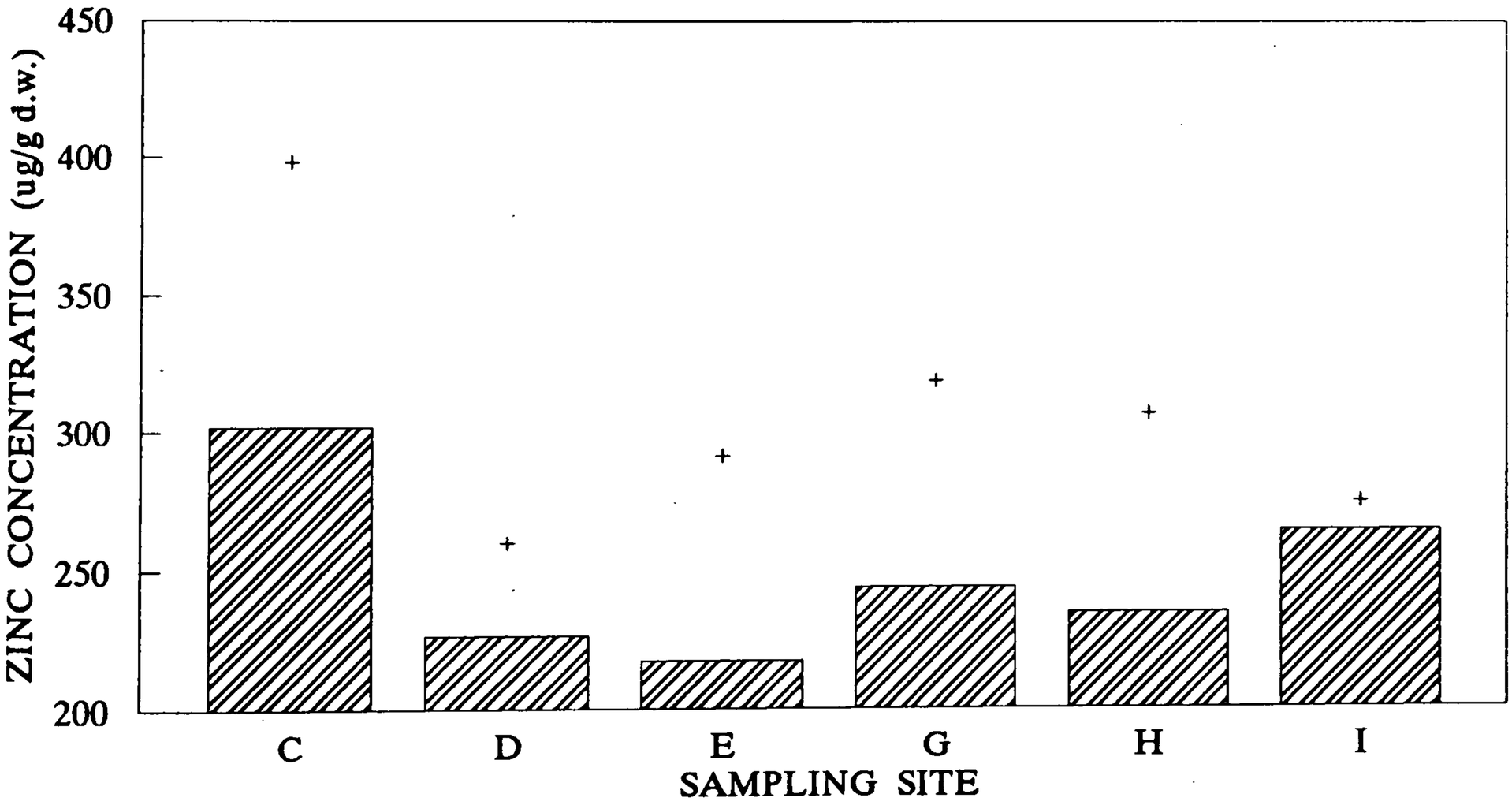



**Figure 3.15** Concentrations of zinc in samples of 3-spined stickleback taken from the River Wandle at various sites. + indicates the value of mean plus S.D. (The original data are listed in Appendix B.4).

easily accessible all year round. Site A was selected as the reference or clean water site, since lower copper concentration in the water was found here (Figures 3.10 and 3.11), and although hitherto not routinely sampled for them, it was found that sticklebacks were plentiful. Site A is a spring fed pond from which the Croydon arm of the Wandle emerges.

Samples were collected each season from each of the three sites at about three monthly intervals. This was geared to cover one year in the life-cyle of the 3-spined stickleback. The sampling dates were 21st July 1989, 12th October 1989, 10th January 1990 and 25th April 1990.

The samples collected included: water, sediment, flocs (fine film of organic detritus), algae and aquatic plants and representatives of the aquatic invertebrate community. Thus every component of the ecosystem including potential stickleback foods would be covered and investigated.

The sampling procedures adopted were carefully carried out in order to minimise contamination between the various types of samples.

(i) 6 replicate water samples were collected in 100 ml acid-washed bottles and immediately acidified with ca 0.5 ml of concentrated $HNO_3$ (BDH, "AristaR" grade) for total metal analysis.

(ii) Six replicates of filtered water samples were collected by filtering 100 to 200 ml of water through 0.45 um membrane filter papers (Whatman, 45 mm diameter) into 6 x 100 ml acid-washed bottles and immediately acidified as well.

(iii) By disturbing the surface of river bed, buckets of turbid water were collected and allowed to settle, for 20-30 mins. The fine particulate matter which precipitated out was transferred into 3 l bottles.

(iv) Water plants were collected, where possible. They were washed clean in river water, and then, placed in a 21 cm x 30 cm polythene bag, one for each species.

(v) Aquatic invertebrates were obtained from 3 to 4 previously planted basket samplers at each site (Plate 3.12). All the animals in the basket were shaken and washed out into a white tray, where they were sorted out into different species. The individual species were then rinsed with double distilled water (DDW) and transferred into polythene bags.

(vi) Fish were caught using a seine net (1 metre wide by 3 metre length, mesh size 1 mm). The fish were transported live to the laboratory and killed by striking their heads on the bench. Before being frozen at -20$^{o}$C for later study, they were randomly separated into two subsamples, handling with acid-washed white plastic forceps, and rinsed three times with DDW and put in clean polythylene bags. When possible, the half of the samples of 20 to 30 fish were obtained from each site were used for metal burden studies (see Table 4.1 in chapter 4) and the other half of the samples were saved for dietary studies (see Table 5.1 in Chapter 5).

(vii) Sometime, aquatic invertebrates, such as snails and *Gammarus* were caught in the seine net. They were also collected for further examination. Similarly, chironomid larvae were occasionally collected from the surface sediment.

(viii) The surface sediment was collected by using a pond net scraping into the surface of river bed to a depth of about 10-20 cm. At site B, where the sediment was more gravelly, a spade was used to dig out the sediment. The sediments were filtered through a stainless steel filter (mesh size 1 cm) in the field.

The further treatment of samples will be described accordingly in the following chapters.

The treatment for metal analysis followed that detailed in Chapter 2, section 2.2.

Plate A

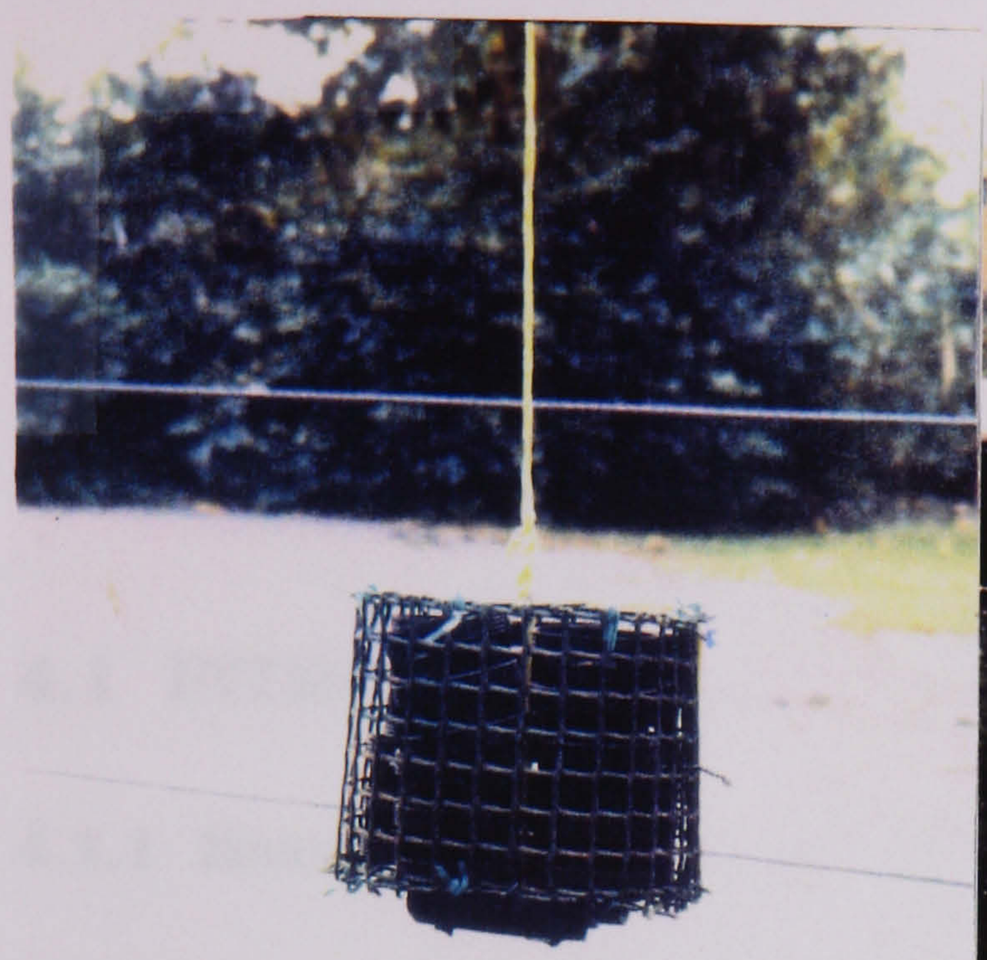


Plate B



Plate C

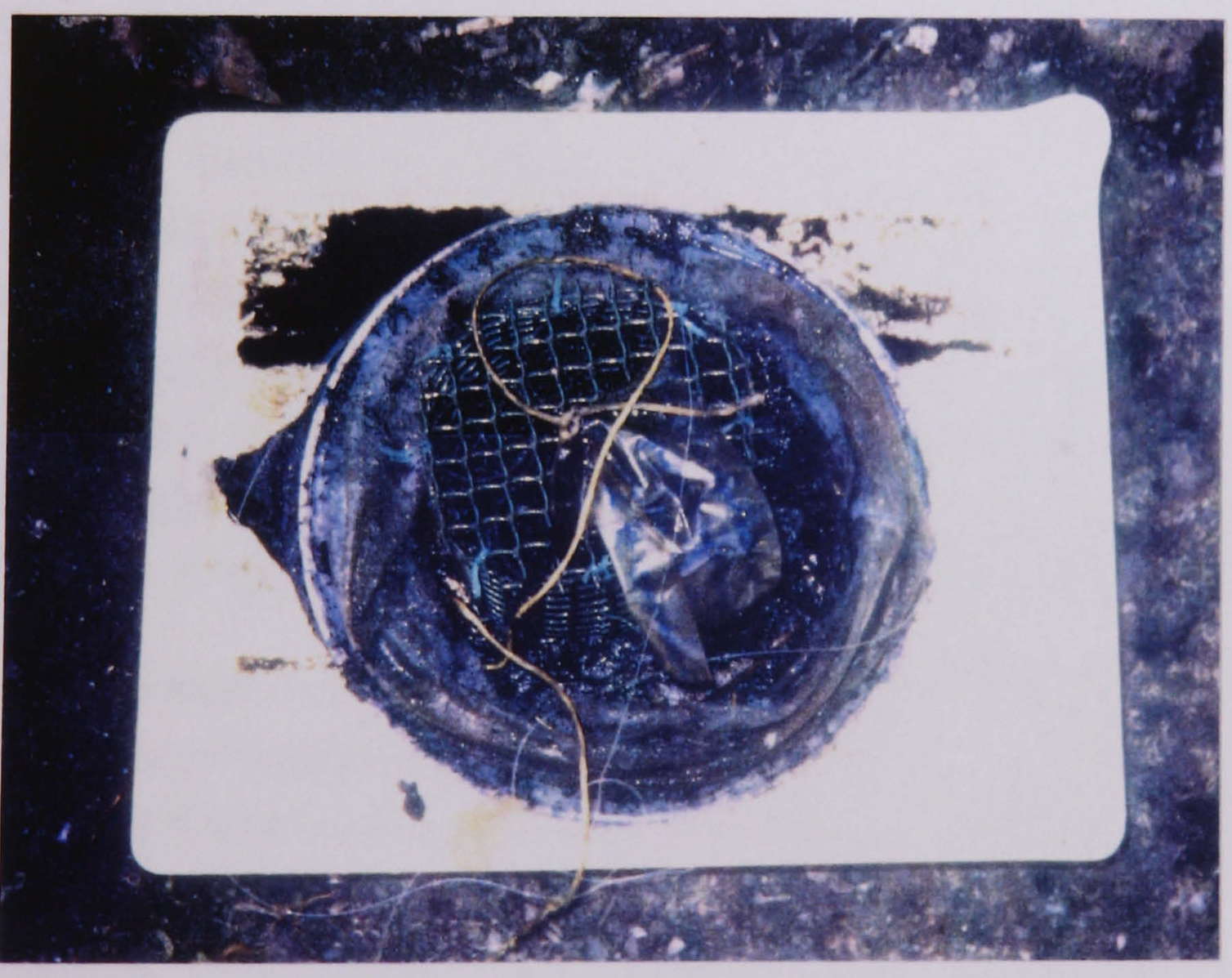


Plate 3.12 Showing two types of basket samplers (A & B) used in the study, and the settled community after more than 3 weeks planting (C).

# CHAPTER FOUR

# EVALUATION OF THE RELATIONSHIPS BETWEEN GROWTH, *SCHISTOCEPHALUS* INFECTION, AND COPPER AND CADMIUM IN THREE-SPINED STICKLEBACK POPULATIONS IN THE RIVER WANDLE

## 4.1 INTRODUCTION

### 4.1.1 Heavy Metal Accumulation in Fish

Metal concentrations in fish have been commonly used as indicators of metal levels in marine and freshwater environments (Uthe & Bligh 1971, Portmann 1972, Penden et al. 1973, Winder et al.1973, Eustace 1974, MacKay et al. 1975, Mathis & Kevern 1975, Bebbington et al. 1977, Murphy et al. 1978, Wiener & Giesy 1979, Lewis 1980, McFarlane & Franzin 1980, Mason et al. 1982, Binnemann et al. 1983, Taylor 1983, Janben & Brune 1984, Szefer & Falandysz 1985, Weber 1985, Bradley & Morris 1986, Maher 1986, Reash & Berra 1986, Zamojski et al. 1986, Mason 1987, Badcha & Goldspink 1988, Law & Singh 1988, Pesch et al. 1988, Sapunar et al. 1989, Tsoukali-Papadopoulou et al. 1989, Barak & Mason 1990a&b, Carpene et al. 1990, Harrison & Klaverkamp 1990, Mason & Barak 1990, Singh et al. 1990, Szefer et al. 1990, Winger et al. 1990, Giordano et al. 1991, Law & Singh 1991, Mclean et al. 1991, Singh et al. 1991, Van 1991).

Reported average concentrations of Cu and Cd for whole fish from relatively uncontaminated aquatic ecosystems are in the range of 0.01-0.29 ug Cd/g and 1.8-7.5 ug Cu/g in dry weight (in Lucas et al. 1970, Uthe & Bligh 1971, Lovett et al. 1972, Eustace 1974, Kelso & Frank 1974, Armstrong & Lutz 1977a&b, Giesy & Wiener 1977, Wharfe & Van Den Broek 1977, Murphy et al. 1978, Abo-Rady 1979, Lowe et al. 1985, Falandysz 1985, Denton & Burdon-Jones 1986, Harding & Goyette 1989, Sapunar et al. 1989, Hernandez et al. 1990).

In contaminated environments much higher concentrations were found. For example, in the Severn estuary which is contaminated by a nearby lead and zinc smelter, Hardisty et al. (1974a) reported Cd

concentrations of 4.7 ug/g in flounder (*Platichthyes flesus*), 3.3 ug/g in sand goby (*Pomatoschistys minutus*), 0.5 ug/g in river lamprey (*Lampetra fluviatilis*) and 0.6 ug/g in brook lamprey (*Lampetra planeri*). In another study by the same authors (Hardisty et al. 1974b) in fish from near Oldbury-on-Severn (U.K.), Cd and Cu concentrations were 20 ug/g Cu and 8.5 ug/g Cd in poor cod, 25 ug/g Cu and 5.6 ug/g Cd in flounder, 24 ug/g Cu and 6.2 ug/g Cd in whiting, 19 ug/g Cu and 3 ug/g Cd in grey mullet, and 18 ug/g Cu and 3.2 ug/g Cd in sandy goby. Murphy et al. (1978) examined 11 species of freshwater fishes from the west basin of Palestine Lake which receives wastes from an electroplating factory. Cadmium concentrations in bluegill, redear, warmouth, orangespot sunfish, largemouth bass, black crappie, golden shiner, white sucker, brown bullhead, bowfin, and yellow perch were 3.1, 8.0, 1.4, 6.7, 1.1, 0.6, 0.7, 2.0, 2.8, 0.06 and 1.1 in ug/g dry weight, respectively. Badsha & Goldspink (1982) in fish from rivers of NW England, reported whole body Cd concentrations in roach (*Rutilus rutilus*), bream (*Abramis brama*), perch (*Perca fluviatilis*), and pike (*Esox lucius*) which ranged between 9.3-29.0, n.d.-11.3, 12.3-28.7, and 22.5 in ug/g dry weight, respectively. Whole body concentrations of Cd and Cu in sticklebacks (*Gasterosteus aculeatus*) from Cambell River drainage in Vancouver island, Canada, which is contaminated with mining effluent were 2 and 23 ug/g dry weight, respectively (Nordin et al. 1984 in Roch et al. 1985).

An alternative method of assessing the metal content of fish is to determine the whole body burden of a metal. Moriarty et al. (1984) studied Cd and Pb total body burdens of the bullhead, *Cottus gobio*, in the river Ecclesbourne, Derbyshire, U.K. and Douben (1989) studied the same total metal burdens in stone loach, *Noemacheilus barbatulus* L, in three Derbyshire rivers. In each case regression models relating metal body burden to the size of fish were established.

Thus, there are many factors in addition to total metal loading which can influence the accumulation of metals in fish. These include differences between species, as well as differences between age/size classes of fish and differences in feeding habits. In the environment, the bioavailability of metals will further vary, depending on the physical and chemical characteristics of the water such pH (Bendell-

Young et al. 1986) and the degree of hardness (Carroll et al. 1979, Calamari et al. 1979).

### 4.1.2 The Infection of *Schistocephalus solidus* in the Three Spine Stickleback

During investigation of natural populations of sticklebacks it is impossible to ignore the fact that they are often infected by *Schistocephalus solidus*, with for examples 3 to 16% of incidence of parasitism in the River Wandle (see Chapter 3, section 3.2.2). Among the 40 or so of parasitic species that are known to infect stickleback in Britain (Kennedey 1974), infestation of *Schistocephalus solidus* has attracted the most attention since the plerocercoid stage of this parasitic cestode is quite large and may occupy much of the body cavity causing an extended abdomen, that is unmistakable (Wootton 1976).

The infestation of *S. solidus* rarely causes severe mortality in the stickleback populations, although sub-lethal effects are quite common. These include inhibition of growth, physiological weakness and increased energy demands. Often, females fail to sexually mature, they are unresponsive during courtship and they have been known to destroy the nest. Infected males show altered behaviour and often they do not complete nest building (Arme & Owen 1967, Pennycuick 1971a, b & c). Moreover the distended abdomen can affect swimming ability which in turn increases the risk of predation. Infected fish have higher food and oxygen consumption (Lester 1970, Godin & Sproul 1988). Therefore in poor water quality environments infected fish are less able to survive (Meakins & Walkey 1975); they die sooner from the starvation than uninfected fish (Walkey and Meakins 1970).

Epidemiological aspects of infestation in *G. aculeatus* by *S. solidus* have not been studied very much. Arme & Owen (1976) examined the infestation of fish in Farnley pond, Leeds, U.K., and Pennycuick (1971a, b, c & d) of Priddy pool, Somerset, U.K. In running water systems particularly however this type of research is rare.

Moreover, there are no reports of investigations of heavy metal accumulation in cestodes within their hosts although Pascoe and Cram (1977) studied the cadmium toxicity of stickleback with and

without infestations of S. *solidus*. They found that the parasitised sticklebacks were less able to tolerate water containing cadmium.

The stickleback populations identified at the various locations in the River Wandle provided an ideal opportunity for studying specific aspects of growth/age patterns, and parasite infection and relating these to the concentration and body burdens of copper and cadmium. This approach bringing together considerations of growth, metal accumulation and interaction with natural stress has not previously been investigated to any extent.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Fish Sampling and Handling

Four surveys covering the seasons of the year were undertaken during which samples of sticklebacks were collected from each of three sites selected for further more intensive study (see Chapter 3, section 3.3). During the period over 600 fish were caught. The size and the weight of samples were determined. Fish were divided into non-parasitised and parasitised. Also where possible, each batch of fish was divided into 2 groups; one was for metal analysis and the other was used for gut analysis.

### 4.2.2 Estimation of the Degree of *Schistocephalus solidus* Infestation in the Stickleback Populations

To quantify the infestation of S. *solidus* in the stickleback populations two parameters were calculated; (1) incidence of parasitism, which is the percentage of the number of infected fish to the total number of fish examined. (2) intensity of parasitism which is determined as the mean number of cestodes found in each batch of infected fish.

In addition to estimating the parasite loading which is related to the size of individual host, a parasite index (PI) was calculated according to the formula of Arme & Owen (1967):

(Total weight of parasites/Total weight of host plus parasites) x 100

A PI of 50 thus refers to an infection where the total weight of parasites is equal to the net weight of the host.

### 4.2.3 Determination of Age Structure of Stickleback Populations

There are two methods available for ageing natural populations of sticklebacks. These methods are: (1) measuring the annual rings in the ear otoliths, and (2) the use of frequency distributions of the lengths of the fish in the population. In many studies the ages of the stickleback were deduced from the frequency distribution of their lengths. In some cases otoliths were taken from a relatively small number of fish in order to confirm the ageing scheme obtained from the length data (Wootton 1976). However the otoliths of sticklebacks are extremely small and with the large number of fish used in this study, it was decided to use the length frequency for the examination of the age of fish.

Each fish was allocated to an age group according to the methods of Harding (1949) and Cassie (1954). This involved plotting the accumulative percentage of length frequency distribution on probability paper. While the sample size was less than twenty-five, the data were arranged in ascending order of magnitude and plotted in such a way that there were equal percentage intervals between each observation, (for an example, see Table 4.1). The next stage was to plot the lengths of the fish against the accumulative frequency on probability paper, to produce the so-called 'Cassie curve', an example of which is shown in Figure 4.1. It can be seen that the plot is made up of series of steps and the inflexion points are used to separate different age groups. In the example there are three inflexion points at 38, 66 and 86 per cent. Separate age groups were identified by determining the relative accumulative percentage length frequency distribution within each of these groups (see Table 4.1). These were plotted on probability paper and each group was fitted with straight line (Figure 4.1). To estimate the number, mean, and standard deviation in each group, the mean is obtained from the 50% accumulative percentage to the length, and the standard deviation can be determined by measuring the difference between the mean and the 15.86 % or 84.13 % level on the graph, or measuring to the 99.99 %

**Table 4.1** An example of length-frequency data used to construct a Cassie curve and to identify the different age groups of sticklebacks at site E in 21st July 1989.

| Sample Sequence No. | Total Length (mm) | Accumulative % | Inflexion Point | 0+ Year Age | 1+ Year Age | 2+ Year Age | 3+ Year Age |
|---|---|---|---|---|---|---|---|
| 1 | 15.0 | 2.00 | | 5.26 | | | |
| 2 | 18.0 | 6.00 | | 15.79 | | | |
| 3 | 20.0 | 10.00 | | 26.32 | | | |
| 4 | 20.5 | 14.00 | | 36.84 | | | |
| 5 | 21.5 | 18.00 | | 47.37 | | | |
| 6 | 22.0 | 22.00 | | 57.89 | | | |
| 7 | 23.5 | 26.00 | | 68.42 | | | |
| 8 | 25.5 | 30.00 | | 78.95 | | | |
| 9 | 26.5 | 34.00 | | 89.47 | | | |
| 10 | 26.5 | 38.00 | * | 100.00 | | | |
| 11 | 29.0 | 42.00 | | | 14.29 | | |
| 12 | 31.0 | 46.00 | | | 28.57 | | |
| 13 | 31.5 | 50.00 | | | 42.86 | | |
| 14 | 35.0 | 54.00 | | | 57.14 | | |
| 15 | 38.0 | 58.00 | | | 71.43 | | |
| 16 | 38.0 | 62.00 | | | 85.71 | | |
| 17 | 43.0 | 66.00 | ** | | 100.00 | 20.00 | |
| 18 | 44.0 | 70.00 | | | | 40.00 | |
| 19 | 44.0 | 74.00 | | | | 60.00 | |
| 20 | 45.0 | 78.00 | | | | 70.00 | |
| 21 | 46.0 | 82.00 | | | | 80.00 | |
| 22 | 52.0 | 86.00 | *** | | | 100.00 | |
| 23 | 55.0 | 90.00 | | | | | 33.33 |
| 24 | 57.0 | 94.00 | | | | | 66.67 |
| 25 | 67.0 | 98.00 | | | | | 100.00 |

levels at the edge of the paper subtracted by the levels at 50 % and divided by 3.72 e.g. for the $0^+$ year group (37-22)/3.72=4.03.

Therefore, fish were allocated into one of five age groups; fish less than 1 year old (0+ fish); fish less than 1 year old but born late in the spawning season ($^-0$ fish); fish between 1 and 2 years old ($1^+$ fish); fish between 2 and 3 years old ($2^+$ fish); fish between 3 and 4 years old ($3^+$ fish).

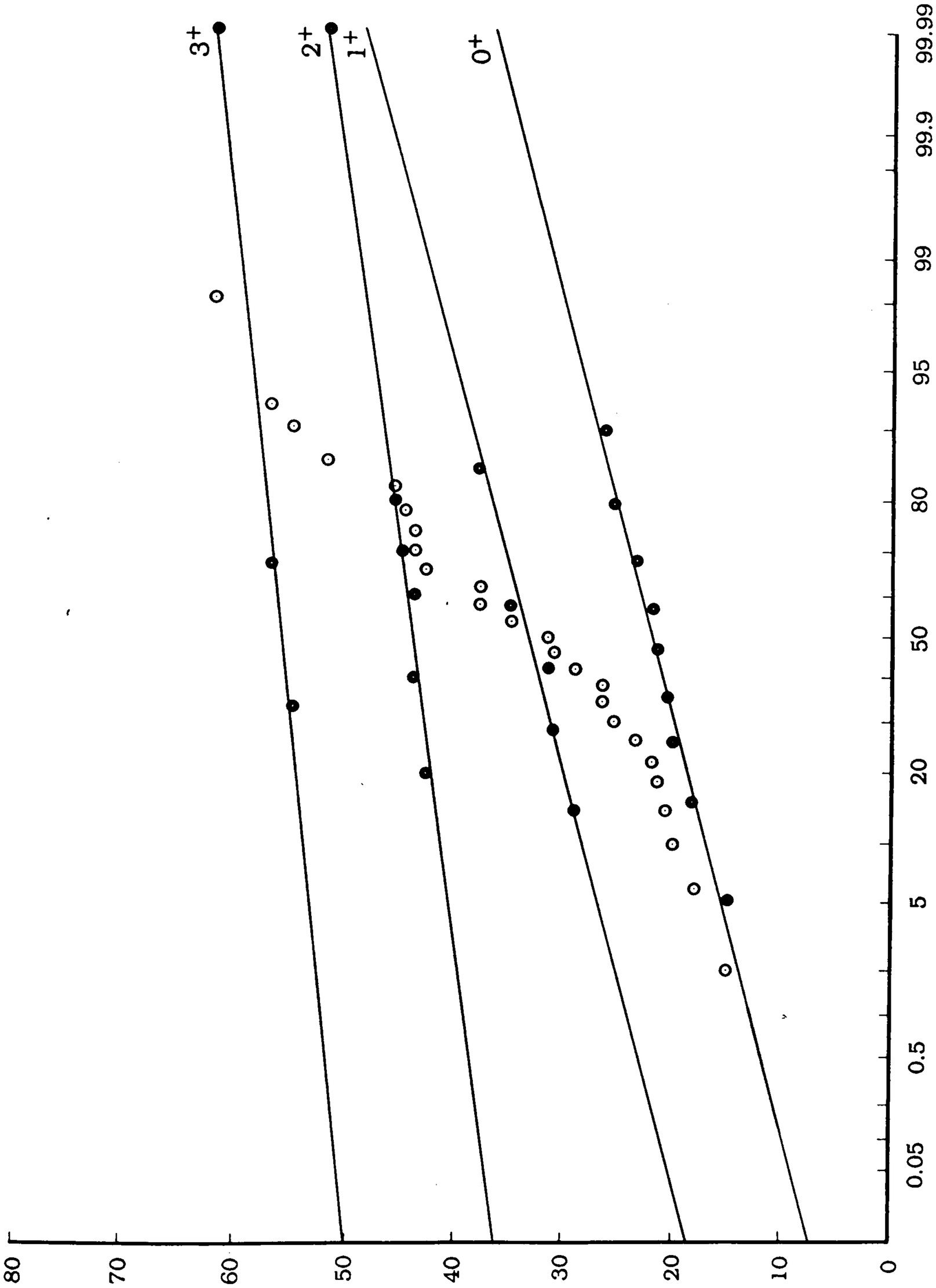



**Figure 4.1** An example of a Cassie curve using length-frequency data from sticklebacks at site E in 21st July 1989.

### 4.2.4 Estimations of the Growth of Wandle Sticklebacks

The growth (the increments of total length and dry body weight) of stickleback was calculated as the annual increments in length or weight between the different age groups and the seasonal growth of $0^+$ group of fish.

The absolute growth and the absolute growth rate were calculated as following formula;

$w_2$-$w_1$, and $w_2$-$w_1/t_2$-$t_1$

The relative growth and the relative growth rate were calculated as;

$w_2$-$w_1/w_1$, and $w_2$-$w_1/w_1(t_2$-$t_1)$

where the $w_2$ and $w_1$ represent either total length or dry body weight at the final time $t_2$ and the initial time $t_1$.

### 4.2.5 Determination of Copper and Cadmium in the Fish and Their Cestode Parasites

Each fish was placed in a sterilised disposable petri dish and its total length was measured to within 0.5 mm. It was then dissected to determine the sex, to find evidence of parasitic infection, and finally the gut contents were squeezed out. Individual body weight (fresh weight) was determined to $\pm$ 0.001 g. In cases where cestode parasites, *Schistocephalus solidus*, were present these were first removed before the other parameters were determined.

Fish and cestodes were dried at 70$^{o}$C in a stainless steel oven (LTE, Laboratory Thermal Equipment Ltd, or equivalent) for at least 4 days to a constant weight, and the dry weights were obtained. For very tiny fish and cestode parasites the weighing procedure was refined (Kwan 1988). Essentially this involved weighing the samples on acid-washed pre-weighed cover slips and transferring the glass cover slip and sample to the digestion vessels.

The dried fish were prepared for Cu and Cd determination according to the procedures described in Chapter 2, section 2.2.

## 4.3 RESULTS

### 4.3.1 The Infestation of *Schistocephalus solidus* in Wandle Sticklebacks.

The degree of infection of *Schistocephalus solidus* in the Wandle's stickleback at the three sites is shown in Table 4.2. It can be seen that the overall incidences of parasitism are 22%, 36% and 24% at the each of the sites A, B, and E. At sites A and E, 76% of parasitised fish had only one worm but at site B just over 50% had one worm and over 30% of the fish were infected with two worms giving the highest intensity at this site of 1.68. The maximum number of parasites found in a fish was four. The total lengths and dry weights of the cestode parasites were significantly different between the three sites ($P<0.0001$) and being notably greater at site B than at the other two sites. The parasite index covered a fairly wide range but gave similar mean values of 23 and 22 at site A and E, respectively and a raised value of 29 at site B. There were no distinguishable differences of the infections between the reference site and the contaminated site E, but the fish at site B had the highest incidence, intensity, and parasite index (PI) of *S. solidus* infection.

Figure 4.2 examines the relationship between the size of the stickleback host and the weight of the parasite. At site B and E excellent correlations were found between both the dry weight and the length of the host fish and the dry weight of the parasite; correlation coefficients were 0.8681 and 0.7974 for length of host versus weight of parasite and 0.8848 and 0.6283 for weight of host versus weight of parasites. The relationships at site A were not significant.

Figure 4.3 shows the relationship between the parasite index (PI) and the size of the host fish. There is an inverse trend between PI and size of host fish at site A , but no trend is seen at the two contaminated sites.

Bearing in mind the prevalence of parasite infection, it was decided early on in the study to treat the parasitised fish as a separate group and to evaluate the various relationships between length, weight and therefore age of the fish and to examine metal burdens and metal concentrations of both the host fish and the parasites.

**Table 4.2** Summary of the infestation of *Schistocephalus solidus* in the parasitised stickleback at the three sampling site in the River Wandle.

| Parameter | Site A | Site B | Site E |
|---|---|---|---|
| No. of parasitised fish | 21 | 35 | 25 |
| Incidence, % | 21.9 | 35.7 | 24.0 |
| Intensity* | 1.28 | 1.68 | 1.36 |
| **Frequency distribution of the cestode parasites in parasitised fish** | | | |
| No. of Cestode | No. % | No. % | No. % |
| 1 | 16 76.2 | 18 51.4 | 19 76.0 |
| 2 | 4 19.0 | 11 31.4 | 4 16.0 |
| 3 | 1 4.8 | 5 14.3 | 1 4.0 |
| 4 | 0 0.0 | 1 2.9 | 1 4.0 |
| Mean length (mm) | 36.76 | 71.30 | 49.20 |
| ANOVA analysis | **** | | |
| Multiple range test | a | b | a |
| Mean dry wt (g) | 0.02 | 0.12 | 0.06 |
| ANOVA analysis | **** | | |
| Multiple range test | a | b | a |
| Parasite Index** | 22.90 (5.7–67.7) | 29.00 (8.8–40.3) | 22.14 (7.6–39.9) |

Intensity = Mean number of cestodes in parasitised fish
Parasite Index = (Weight of cestode / Wt. of cestode + Wt. of fish) x 100

(The significance abbreviations in Tables of this chapter are shown: **** = P<0.0001; *** = P<0.001; ** = P<0.01; * = P<0.05; + = P<0.1; n.s. = not significant; a, b, c, & d indicate the grouping of the means in ascending order from the multiple range test).

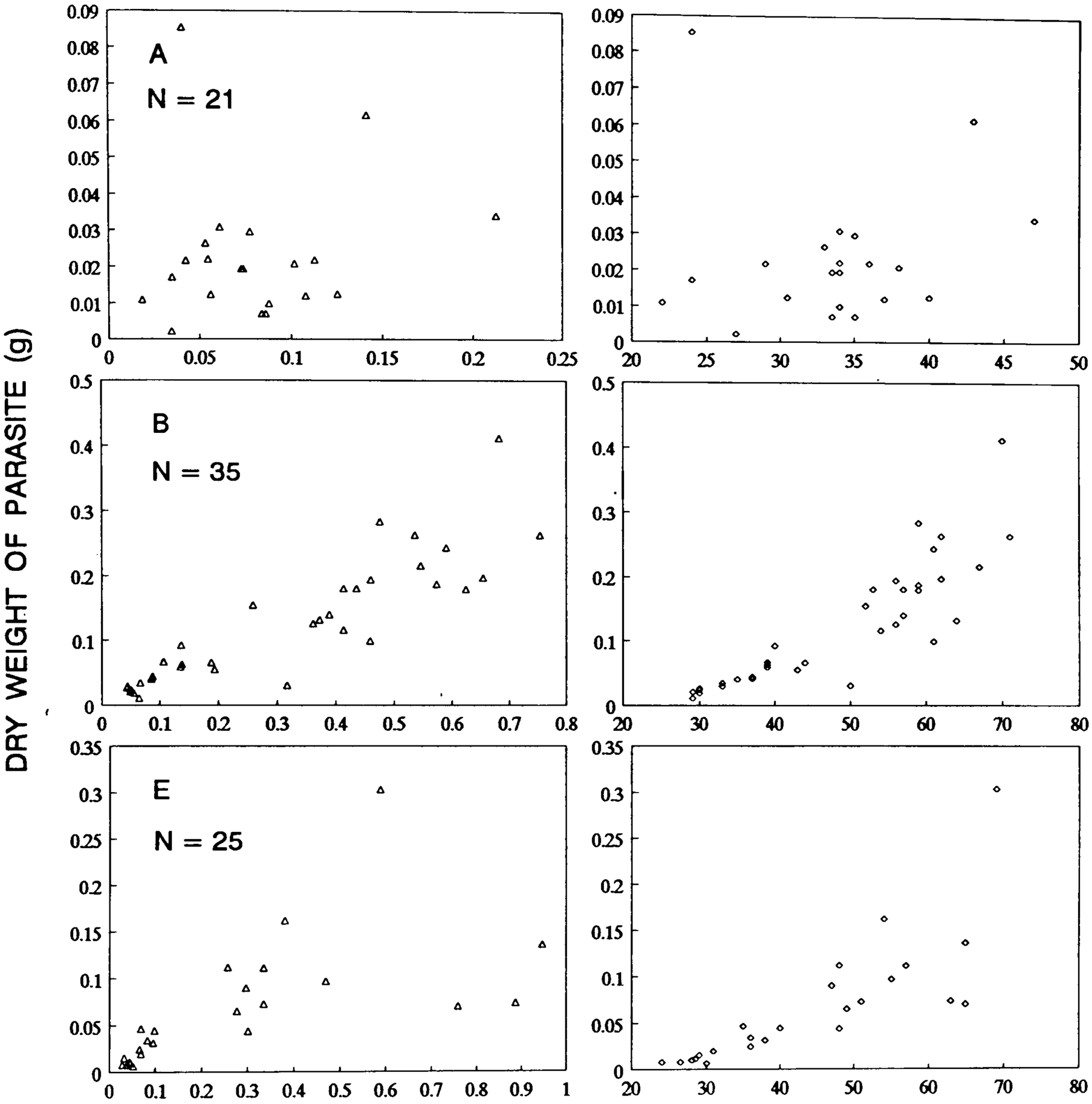



**Figure 4.2** The relationship between the dry weight of parasites and the dry weight, or the total length of the host fish at the three sampling sites (A, B, & E) in the River Wandle.

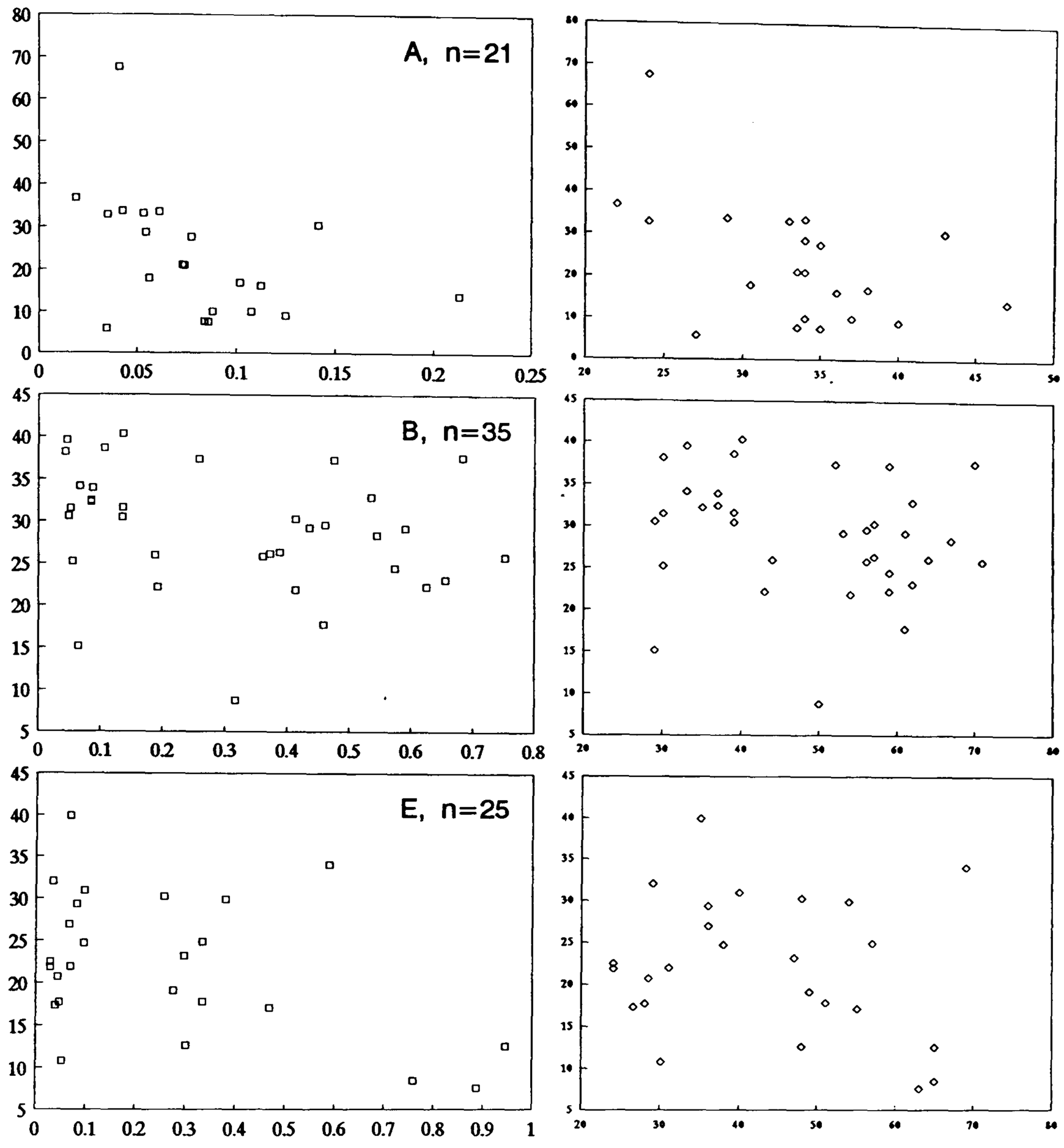



**Figure 4.3** The relationship between the parasite index and the dry weight, or the total length of the host fish at the three sampling sites (A, B, & E) in the River Wandle.

### 4.3.2 Age Groups of the Stickleback at Each Site

Using the Cassie curve method, each batch of fish were divided into age groups and the results are given in Table 4.3. These data have been used in the construction to the Figures 4.4-4.8. The frequency of successive size classes and the assigned age groups at each site are presented in Figures 4.4, 4.5 and 4.6. It can be seen that the populations of stickleback had almost a 3 year life span at site A and nearly 4 years at the contaminated sites of B and E. The smallest fish appeared in July 1989, and were and an average of 18 mm, 13 mm, and 15 mm, at site A, B and E, respectively. At site A, the main trend exhibited by the non-parasitised fish in the progressive increase in size of the $0^+$ age group over the sampling period. At the same time the first and second year age groups understandably decrease in frequency. At site B a similar trend is seen except that for some reason the population had all but disappeared by April 1990. The age structure at site E is more complex. Age groups 1, 2 and 3 make a significant contributions in the first two batches of samples but by April 1990 the population is essentially made up of only the $0^+$ age group.

Figure 4.7 shows the percentage number of fish in each age group for non-parasitised and parasitised sticklebacks at the three sites. By looking at the pooled seasonal samples it can be seen that $0^+$ age group fish were the major component, accounting for more than 50% of the population although in the parasitised fish at site B the $1^+$ age group was the dominant age group accounting for 37% of the population, the $2^+$ age group made up a further 34% of the population.

The above mentioned trends are further borne out by examining the percentage number of fish in each age group on a survey by survey basis. Figure 4.8 shows that the $0^+$ age groups were the major component in all samples from each of the seasonal surveys of non-parasitised fish at the three sites and in the sample of parasitised fish at site E. In fact the $0^+$ age group became an increasingly significant part of the samples in the later survey. This is understandable as the older members of the population from previous recruiting seasons died off as the next breeding season approached. There are similarities

**Table 4.3** Cassie curve estimates of the number (No.), percentage of total (%), mean and standard deviation (S.D.) for different age groups of non-parasitised and parasitised sticklebacks.

| | | | $^{-}$0 age class | | | | $0^{+}$ age class | | | | $1^{+}$ age class | | | | $2^{+}$ age class | | | | $3^{+}$ age class | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SITE | | DATE | No. | % | Mean | S.D. | No. | % | Mean | S.D. | No. | % | Mean | S.D. | No. | % | Mean | S.D. | No. | % | Mean | S.D. | TOTAL |
| | A | 21/07/89 | | | | | 12 | 48.0 | 21.2 | 2.12 | 4 | 17.0 | 29.0 | 0.81 | 8 | 35.0 | 35.0 | 1.88 | | | | | 24 |
| N | A | 12/10/89 | | | | | 10 | 61.0 | 28.0 | 2.02 | 4 | 24.0 | 35.0 | 1.88 | 3 | 15.0 | 45.0 | 0.27 | | | | | 17 |
| O | A | 10/01/90 | 1 | 10.0 | 24.0 | 1.08 | 10 | 72.0 | 34.0 | 3.44 | 3 | 18.0 | 43.7 | 1.48 | | | | | | | | | 14 |
| N | A | 25/04/90 | 1 | 7.0 | 35.0 | 2.69 | 11 | 55.0 | 49.0 | 2.63 | 6 | 30.0 | 54.0 | 1.29 | 2 | 8.0 | 62.0 | 0.48 | | | | | 20 |
| \| | | (Sum) | (2) | 2.6 | | | (43) | 57.3 | | | (17) | 22.3 | | | (13) | 17.3 | | | | | | | (75) |
| P | | | | | | | | | | | | | | | | | | | | | | | |
| A | B | 21/07/89 | 2 | 16.0 | 14.0 | 1.08 | 6 | 33.0 | 20.8 | 2.74 | 7 | 50.0 | 31.2 | 2.04 | | | | | | | | | 15 |
| R | B | 12/10/89 | | | | | 15 | 58.0 | 28.3 | 2.10 | 10 | 42.0 | 38.7 | 4.68 | | | | | | | | | 25 |
| A | B | 10/01/90 | | | | | 17 | 75.0 | 36.5 | 4.03 | 5 | 25.0 | 49.0 | 2.42 | | | | | | | | | 22 |
| S | B | 25/04/90 | | | | | 1 | | 47.0 | | | | | | | | | | | | | | 1 |
| I | | (Sum) | (2) | 3.2 | | | (39) | 61.9 | | | (22) | 34.9 | | | | | | | | | | | (63) |
| T | | | | | | | | | | | | | | | | | | | | | | | |
| I | E | 21/07/89 | | | | | 10 | 40.0 | 22.0 | 4.03 | 6 | 24.0 | 33.8 | 4.03 | 5 | 20.0 | 44.0 | 2.42 | 4 | 16.0 | 56.0 | 2.96 | 25 |
| S | E | 12/10/89 | 1 | 4.0 | 18.0 | 1.61 | 11 | 48.0 | 29.2 | 3.71 | 6 | 26.0 | 40.0 | 2.42 | 3 | 13.0 | 51.1 | 2.39 | 2 | 9.0 | 63.0 | | 23 |
| E | E | 10/01/90 | 2 | 8.0 | 20.5 | 1.28 | 10 | 56.0 | 31.9 | 1.91 | 4 | 22.0 | 43.0 | 2.96 | 2 | 11.0 | 62.0 | | | | | | 18 |
| D | E | 25/04/90 | 1 | 8.0 | 18.5 | | 12 | 92.0 | 39.8 | 3.95 | | | | | | | | | | | | | 13 |
| | | (Sum) | (4) | 5.1 | | | (43) | 54.4 | | | (16) | 20.3 | | | (10) | 12.7 | | | (6) | 7.6 | | | (79) |
| | A | 21/07/89 | | | | | | | | | 1 | | 33.3 | | | | | | | | | | 1 |
| P | A | 12/10/89 | | | | | 5 | 45.0 | 27.1 | 3.95 | 5 | 45.0 | 36.3 | 1.77 | 1 | 10.0 | 47.0 | | | | | | 11 |
| A | A | 10/01/90 | | | | | 6 | 75.0 | 34.0 | 0.54 | 2 | 25.0 | 40.0 | 0.81 | | | | | | | | | 8 |
| R | A | 25/04/90 | 1 | | 24.0 | | | | | | | | | | | | | | | | | | 1 |
| A | | (Sum) | (1) | 4.8 | | | (11) | 52.4 | | | (8) | 38.1 | | | (1) | 4.8 | | | | | | | (21) |
| S | | | | | | | | | | | | | | | | | | | | | | | |
| I | B | 21/07/89 | | | | | | | | | 2 | 12.5 | 29.0 | 0.27 | 12 | 75.0 | 57.5 | 3.36 | 2 | 12.5 | 70.0 | 0.27 | 16 |
| T | B | 12/10/89 | | | | | 3 | 19.0 | 29.0 | 0.27 | 10 | 62.0 | 38.1 | 4.32 | | | | | 3 | 19.0 | 58.8 | 5.30 | 16 |
| I | B | 10/01/90 | | | | | 2 | 66.7 | 38.0 | | 1 | 33.3 | 50.0 | | | | | | | | | | 3 |
| S | B | 25/04/90 | | | | | | | | | | | | | | | | | | | | | 0 |
| E | | (Sum) | | | | | (5) | 14.3 | | | (13) | 37.1 | | | (12) | 34.3 | | | (5) | 14.3 | | | (35) |
| D | | | | | | | | | | | | | | | | | | | | | | | |
| | E | 21/07/89 | | | | | 4 | 29.0 | 27.0 | 2.28 | | | | | 7 | 50.0 | 51.8 | 4.22 | 3 | 21.0 | 64.5 | 1.48 | 14 |
| | E | 12/10/89 | | | | | 3 | 60.0 | 28.0 | | | | | | 1 | 20.0 | 47.0 | | 1 | 20.0 | 65.0 | | 5 |
| | E | 10/01/90 | | | | | 1 | | 30.0 | | | | | | | | | | | | | | 1 |
| | E | 25/04/90 | | | | | 5 | | 37.0 | | | | | | | | | | | | | | 5 |
| | | (Sum) | | | | | (13) | 52.0 | | | | | | | (8) | 32.0 | | | (4) | 16.0 | | | (25) |

$^{-}$0 age group fish born in late summer at the end of spawning season.
$0^{+}$ age group fish born in spawning season, in late spring and early summer.
$1^{+}$ age group fish have passed the 2nd summer, 1 year old.
$2^{+}$ age group fish have passed the 3rd summer, 2 years old.
$3^{+}$ age group fish have passed the 4th summer, 3 years old

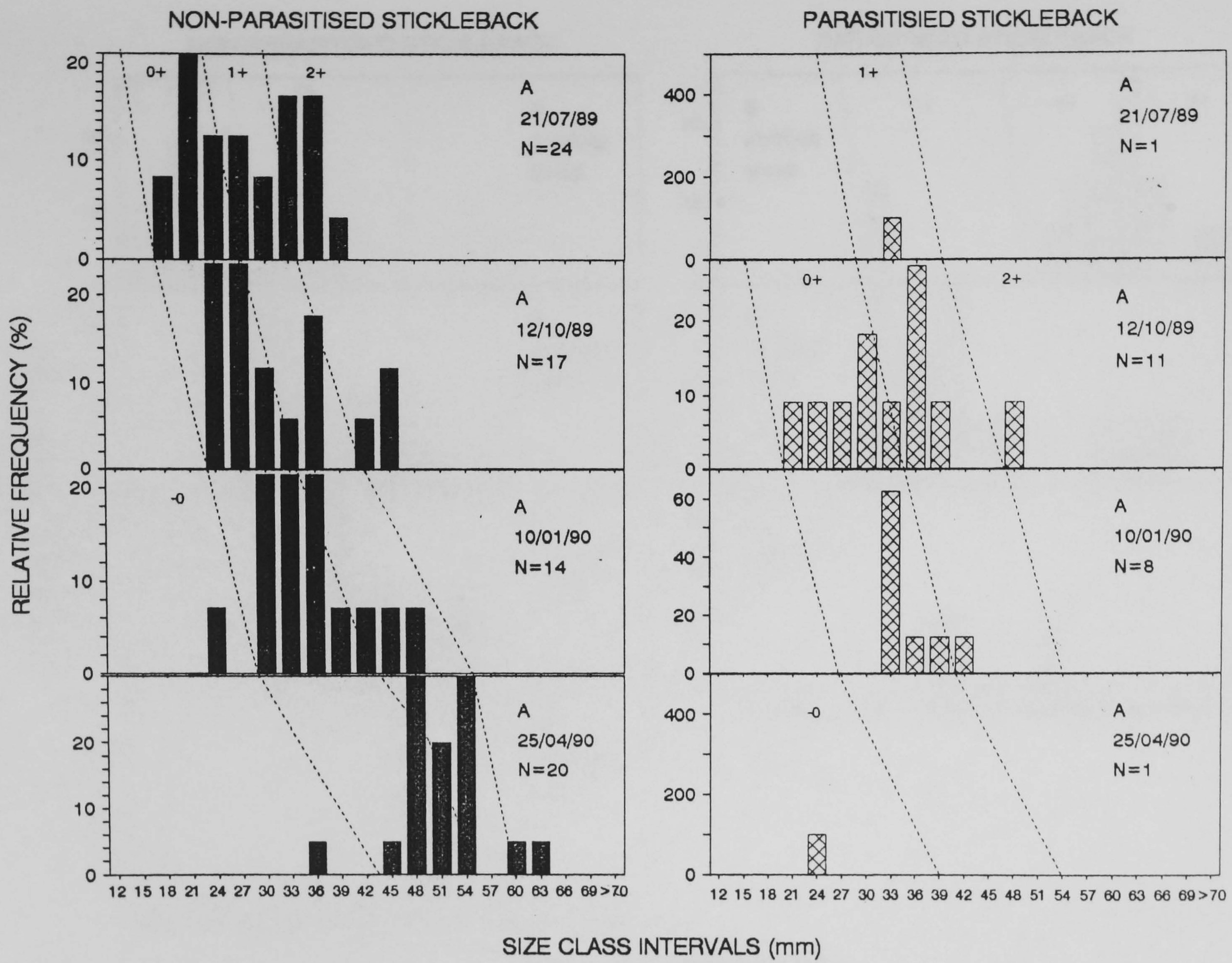



**Figure 4.4** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site A in the River Wandle.

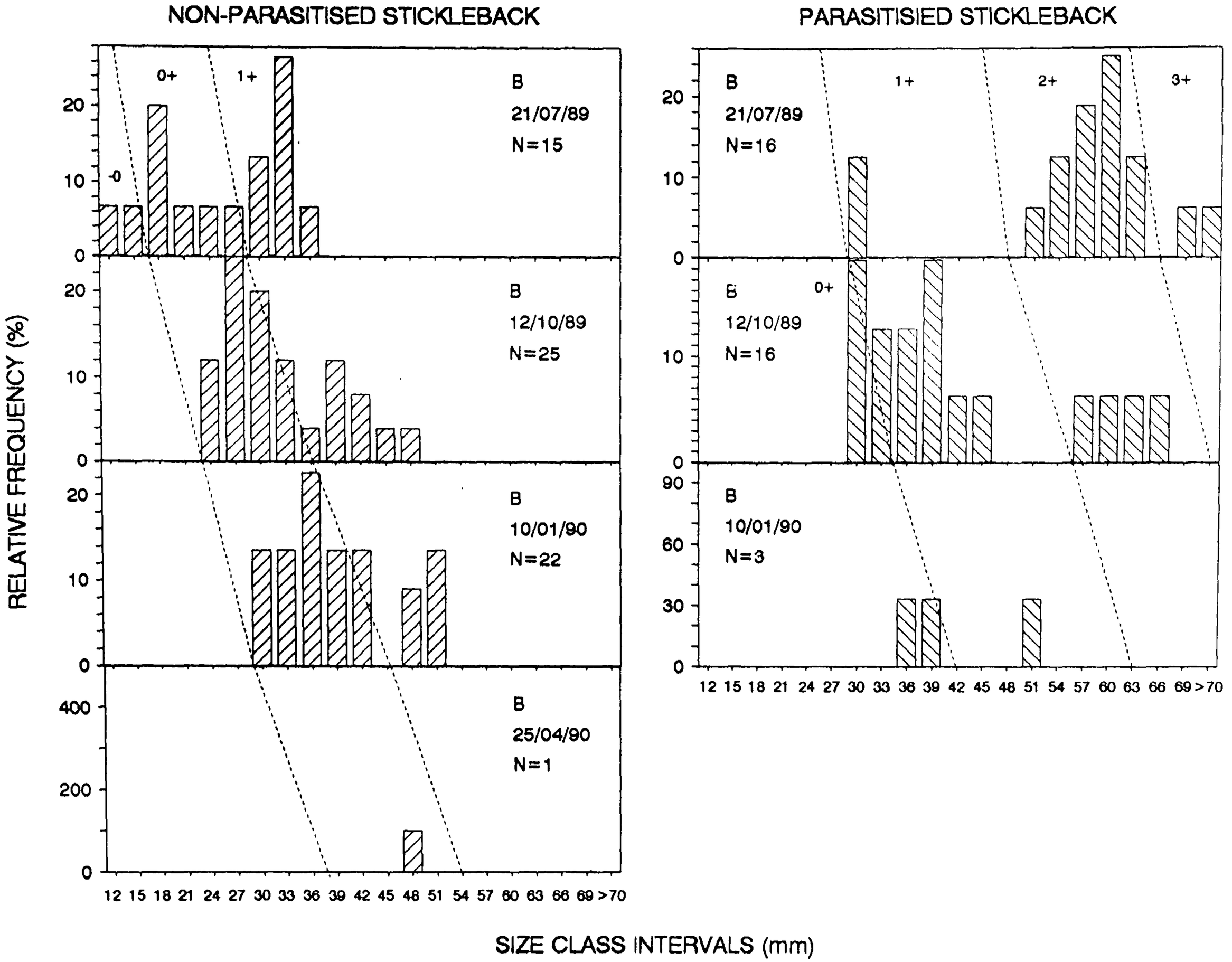



**Figure 4.5** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site B in the River Wandle.

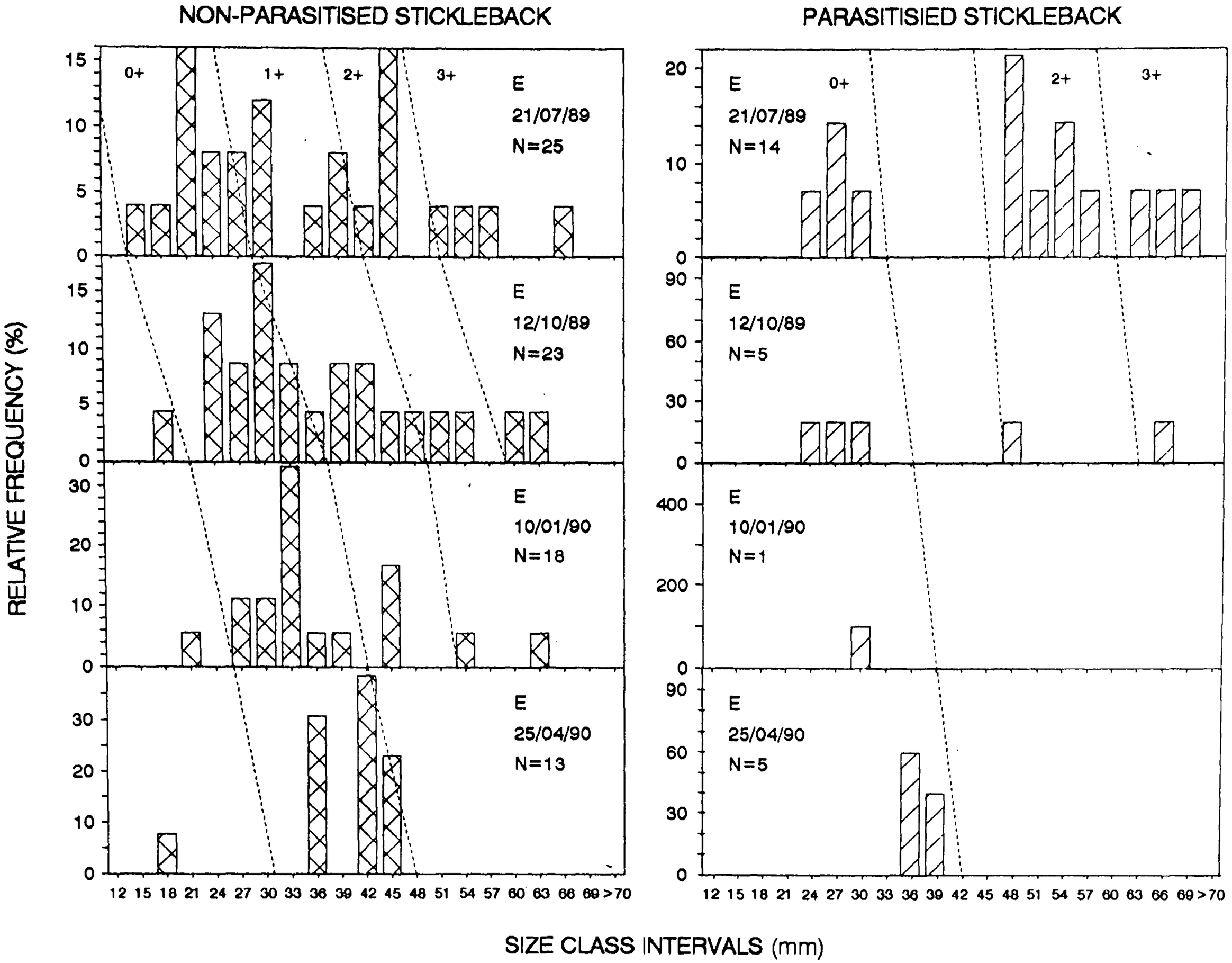



**Figure 4.6** The relative frequency distributions of the total length and the age-group compositions in each seasonal sample of non-parasitised and parasitised sticklebacks at site E in the River Wandle.

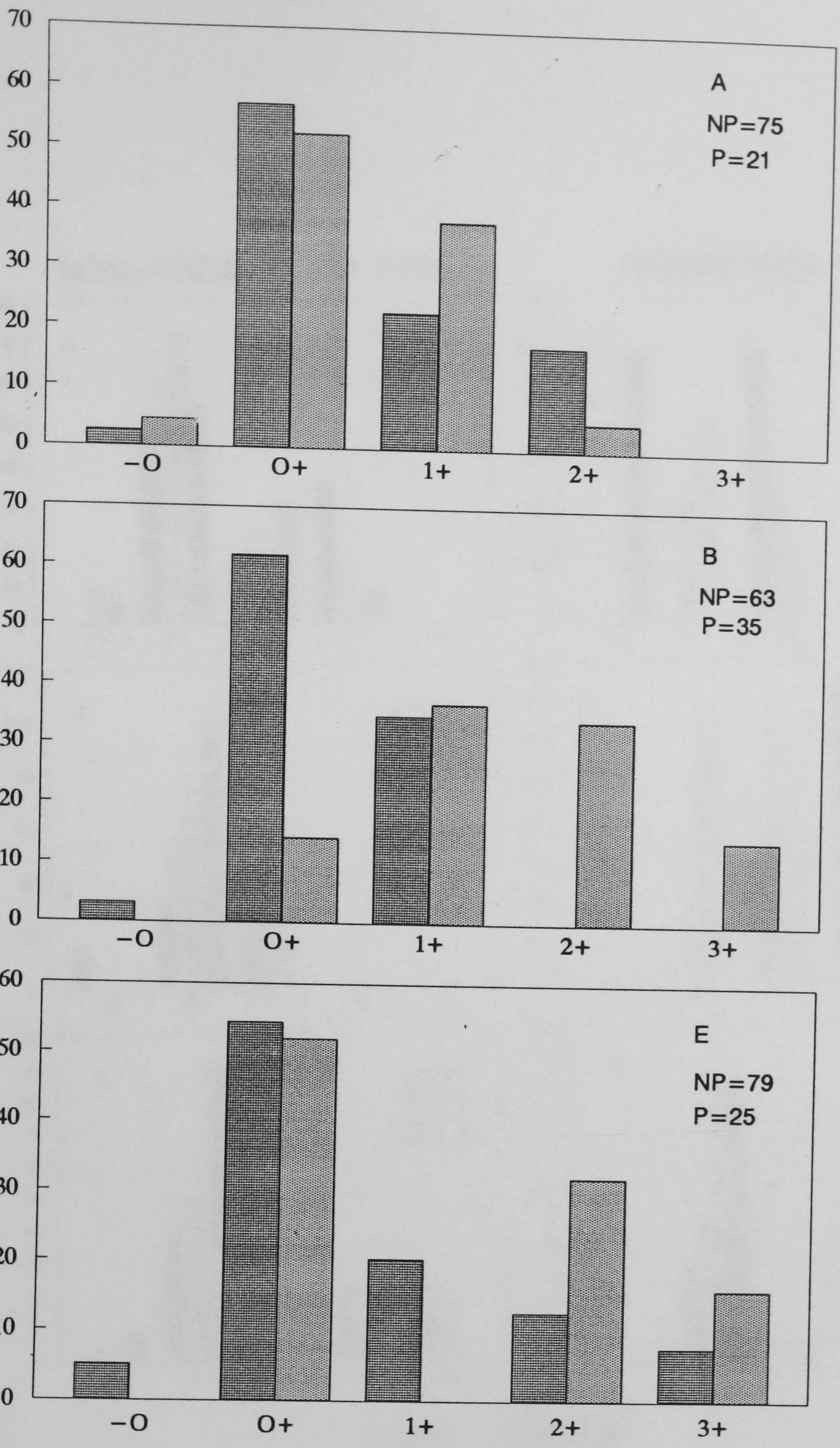



**Figure 4.7** The relative percentage of different age groups in non-parasitised (NP) and parasitised (P) sticklebacks at the three sites (A, B, & E) in the River Wandle.

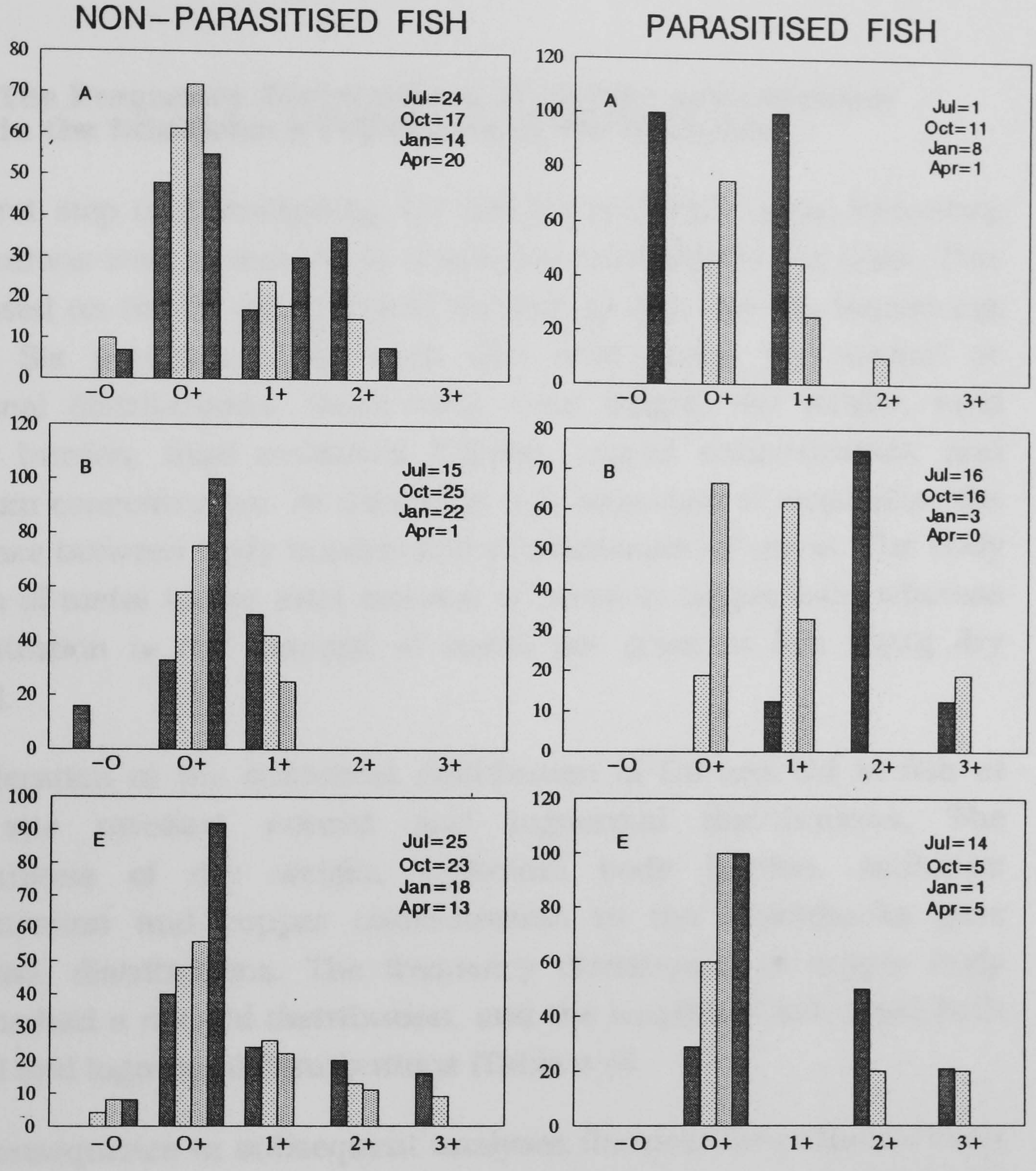



**Figure 4.8** The relative percentage of different age groups in four seasonal samples (Jul., Oct., Jan., & Apr.) at the three sites (A, B, & E) in the River Wandle.

in the distribution at sites A and E, but at B there is an absence of $2^+$ and $3^+$ age groups in the non-parasitised fish, and the only sticklebacks present in the early surveys were infected with the parasite.

### 4.3.3 The Frequency Distributions of Copper and Cadmium in the Stickleback Population at the Each Site.

As a first step in investigating Cu and Cd in sticklebacks, frequency distributions were examined to check the normality of the data. This was based on the $0^+$ age group of the fish as this was the largest age group. Six parameters for each site were tested for normal or lognormal distributions, these were, total length, dry weight, total copper burden, total cadmium burden, copper concentration, and cadmium concentration. At this stage it is important to emphasise the difference between body burden and concentration of metal. The body burden of metal in the total amount of metal in ug per fish, whereas concentration is the amount of metal per gram of fish (ug/g dry weight).

Consideration of the statistical distribution of Cu and Cd in fish at each site revealed normal and lognormal distributions. The distributions of dry weight, cadmium body burden, cadmium concentration and copper concentration in the sticklebacks gave lognormal distributions. The frequency distribution of copper body burdens had a normal distribution, and the lengths of fish fitted both normal and lognormal distributions (Table 4.4).

As a consequence in subsequent analyses the data for cadmium body burden, copper and cadmium concentrations, and dry weight were transformed into logarithmic values so that the residual variations in the data conformed more nearly to normal distribution. The rest were not transformed, and these included all the data of cestode parasites.

### 4.3.4 Fresh Weight-Dry Weight and Length-Weight Relationships and Growth of Stickleback Populations

The total length of the sticklebacks in this study generally ranged from 13 mm to 71 mm, the fresh weights were from 0.01-3.5 g, and

**Table 4.4** Results of the Chi-square tests of the various parameters on the fitness of normal and lognormal distributions of the $0^+$ age groups of sticklebacks at the three sites in the River Wandle (for the significance abbreviations see Table 4.2).

| | | Site A | | | | Site B | | | | Site E | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Para-metre | Distri-bution | Sample Size | Chi-Value | d.f. | Sig. Level | Sample Size | Chi-Value | d.f. | Sig. Level | Sample Size | Chi-Value | d.f. | Sig. Level |
| Total Length | Normal | 43 | 7.2523 | 3 | N.S. | 39 | 1.9831 | 3 | N.S. | 43 | 3.60253 | 3 | N.S. |
| | Lognormal | | 3.7291 | 3 | N.S. | | 1.1035 | 2 | N.S. | | 7.36714 | 4 | N.S. |
| Dry Weight | Normal | 43 | 37.3711 | 3 | *** | 39 | 7.4440 | 2 | + | 43 | 10.9106 | 3 | + |
| | Lognormal | | 9.0418 | 3 | + | | 3.8195 | 2 | N.S. | | 3.62912 | 3 | N.S. |
| Total Copper | Normal | 43 | 10.9595 | 3 | + | 39 | 14.0834 | 2 | *** | 43 | 4.47156 | 2 | N.S. |
| | Lognormal | | 11.9425 | 3 | *** | | 15.9323 | 2 | *** | | 11.9341 | 4 | + |
| Total Cadmium | Normal | 43 | 19.4611 | 3 | *** | 39 | 4.9599 | 2 | N.S. | 43 | 8.98351 | 3 | + |
| | Lognormal | | 2.2580 | 3 | N.S. | | 0.8543 | 2 | N.S. | | 8.84205 | 2 | + |
| Copper Concen-tration | Normal | 43 | 19.8611 | 2 | *** | 39 | 2.4651 | 2 | N.S. | 43 | 6.69819 | 3 | N.S. |
| | Lognormal | | 2.6158 | 2 | N.S. | | 1.7250 | 2 | N.S. | | 3.9033 | 4 | N.S. |
| Cadmium Concen-tration | Normal | 43 | 5.3759 | 3 | N.S. | 39 | 4.5956 | 2 | N.S. | 43 | 10.6793 | 3 | + |
| | Lognormal | | 5.4207 | 3 | N.S. | | 2.5139 | 2 | N.S. | | 2.14419 | 4 | N.S. |

the dry weights were from 0.003 g to about 0.75 g. Regression analyses were undertaken between these parameters at each to the three sites.

Table 4.5 presents the means and standard deviations of the lengths and dry weights of non-parasitised and parasitised sticklebacks at each of the three sites. For the non-parasitised group there is no significant difference in the lengths and weights of fish at the different sites. The fish have average lengths of between 33 - 37 mm and average dry weights of between 0.08-0.09 g. On the other hand parasitised sticklebacks are significantly longer and heavier at site B than the other sites, and those at site E are longer that at site A. Comparing the two groups of fish at each site by t-tests shows the main difference is that the parasitised fish at site B are longer and heavier than the non-parasitised fish ($P<0.0001$). It can be seen from Figures 4.7 and 4.8 that the parasitised fish at site B have a greater proportion of $1^+$ and $2^+$ age groups and relatively few of the $0^+$ age group and this could explain the differences found at site B.

**Table 4.5** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total length and dry-weight in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.), as well as t-tests on the parasitic effects (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.4).

| | NON-PARASITISED STICKLEBACK | | | | | | PARASITISED STICKLEBACK | | | | | | t-statistic of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level | Parasitism H∝NP-P=0 |
| Total Length (AM) | | | | | | n.s. | | | | | | **** | |
| Site A | 75 | 36.37 | 11.38 | 33.92 | 38.81 | | 21 | 33.50 | 6.10 | 28.22 | 38.78 | a | 1.11 n.s. |
| Site B | 63 | 33.47 | 8.83 | 30.79 | 36.14 | | 35 | 48.49 | 13.27 | 44.39 | 52.58 | b | −6.71 **** |
| Site E | 79 | 36.67 | 11.49 | 34.28 | 39.05 | | 25 | 43.08 | 14.15 | 38.24 | 47.92 | a b | −2.30 * |
| Dry Weight (GM) | | | | | | n.s. | | | | | | **** | |
| Site A | 75 | 0.0869 | 2.9300 | 0.0682 | 0.1106 | | 21 | 0.0614 | 2.1447 | 0.0382 | 0.0986 | a | 1.38 n.s. |
| Site B | 63 | 0.0771 | 2.6730 | 0.0592 | 0.1004 | | 35 | 0.2022 | 3.0199 | 0.1401 | 0.2918 | b | −4.45 **** |
| Site E | 79 | 0.0898 | 3.0314 | 0.0710 | 0.1137 | | 25 | 0.1374 | 3.6089 | 0.0890 | 0.2121 | a b | −1.61 n.s. |

## (A) Fresh weight - Dry weight Relationships

The results of linear regression analyses of fresh body weight verses dry body weight carried out on the stickleback populations from each site are shown in Table 4.6. Highly significant relationships were found for non-parasitised, parasitised fish and the cestode parasites at each of the sites. Correlation coefficients ranged between 0.945 to 0.997 ($P<0.0001$) and all were significant at $P<0.0001$.

Analysis of covariance was used to analyse the site differences of fresh weight verses dry weight for non-parasitised and parasitised sticklebacks and their cestode parasites. The results revealed that the regression slopes and intercepts in non-parasitised fish and cestode parasites were significantly different between sites ($P<0.01$), but were not significantly different in the parasitised fish (Table 4.7).

## (B) Length-Weight Relationships

The length-weight relationships of the different age groups of non-parasitised sticklebacks at the three sites were calculated. They were compared on the basis of their age by analysis of covariance. The

**Table 4.6** Results of linear regressions between dry body weights and fresh body weights, and the linearity tests on the non-parasitised and parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | 0.0068 | 0.0029 | 0.2131 | 0.0033 | 0.9913 | 1.2510 | 1 | 1.2510 | 4115.8 | **** |
| | | | | | | | 0.0222 | 73 | 0.0003 | | |
| Site B | 63 | −0.0051 | 0.0024 | 0.2789 | 0.0047 | 0.9914 | 0.4522 | 1 | 0.4522 | 3484.3 | **** |
| | | | | | | | 0.0079 | 61 | 0.0001 | | |
| Site E | 79 | −0.0033 | 0.0069 | 0.2320 | 0.0074 | 0.9657 | 2.0767 | 1 | 2.0767 | 1064.8 | **** |
| | | | | | | | 0.1502 | 77 | 0.0020 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | 0.0083 | 0.0026 | 0.2102 | 0.0066 | 0.9909 | 0.0384 | 1 | 0.0384 | 1022.3 | **** |
| | | | | | | | 0.0007 | 19 | 0.0000 | | |
| Site B | 35 | 0.0218 | 0.0128 | 0.2171 | 0.0077 | 0.9797 | 1.6328 | 1 | 1.6328 | 790.0 | **** |
| | | | | | | | 0.0682 | 33 | 0.0021 | | |
| Site E | 25 | −0.0081 | 0.0269 | 0.2479 | 0.0179 | 0.9449 | 1.6332 | 1 | 1.6332 | 191.5 | **** |
| | | | | | | | 0.1962 | 23 | 0.0085 | | |
| Cestode Parasites of Stickleback | | | | | | | | | | | |
| Site A | 21 | 0.0055 | 0.0015 | 0.1971 | 0.0116 | 0.9687 | 0.0069 | 1 | 0.0069 | 289.3 | **** |
| | | | | | | | 0.0005 | 19 | 0.0000 | | |
| Site B | 35 | −0.0000 | 0.0002 | 0.2949 | 0.0038 | 0.9973 | 0.3115 | 1 | 0.3115 | 6089.1 | **** |
| | | | | | | | 0.0017 | 33 | 0.0001 | | |
| Site E | 25 | −0.0004 | 0.0033 | 0.2658 | 0.0096 | 0.9852 | 0.1025 | 1 | 0.1025 | 763.1 | **** |
| | | | | | | | 0.0031 | 23 | 0.0001 | | |

**Table 4.7** The ANOCOVA tables of site effects on the relationships between the dry weights and fresh weights in non-parasitised and parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Non− | Equality of Slopes | 2 | 0.0260219 | 0.0130110 | 15.2284 | ** |
| parasitised | Residual Error | 211 | 0.1802760 | 0.0008544 | | |
| Stickleback | Equality of Adj. Means | 2 | 0.0132479 | 0.0066240 | 6.8390 | ** |
| | Residual Error | 213 | 0.2062979 | 0.0009685 | | |
| Parasitised | Equality of Slopes | 2 | 0.0146060 | 0.0073030 | 2.0662 | n.s. |
| Stickleback | Residual Error | 75 | 0.2650950 | 0.0035346 | | |
| | Equality of Adj. Means | 2 | 0.0011645 | 0.0005823 | 0.1600 | n.s. |
| | Residual Error | 77 | 0.2797010 | 0.0036325 | | |
| Parasites | Equality of Slopes | 2 | 0.0022394 | 0.0011197 | 16.0623 | ** |
| | Residual Error | 75 | 0.0052280 | 0.0000697 | | |
| | Equality of Adj. Means | 2 | 0.0011811 | 0.0005906 | 6.0900 | ** |
| | Residual Error | 77 | 0.0074674 | 0.0000970 | | |

(for significance abbreviations see Table 4.2)

results of the analysis for log dry body weight (DW) versus log total length (TL) are shown in Table 4.8. The relationships for each age group at each site were not significantly different except those at site B ($P<0.05$).

The site difference were examined by pooling together the different age groups of sites A and E, but taking only the $0^+$ age group of site B. There were no significant differences between the slopes or the intercepts between sites. The analysis was repeated with the $1^+$ age group of site B fish to the $0^+$ age group of B and the populations of A and E. Significant differences were found for the slopes ($P<0.1$) and the intercepts ($P<0.0001$; Table 4.8).

Figure 4.9 shows the length-weight relationships, together with the regression models for the non-parasitised fish at site A, E and $0^+$ age at site B. For the pooled samples the regression line was $DW = 6.7638 \times 10^{-7} TL^{3.3245}$ ($r=0.9845$), and for the $1^+$ age at site B it was DW =

**Table 4.8** The ANOCOVA tables of the effects of age on the relationships between logarithmic dry weights and the logarithmic total lengths at the each sites and between sites in the non-parasitised sticklebacks.

| Source | | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|---|
| Dif. age | | Equality of Slopes | 2 | 0.2018240 | 0.0100912 | 3.1303 | + |
| group at | Site A | Residual Error | 67 | 0.2159870 | 0.0032237 | | |
| | | Equality of Adj. Means | 2 | 0.0104970 | 0.0052485 | 1.5330 | n.s. |
| | | Residual Error | 69 | 0.2361694 | 0.0034227 | | |
| Dif. age | | Equality of Slopes | 1 | 0.0022472 | 0.0224720 | 4.7163 | * |
| group at | Site B | Residual Error | 57 | 0.2715900 | 0.0047647 | | |
| | | Equality of Adj. Means | 1 | 0.0270006 | 0.0270006 | 5.3260 | * |
| | | Residual Error | 58 | 0.2940620 | 0.0050700 | | |
| Dif. age | | Equality of Slopes | 4 | 0.0655979 | 0.0163995 | 1.8048 | n.s. |
| group at | Site E | Residual Error | 69 | 0.6269654 | 0.0090865 | | |
| | | Equality of Adj. Means | 4 | 0.0745041 | 0.0186260 | 1.9630 | n.s. |
| | | Residual Error | 73 | 0.6925633 | 0.0094873 | | |
| Compare | Equality of Slopes | | 2 | 0.0012157 | 0.0006079 | 0.0955 | n.s. |
| Pooled A | Residual Error | | 187 | 1.1902500 | 0.0063640 | | |
| Pooled E | Equality of Adj. Means | | 2 | 0.0333504 | 0.0166752 | 2.6450 | + |
| B (0+ age) | Residual Error | | 189 | 1.1914657 | 0.0063041 | | |
| Compare | Equality of Slopes | | 1 | 0.0211017 | 0.0211017 | 3.3719 | + |
| Pooled All | Residual Error | | 211 | 1.3204520 | 0.0062581 | | |
| & | Equality of Adj. Means | | 1 | 0.0994793 | 0.0994793 | 15.7200 | **** |
| B (1+ age) | Residual Error | | 212 | 1.3415537 | 0.0063282 | | |

(for significance abbreviations see Table 4.2)

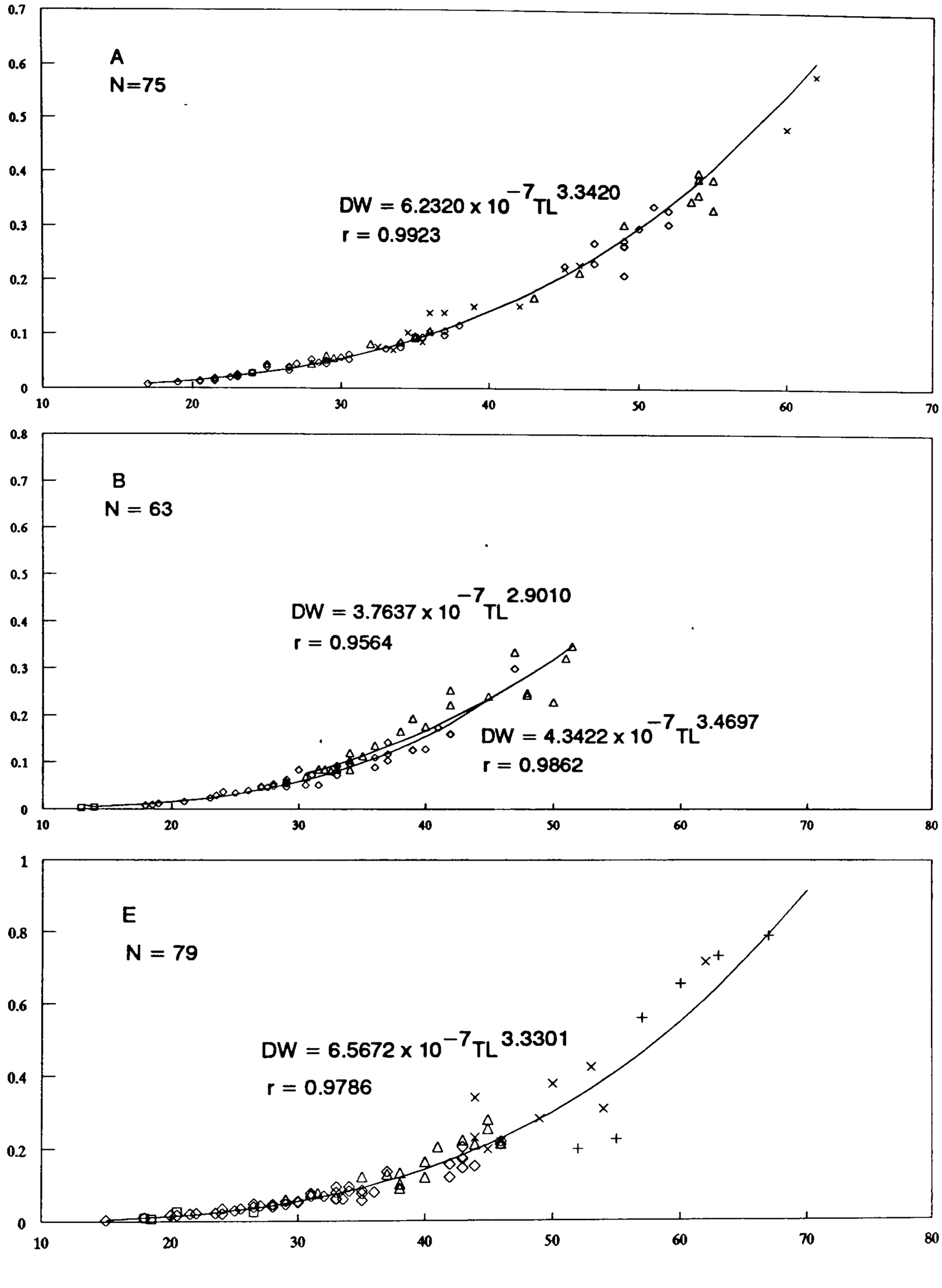



**Figure 4.9** The relationship of dry weight (DW) and total length (TL) in non-parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.1).

$3.7637 \times 10^{-7}$ TL $^{2.9010}$ (r=0.9564). The regression slopes were between 3.3301 to 3.4697, and the correlation coefficients were between 0.9786 to 0.9923.

Regression models of length-weight relationships for parasitised stickleback were developed as described above. Significant differences were found between $0^+$ and $1^+$ age groups at site A ($P<0.05$) and between $>2^+$ age fish and other age groups at site B ($P<0.01$). For all other relationships no significant difference was found (Table 4.9).

For the parasitised fish the regression models were DW = $3.3413 \times 10^{-7}$ TL $^{3.4943}$ (r=0.9895) for the most of the fish, except DW = $7.2611 \times 10^{-6}$ TL $^{2.5899}$ (r=0.9369) for the $0^+$ age fish at site A, DW = $4.0413 \times 10^{-8}$ TL $^{3.9113}$ (r=0.8589) for the $3^+$ age fish at site B. The regression slopes were between 3.4756 to 3.5237, and correlation coefficient were between 0.9861 to 0.9913 (Figure 4.10).

A comparison of the difference of length-weight relationship between non-parasitised and parasitised sticklebacks were made using the groups of fish which showed no significant difference in the above statistical analyses. Significant differences ($P<0.05$) were found between slopes and intercepts of non-parasitised and parasitised sticklebacks (Table 4.10). The regression equations were DW = $6.7638 \times 10^{-7}$ TL $^{3.3245}$ for non-parasitised sticklebacks and DW= $3.3413 \times 10^{-7}$ TL $^{3.4943}$ for parasitised sticklebacks, which indicate that parasitised sticklebacks tend to be lighter than the non-parasitised fish while they had same length, or same age.

(C) Growth Rate of the Wandle Sticklebacks

The absolute growth rates and relative growth rates of stickleback were calculated in terms of length and dry body weight. For the fish in the $0^+$ and $0^-$ age groups the absolute growth rate and the relative growth rate were estimated on tri-monthly basis, while for fish in the older age groups these parameters were determined on an annual basis (Figure 4.11). These reveal that the $0^+$ group fish grew quickly from 9 to 12 months old during late spring to early summer just prior to spawning. The relative dry weight growth rate tended to decrease in fish of 6 to 9 months old during the winter months.

**Table 4.9** The ANOCOVA tables of the effects of age on the relationships between logarithmic dry weights and the logarithmic total lengths at the each sites and between sites in the parasitised sticklebacks.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| At Site A | Equality of Slopes | 1 | 0.0261362 | 0.0261362 | 5.8028 | * |
| 0+ age v.s. | Residual Error | 15 | 0.0675610 | 0.0045041 | | |
| 1+ age | Equality of Adj. Means | 1 | 0.0009125 | 0.0009125 | 0.1560 | n.s. |
| | Residual Error | 16 | 0.0936972 | 0.0058561 | | |
| At Site B | Equality of Slopes | 1 | 0.0000423 | 0.0000423 | 0.0092 | n.s. |
| 1+ age v.s. | Residual Error | 21 | 0.0961600 | 0.0045791 | | |
| 2+ age | Equality of Adj. Means | 1 | 0.0000771 | 0.0000771 | 0.0180 | n.s. |
| | Residual Error | 22 | 0.0962023 | 0.0043728 | | |
| At Site B | Equality of Slopes | 1 | 0.0005301 | 0.0005301 | 0.2198 | n.s. |
| 0+ age v.s. | Residual Error | 26 | 0.1095021 | 0.0042116 | | |
| 1+ & 2+ age | Equality of Adj. Means | 1 | 0.0010849 | 0.0010849 | 0.2660 | n.s. |
| | Residual Error | 27 | 0.1100322 | 0.0040753 | | |
| At Site B | Equality of Slopes | 1 | 0.1462549 | 0.1462549 | 16.5398 | ** |
| 0+ age v.s. | Residual Error | 31 | 0.2741200 | 0.0088426 | | |
| >2+ age | Equality of Adj. Means | 1 | 0.0849375 | 0.0849375 | 21.2570 | *** |
| | Residual Error | 32 | 0.1278651 | 0.0039958 | | |
| At Site E | Equality of Slopes | 1 | 0.0000014 | 0.0000014 | 0.0017 | n.s. |
| 0+ age v.s. | Residual Error | 21 | 0.1696364 | 0.0080779 | | |
| 2+ & 3+ age | Equality of Adj. Means | 1 | 0.0365474 | 0.0365474 | 4.7400 | * |
| | Residual Error | 22 | 0.1696378 | 0.0077108 | | |
| Site Dif. | Equality of Slopes | 2 | 0.0006927 | 0.0003464 | 0.0579 | n.s. |
| A 1+ age, | Residual Error | 57 | 0.3408510 | 0.0059798 | | |
| B ≤2+ age, | Equality of Adj. Means | 2 | 0.0010815 | 0.0005408 | 0.0930 | n.s. |
| v.s. E fish | Residual Error | 59 | 0.3415390 | 0.0057888 | | |

**Table 4.10** The ANOCOVA tables of the effects of parasitism on the relationships between logarithmic dry weights and the logarithmic total lengths between the non-parasitised and the parasitised sticklebacks.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Major Groups of | Equality of Slopes | 1 | 0.0372140 | 0.0372140 | 5.9830 | * |
| Non−parasitised v.s. | Residual Error | 252 | 1.5674400 | 0.0062200 | | |
| Parasitised Stickleback | Equality of Adj. Means | 1 | 0.0296509 | 0.0296509 | 4.6750 | * |
| | Residual Error | 253 | 1.6046054 | 0.0063423 | | |

(for significance abbreviations see Table 4.2)

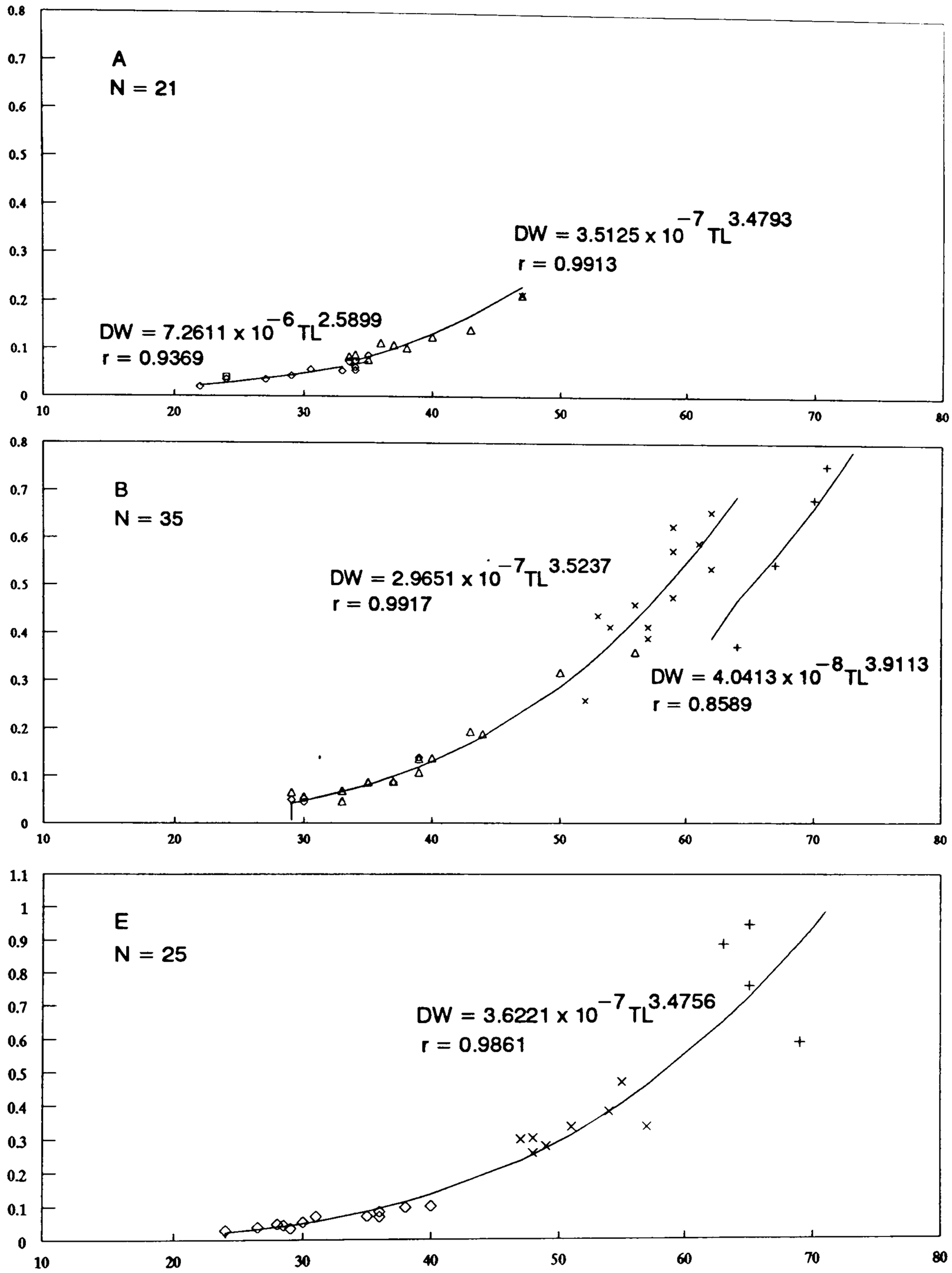



**Figure 4.10** The relationship of dry weight (DW) and total length (TL) in parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.2).

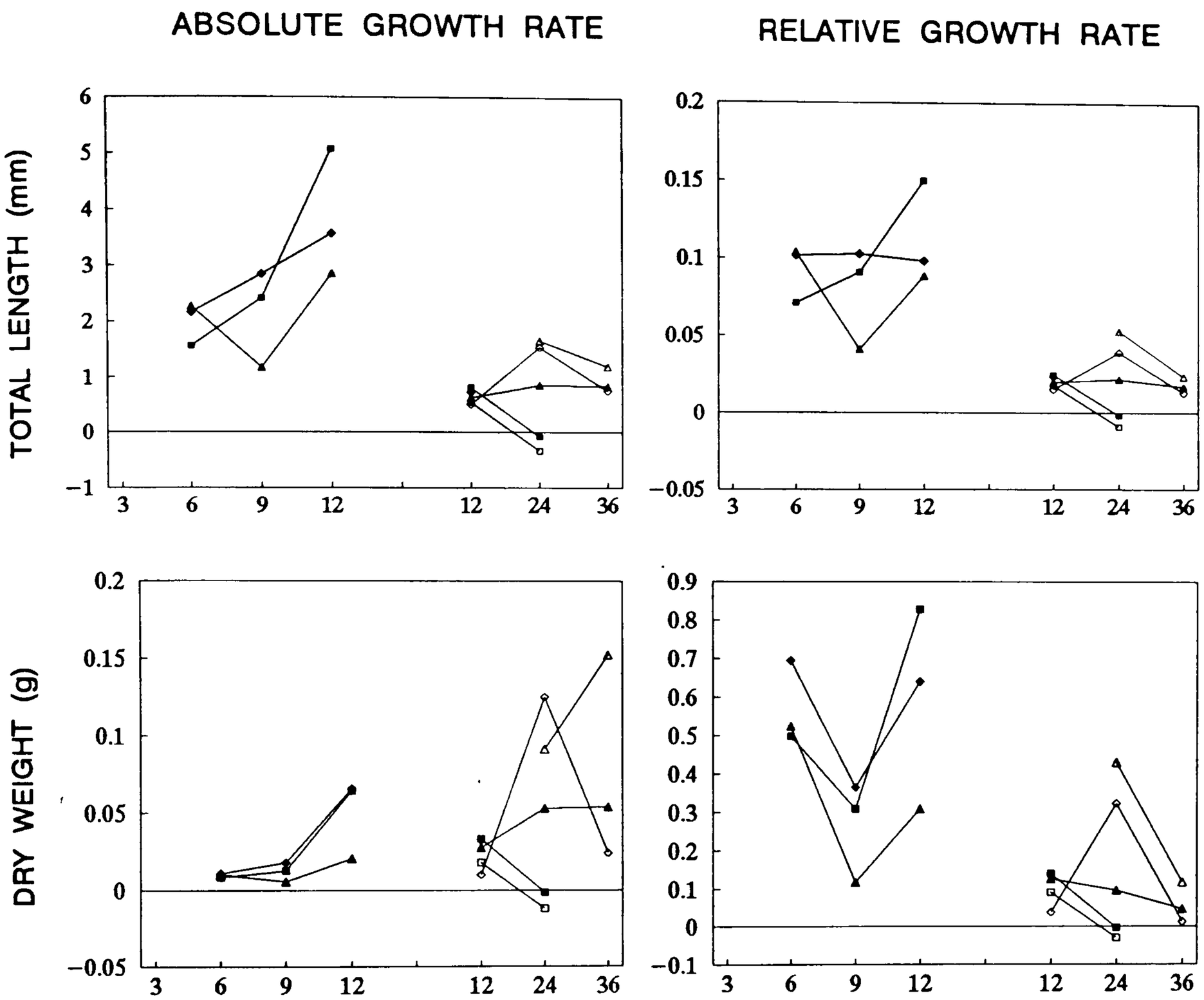



**Figure 4.11** A comparison of growth rates by length and dry-weight of non-parasitised (NP) and parasitised (P) at tri-monthly intervals for the $0^+$ age group (left-hand side) and 12 month intervals for older fish (right-hand site) ate the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.3).

For non-parasitised and parasitised fish over 1 year at site A the absolute growth rates and relative growth rates by length and dry weight showed a marked decrease. At site E the increase in length of non-parasitised fish tended to be constant over the different age groups, however, while the absolute dry weight growth rate increased, the relative growth rate decreased. In parasitised fish of site B and E, there were significant decreases in growth of fish over $2^+$ years old. The only exception was the absolute growth rate in the fish at site E.

### 4.3.5 Copper and Cadmium in Sticklebacks and Their Cestode Parasites at Each Site

Figure 4.12 presents a summary of mean values at each sites, for body burdens of Cu and Cd and concentrations of Cu and Cd in non-parasitised and parasitised sticklebacks. Table 4.11 and 4.12 present the results of the ANOVA analyses which examine the site differences of Cu and Cd concentrations in the two groups of fish, non-parasitised stickleback and parasitised sticklebacks, and the cestode parasites, respectively.

The main point that emerges is the trend in the metal content of the fish between the sites. At site A, which has been classified as the control or reference site, the lowest body burdens and concentrations of the two metals are found. The sticklebacks from sites B and E have elevated levels of Cu and Cd. In the non-parasitised group the body burdens of Cu are 5 and 6 times higher at B and E respectively than at A, for Cd there is a factor of three in the difference. Similar factors were found for the concentrations of Cu (x6 at B and x5 at E) and Cd (x4 at B and x3 at E). Analysis of variance together with the multiple range test shows that sites B and E are significantly different from A ($P<0.0001$; Table 4.11).

For the parasitised group the same trends in the metal content of the fish between sites is shown. At site B, the body burdens in particular are very much more elevated than at the control site. For example the body burden of Cu at site B is x22 greater that at A. Analysis of variance for the data reveals distinct differences ($P<0.0001$) and the multiple range test shows that the values at site B are significantly different than values at the other two sites. The values at site B in

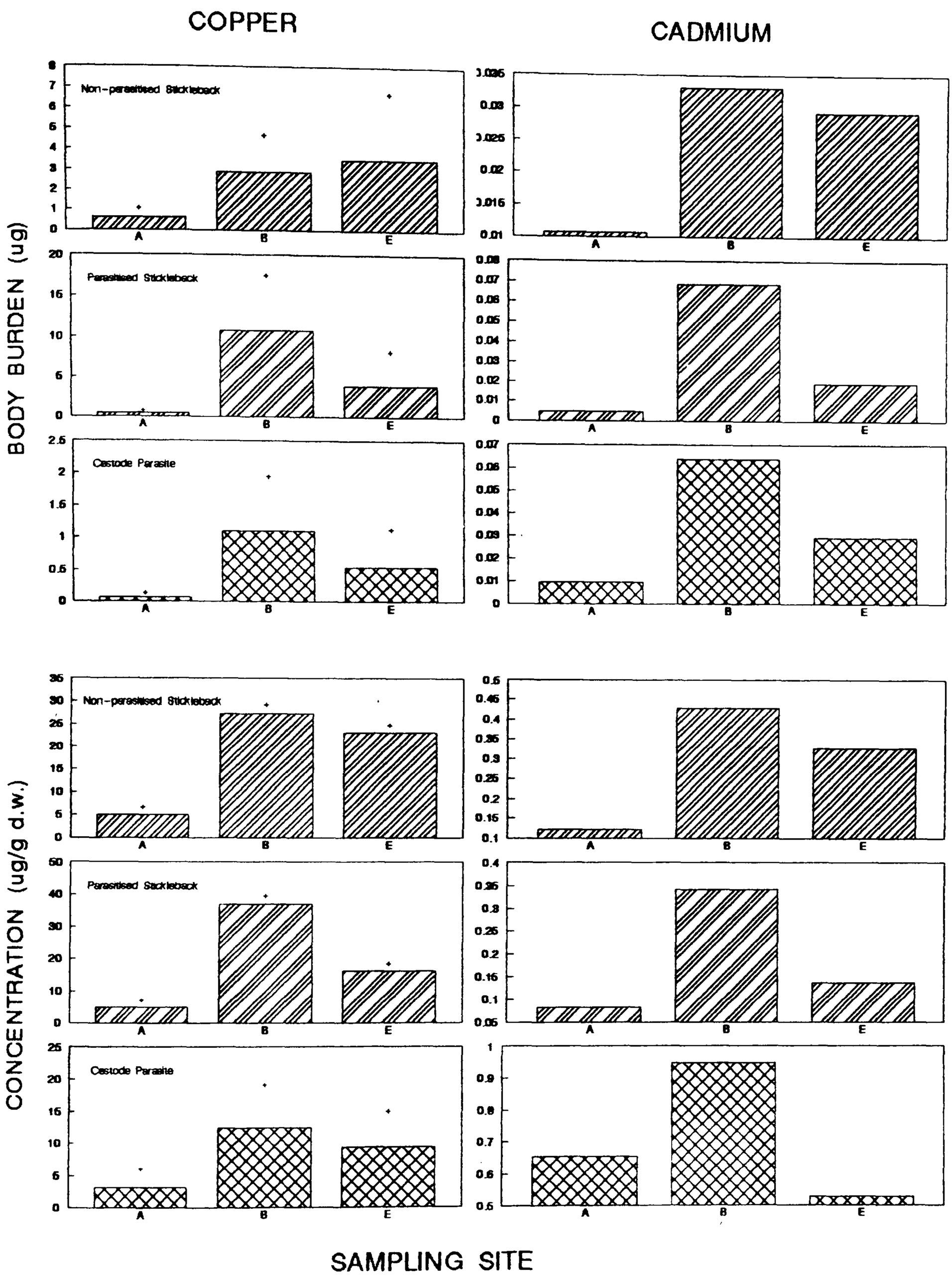



**Figure 4.12** Comparisons of body burdens of Cu and Cd, and concentrations of Cu and Cd of non-parasitised, parasitised sticklebacks, and their cestode parasites at the three sites in the River Wandle. Histograms present the means, and + indicate the standard deviations.

parasitised fish are all significantly different from the values in non-parasitised fish ($P<0.05$) and parasitised fish have the higher metal contents, except in the case of Cd concentration. At site A there are no significantly lower values in parasitised fish at the 5% level. At site E parasitised fish have significantly lower metal concentrations than their non-parasitised counterparts ($P<0.0001$), but no significant difference of metal body burdens was found (Table 4.11).

**Table 4.11** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.int.), as well as t-tests on the parasitic effects (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.4).

| | NON-PARASITISED STICKLEBACK | | | | | | PARASITISED STICKLEBACK | | | | | | t-statistic of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level | Parasitism H$\alpha$NP-P=0 |
| **Total Copper Burden (AM)** | | | | | | **** | | | | | | **** | |
| Site A | 75 | 0.6097 | 0.4346 | 0.1118 | 1.1076 | a | 21 | 0.477 | 0.268 | −1.727 | 2.680 | a | −0.49 n.s. |
| Site B | 63 | 2.8553 | 1.7760 | 2.3120 | 3.3985 | b | 35 | 10.737 | 6.795 | 9.030 | 12.444 | b | −8.72 **** |
| Site E | 79 | 3.4782 | 3.2307 | 2.9930 | 3.9633 | b | 25 | 3.875 | 4.256 | 1.855 | 5.894 | a | 1.33 n.s. |
| **Total Cadmium Burden (GM)** | | | | | | **** | | | | | | **** | |
| Site A | 75 | 0.0107 | 3.4422 | 0.0083 | 0.0137 | a | 21 | 0.0051 | 3.2666 | 0.0030 | 0.0087 | a | 2.46 n.s. |
| Site B | 63 | 0.0331 | 2.6727 | 0.0253 | 0.0434 | b | 35 | 0.0690 | 3.1465 | 0.0455 | 0.1048 | c | −3.34 ** |
| Site E | 79 | 0.0293 | 2.7703 | 0.0230 | 0.0373 | b | 25 | 0.0188 | 4.0628 | 0.0115 | 0.0308 | b | 1.72 n.s. |
| **Copper Concentration (GM)** | | | | | | **** | | | | | | **** | |
| Site A | 75 | 4.970 | 1.737 | 4.383 | 5.635 | a | 21 | 5.140 | 2.100 | 3.596 | 7.348 | a | −2.23 n.s. |
| Site B | 63 | 27.290 | 1.837 | 23.794 | 31.299 | b | 35 | 37.119 | 2.470 | 28.144 | 48.957 | c | −2.01 * |
| Site E | 79 | 23.086 | 1.652 | 20.426 | 26.091 | b | 25 | 16.316 | 2.144 | 9.536 | 18.360 | b | 4.23 **** |
| **Cadmium Concentration (GM)** | | | | | | **** | | | | | | **** | |
| Site A | 75 | 0.1231 | 1.8097 | 0.1089 | 0.1392 | a | 21 | 0.0827 | 2.1936 | 0.0565 | 0.1210 | a | 2.52 * |
| Site B | 63 | 0.4297 | 1.4410 | 2.6614 | 2.0350 | c | 35 | 0.3415 | 2.0566 | 0.2543 | 0.4586 | b | 2.10 * |
| Site E | 79 | 0.3258 | 1.8252 | 3.4602 | 2.7223 | b | 25 | 0.1368 | 3.0489 | 0.0965 | 0.1939 | a | 5.01 **** |

**Table 4.12** Assessment of the differences between sites by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in cestode parasites of stickleback at the three sites in the River Wandle, with the arithmetic means (AM), their standard deviations and 95% confidence limits (C. Int.) (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.5).

| Site | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level |
|---|---|---|---|---|---|---|
| **CESTODE PARASITES** | | | | | | |
| Total Copper Burden (AM) | | | | | | **** |
| Site A | 21 | 0.07519 | 0.06259 | −0.20292 | 0.35461 | a |
| Site B | 35 | 1.09380 | 0.85478 | 0.97456 | 1.40641 | b |
| Site E | 25 | 0.54063 | 0.58956 | 0.28514 | 0.79612 | a |
| Total Cadmium Burden (AM) | | | | | | * |
| Site A | 21 | 0.00966 | 0.01631 | −0.00332 | 0.01790 | a |
| Site B | 35 | 0.06383 | 0.12321 | 0.01693 | 0.03338 | b |
| Site E | 25 | 0.02927 | 0.06706 | 0.00736 | 0.02682 | a b |
| Copper Concentration (AM) | | | | | | **** |
| Site A | 21 | 3.17162 | 2.85382 | 1.07285 | 5.89165 | a |
| Site B | 35 | 12.29510 | 6.80563 | 10.42877 | 14.16139 | b |
| Site E | 25 | 9.33281 | 5.55334 | 7.12456 | 11.54107 | b |
| Cadmium Concentration (AM) | | | | | | n.s. |
| Site A | 21 | 0.65316 | 1.57764 | 0.17867 | 0.43184 | |
| Site B | 35 | 0.94565 | 2.67629 | 0.18559 | 0.38170 | |
| Site E | 25 | 0.52921 | 0.75702 | 0.14276 | 0.37480 | |

Taking the cestode parasite on its own, it can be seen from Table 4.12 that the same overall trends in metal levels occur in the parasite as in their hosts and non-parasitised fish (Figure 4.12). Site B has the highest values followed by E and then A, with the exception of Cd concentration which was exceptionally low at site E.

### 4.3.6 Copper and Cadmium in the Sticklebacks and the Cestode Parasites and Their Relationships to the Size of the Organisms

The relationship of copper and cadmium body burden and the size of fish in terms of the log total length and dry weight are plotted in Figures 4.13 and 4.14. With log transformed data, significant linear relationships were obtained, showing that the body burden of Cu (FTCu) and Cd (FTCd) increased with dry weight (FDW), total length (FTL) and therefore age of the fish. The linear regression relationships between logFTCu and logFTCd versus logFTL and logFDW for both non-parasitised and parasitised sticklebacks at the three sites are listed in Tables 4.13 and 4.14.

By contrast, the relationships using the concentrations of Cu (FSCu) and Cd (FSCd) in fish do not show the same degree of linearity and where it does exist the tendency is for a decrease in metal concentration with increase in the size of fish (Figures 4.15 and 4.16).

The negative linear regression between log copper concentration and log fish size parameters are significant in non-parasitised sticklebacks at site A ($P<0.001$) and site B ($P<0.1$), but not significant in non-parasitised fish at site E and all the parasitised fish (Table 4.15). The log cadmium concentration and log fish size parameters relationships give significant negative correlations at site E only both for non-parasitised fish ($P<0.001$) and for parasitised fish ($P<0.1$; Table 4.16).

The relationships between the actual cestode parasite and its metal content are examined in Figures 4.17 and 4.18, and Tables 4.17 and 4.18 present the regression equations. In Figure 4.17 there is a tendency for Cu and Cd body burden to increase with increases in the length and weight of the parasite. Table 4.17 shows that all relationships are significant apart from total-Cu versus cestode length at site A and total-Cd versus cestode length at sites A and B.

In addition, the relationship between copper and cadmium concentrations and the size of parasites are shown in Figure 4.18. The concentrations of Cu and Cd of parasites appear to show inverse relationships with weight of the parasite, although as Table 4.18 shows, only those at site B were significant.

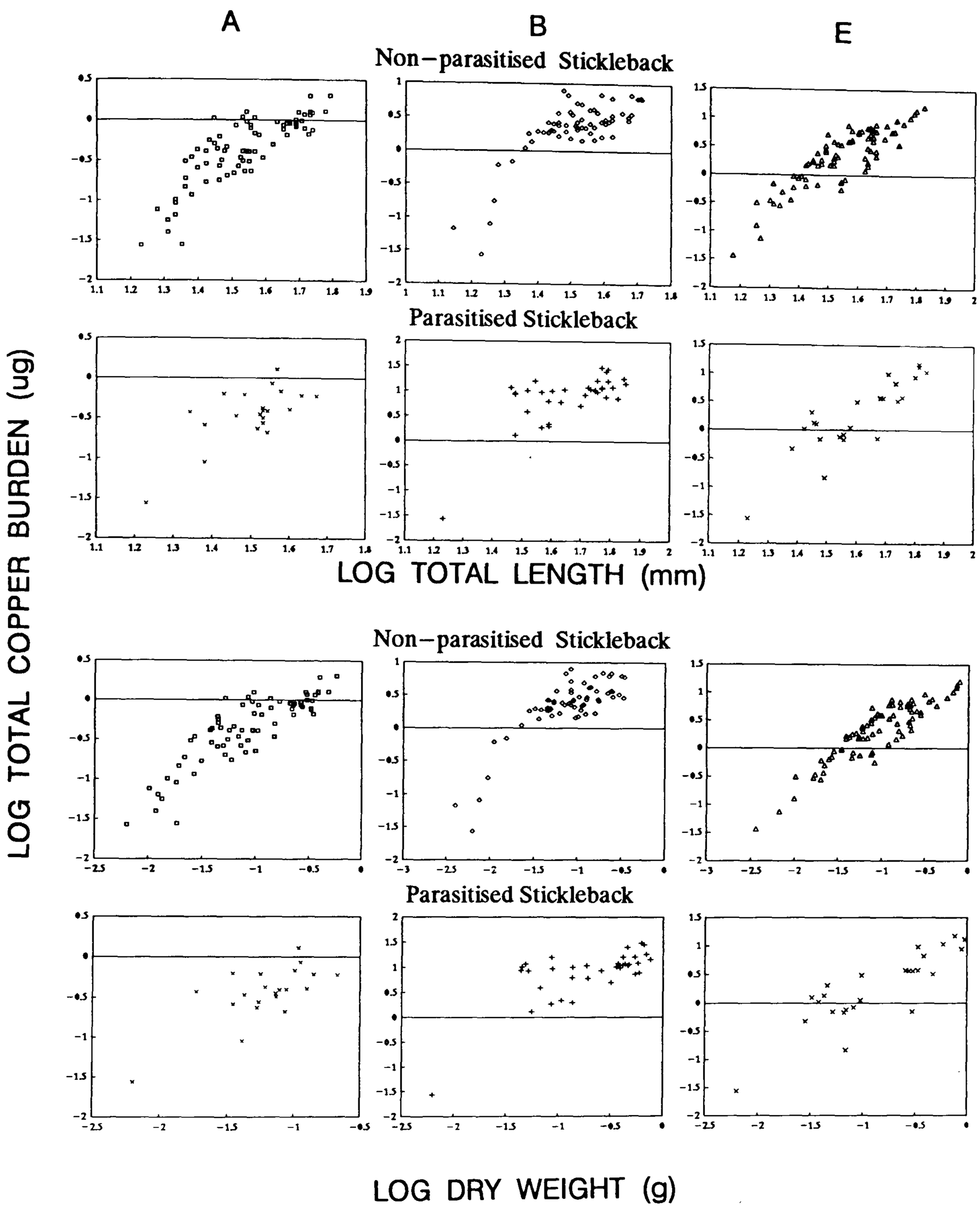



**Figure 4.13** The relationships between log total copper burdens and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.1 and C.2).

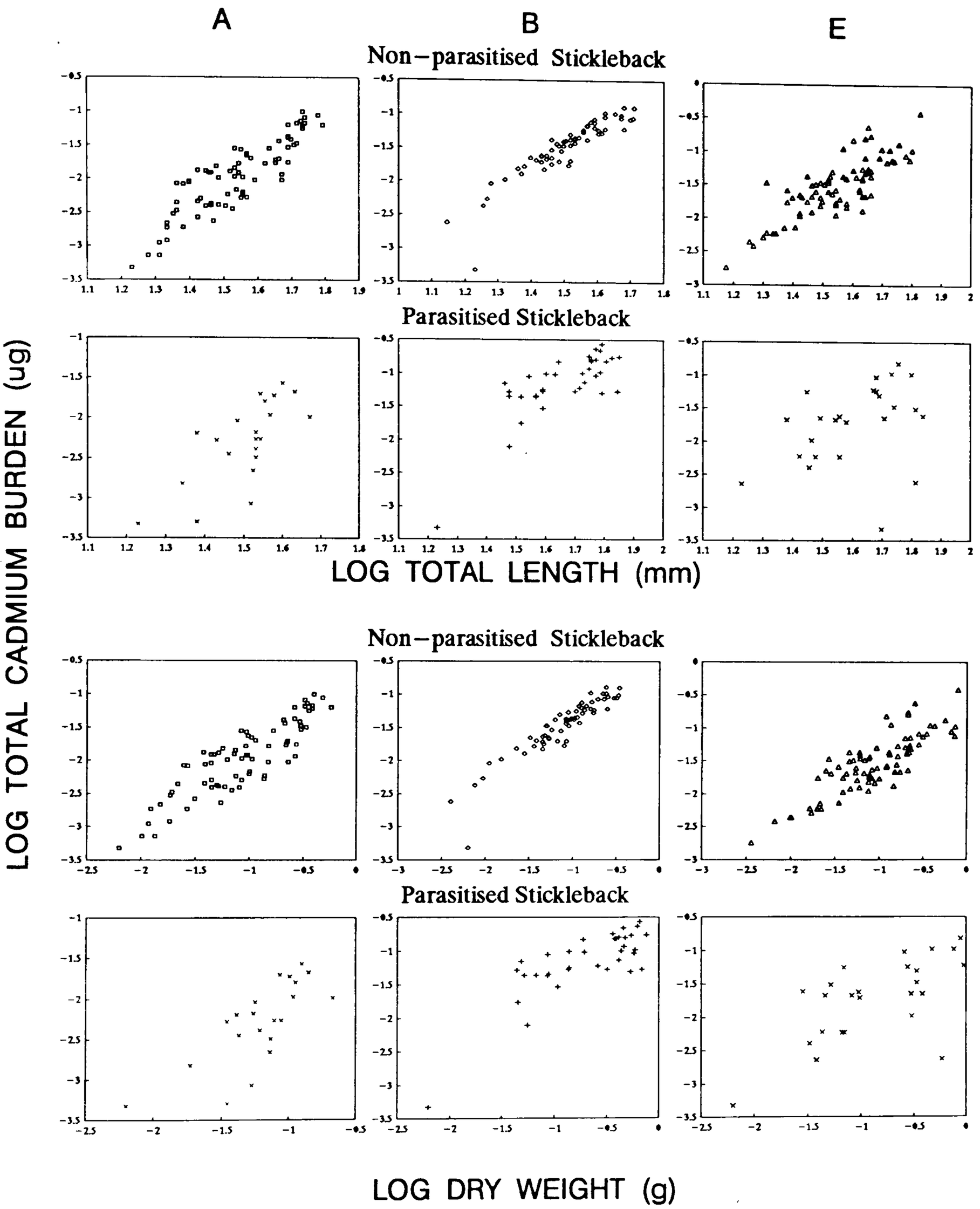



**Figure 4.14** The relationships between log total cadmium burdens and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.1 and C.2).

**Table 4.13** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: logFTCu = a + b logFTL | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | −4.2509 | 0.3051 | 2.5246 | 0.1975 | 0.831449 | 9.0638 | 1 | 9.0638 | 163.5 | **** |
| | | | | | | | 4.0473 | 73 | 0.0554 | | |
| Site B | 63 | −4.0333 | 0.4571 | 2.8856 | 0.3018 | 0.774435 | 7.6347 | 1 | 7.6347 | 91.4 | **** |
| | | | | | | | 5.0951 | 61 | 0.0835 | | |
| Site E | 79 | −4.5894 | 0.3201 | 3.1810 | 0.2067 | 0.868738 | 15.8114 | 1 | 15.8114 | 236.9 | **** |
| | | | | | | | 5.1390 | 77 | 0.0667 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | −4.0640 | 0.8434 | 2.4010 | 0.5596 | 0.701507 | 1.2285 | 1 | 1.2285 | 18.4 | *** |
| | | | | | | | 1.2679 | 19 | 0.0667 | | |
| Site B | 35 | −3.4235 | 0.7816 | 2.5866 | 0.4686 | 0.692846 | 4.5746 | 1 | 4.5746 | 30.5 | **** |
| | | | | | | | 4.9551 | 33 | 0.1502 | | |
| Site E | 25 | −5.3409 | 0.6863 | 3.4890 | 0.4256 | 0.863168 | 7.3054 | 1 | 7.3054 | 67.2 | **** |
| | | | | | | | 2.4998 | 23 | 0.1087 | | |
| Regression model: logFTCu = a + b logFDW | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | 0.4570 | 0.0626 | 0.7745 | 0.0540 | 0.859037 | 9.6752 | 1 | 9.6752 | 205.6 | *** |
| | | | | | | | 3.4358 | 73 | 0.0471 | | |
| Site B | 63 | 1.2932 | 0.0923 | 0.8717 | 0.0775 | 0.821443 | 8.5897 | 1 | 8.5897 | 126.6 | **** |
| | | | | | | | 4.1402 | 61 | 0.0679 | | |
| Site E | 79 | 1.3387 | 0.0593 | 0.9764 | 0.0515 | 0.907459 | 17.2528 | 1 | 17.2523 | 359.22 | **** |
| | | | | | | | 3.6981 | 77 | 0.0480 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | 0.4741 | 0.2136 | 0.7652 | 0.1703 | 0.717718 | 1.2859 | 1 | 1.2859 | 20.18 | *** |
| | | | | | | | 1.2104 | 19 | 0.0637 | | |
| Site B | 35 | 1.4124 | 0.1150 | 0.7736 | 0.1369 | 0.701384 | 4.6880 | 1 | 4.6880 | 31.95 | **** |
| | | | | | | | 4.8416 | 33 | 0.1467 | | |
| Site E | 25 | 1.1053 | 0.1263 | 0.9811 | 0.1238 | 0.855486 | 7.1760 | 1 | 7.1760 | 62.77 | **** |
| | | | | | | | 2.6292 | 23 | 0.1143 | | |

(for significance abbreviations see Table 4.2)

**Table 4.14** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: logFTCd = a + b logFTL | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | −7.7214 | 0.3333 | 3.4060 | 0.2157 | 0.879511 | 16.4970 | 1 | 16.4970 | 249.4 | **** |
| | | | | | | | 4.8296 | 73 | 0.0662 | | |
| Site B | 63 | −6.3448 | 0.2702 | 3.2225 | 0.1784 | 0.917875 | 9.5216 | 1 | 9.5216 | 326.3 | **** |
| | | | | | | | 1.7801 | 61 | 0.0292 | | |
| Site E | 79 | −5.4902 | 0.3155 | 2.5653 | 0.2037 | 0.820481 | 10.2829 | 1 | 10.2829 | 158.6 | **** |
| | | | | | | | 4.9920 | 77 | 0.0648 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | −7.7646 | 1.1763 | 3.6377 | 0.7804 | 0.730393 | 2.8199 | 1 | 2.8199 | 21.7 | *** |
| | | | | | | | 2.4660 | 19 | 0.1298 | | |
| Site B | 35 | −5.7025 | 0.6398 | 2.7326 | 0.3836 | 0.778414 | 5.1057 | 1 | 5.1057 | 50.7 | **** |
| | | | | | | | 3.3206 | 33 | 0.1006 | | |
| Site E | 25 | −5.3852 | 1.0433 | 2.2796 | 0.6470 | 0.592097 | 3.1188 | 1 | 3.1188 | 12.4 | ** |
| | | | | | | | 5.7774 | 23 | 0.2512 | | |
| Regression model: logFTCd = a + b logFDW | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | −0.9003 | 0.0748 | 1.0089 | 0.0646 | 0.877377 | 16.4171 | 1 | 16.4171 | 244.1 | **** |
| | | | | | | | 4.9096 | 73 | 0.0673 | | |
| Site B | 63 | −0.4438 | 0.0557 | 0.9308 | 0.0467 | 0.930954 | 9.7948 | 1 | 9.7948 | 396.5 | **** |
| | | | | | | | 1.5068 | 61 | 0.0247 | | |
| Site E | 79 | −0.7226 | 0.0647 | 0.7749 | 0.0563 | 0.843421 | 10.8659 | 1 | 10.8659 | 189.8 | **** |
| | | | | | | | 4.4090 | 77 | 0.0573 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | −0.8721 | 0.2920 | 1.1735 | 0.2328 | 0.756414 | 3.0244 | 1 | 3.0244 | 25.4 | **** |
| | | | | | | | 2.2615 | 19 | 0.1190 | | |
| Site B | 35 | −0.5881 | 0.0919 | 0.8250 | 0.1094 | 0.795486 | 5.3322 | 1 | 5.3322 | 56.9 | **** |
| | | | | | | | 3.0942 | 33 | 0.0938 | | |
| Site E | 25 | −1.1059 | 0.1749 | 0.7193 | 0.1714 | 0.658533 | 3.8580 | 1 | 3.8580 | 17.6 | *** |
| | | | | | | | 5.0382 | 23 | 0.2191 | | |

(for significance abbreviations see Table 4.2)

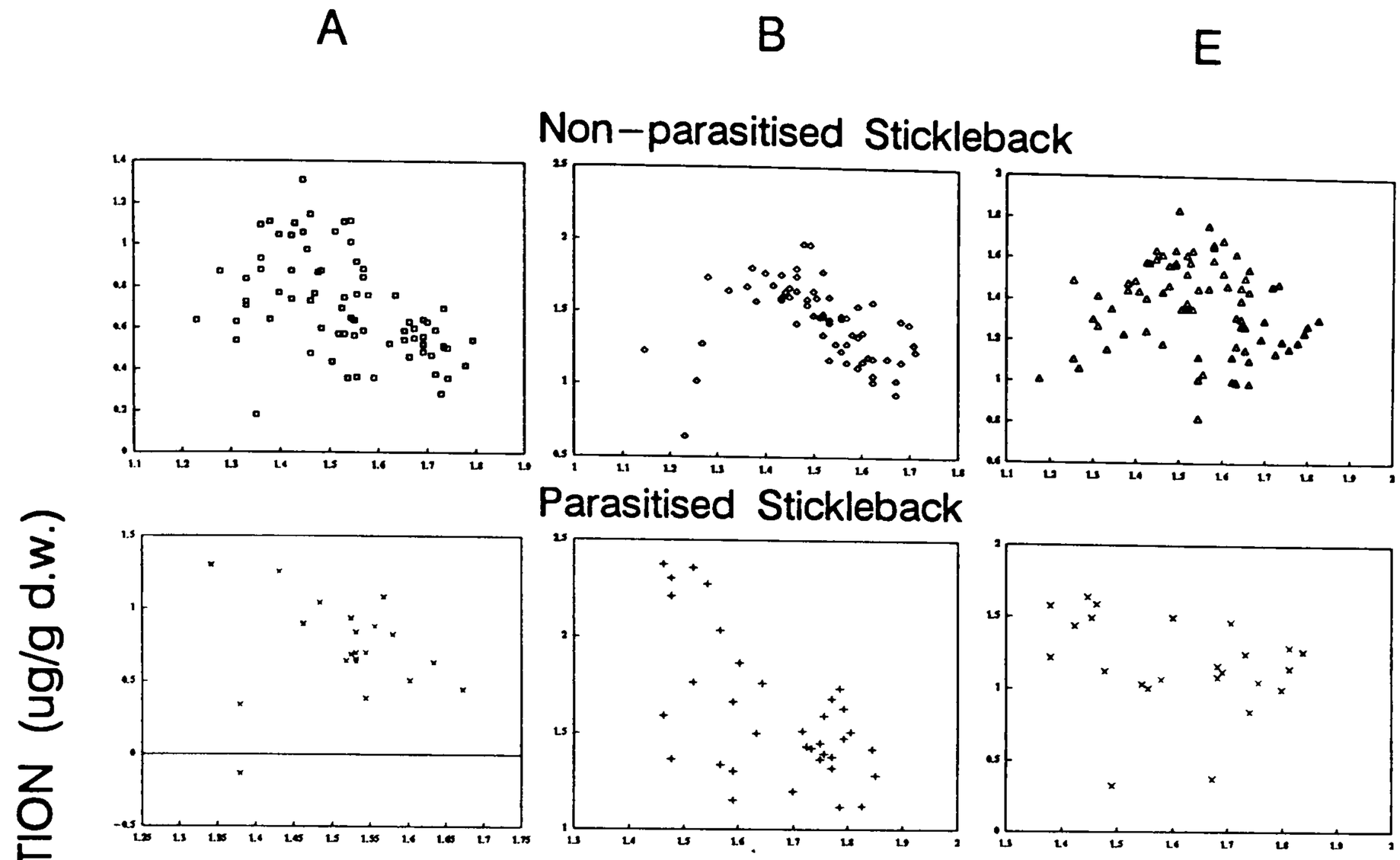



**Figure 4.15** The relationships between log total copper concentrations and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.1 and C.2).

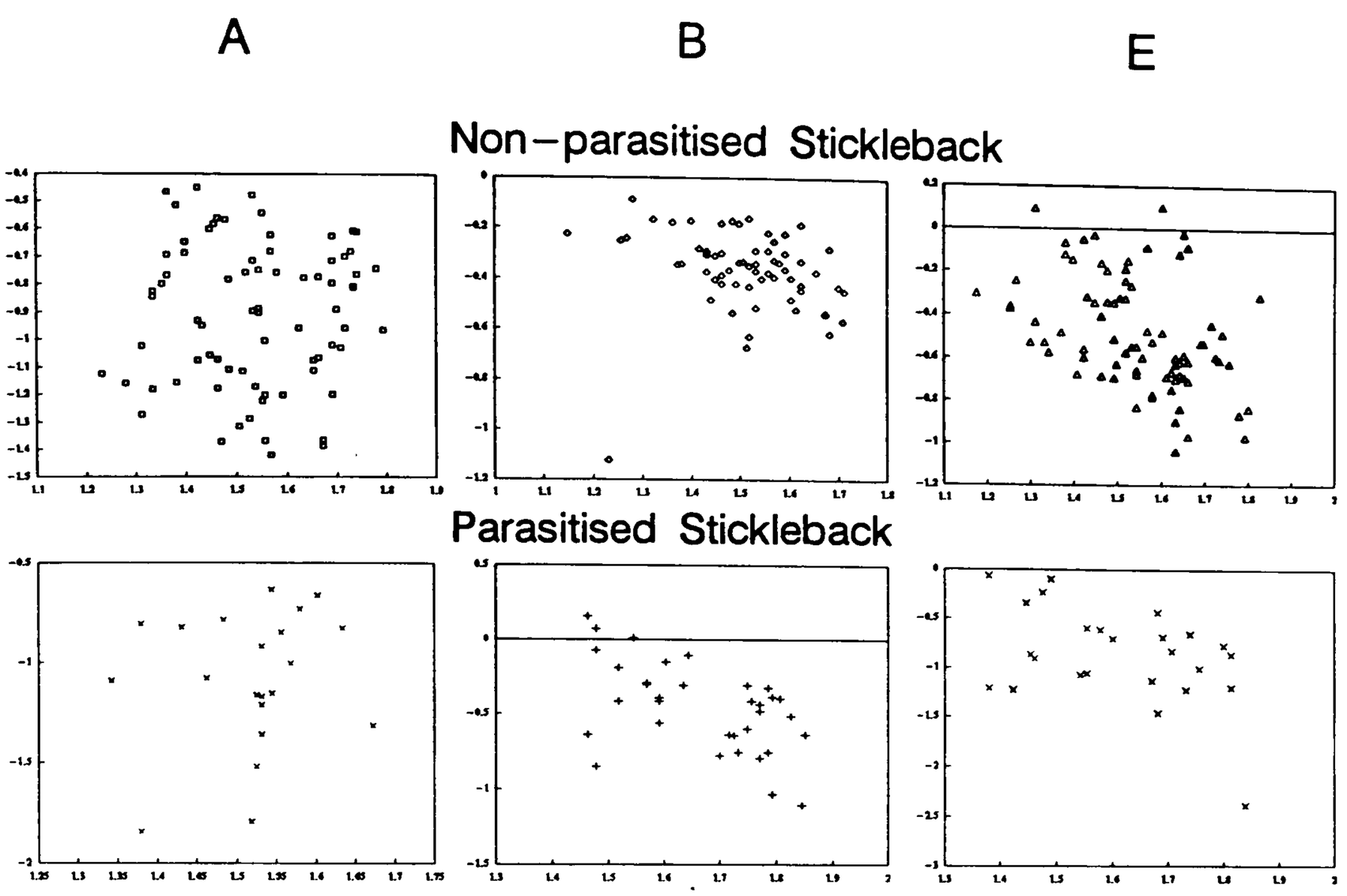



**Figure 4.16** The relationships between log total cadmium concentrations and log total lengths, or log dry weights in non-parasitised and parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.1 and C.2).

**Table 4.15** Results of linear regressions between logarithmic concentrations of copper (logFSCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: logFSCu = a + b logFTL | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | 1.9545 | 0.2758 | −0.8173 | 0.1785 | −0.4725 | 0.9500 | 1 | 0.9500 | 20.98 | **** |
| | | | | | | | 3.3061 | 73 | 0.0453 | | |
| Site B | 63 | 2.3002 | 0.4063 | −0.5724 | 0.2683 | −0.2635 | 0.3004 | 1 | 0.3004 | 4.55 | * |
| | | | | | | | 4.0258 | 61 | 0.0660 | | |
| Site E | 79 | 1.5932 | 0.2706 | −0.1490 | 0.1747 | −0.0967 | 0.0347 | 1 | 0.0347 | 0.73 | N.S. |
| | | | | | | | 3.6734 | 77 | 0.0477 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | 1.2469 | 1.3766 | −0.3437 | 0.9057 | −0.0867 | 0.0159 | 1 | 0.0159 | 0.14 | N.S |
| | | | | | | | 2.0988 | 19 | 0.1105 | | |
| Site B | 35 | 2.8192 | 0.7738 | −0.7519 | 0.4639 | −0.2715 | 0.3865 | 1 | 0.3865 | 2.63 | N.S |
| | | | | | | | 4.8566 | 33 | 0.1472 | | |
| Site E | 25 | 2.0498 | 0.7335 | −0.5528 | 0.4534 | −0.2464 | 0.1557 | 1 | 0.1557 | 1.49 | N.S |
| | | | | | | | 2.4091 | 23 | 0.1047 | | |
| Regression model: logFSCu = a + b logFDW | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | 0.4570 | 0.0626 | −0.2255 | 0.0540 | −0.4390 | 0.8203 | 1 | 0.8203 | 17.43 | **** |
| | | | | | | | 3.4358 | 73 | 0.0471 | | |
| Site B | 63 | 1.2932 | 0.0923 | −0.1283 | 0.0775 | −0.2074 | 0.1861 | 1 | 0.1861 | 2.74 | + |
| | | | | | | | 4.1402 | 61 | 0.0679 | | |
| Site E | 79 | 1.3387 | 0.0593 | −0.0236 | 0.0515 | −0.0520 | 0.0100 | 1 | 0.0100 | 0.21 | N.S. |
| | | | | | | | 3.6981 | 77 | 0.0480 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | 0.3971 | 0.3554 | −0.2832 | 0.3007 | −0.2112 | 0.0943 | 1 | 0.0943 | 0.89 | N.S. |
| | | | | | | | 2.0203 | 19 | 0.1063 | | |
| Site B | 35 | 1.4124 | 0.1150 | −0.2264 | 0.1369 | −0.2767 | 0.4015 | 1 | 0.4015 | 2.74 | + |
| | | | | | | | 4.8416 | 33 | 0.1467 | | |
| Site E | 25 | 1.0049 | 0.1256 | −0.1845 | 0.1292 | −0.2853 | 0.2088 | 1 | 0.2088 | 2.04 | N.S. |
| | | | | | | | 2.3560 | 23 | 0.1024 | | |

(for significance abbreviations see Table 4.2)

**Table 4.16** Results of linear regressions between logarithmic concentrations of cadmium (logFTCu) and logarithmic total lengths (logFTL) and logarithmic dry weights (logFDW), and the linearity tests in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: logFSCd = a + b logFTL | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | −1.0082 | 0.3359 | 0.0640 | 0.2174 | 0.0345 | 0.0058 | 1 | 0.0058 | 0.09 | N.S. |
| | | | | | | | 4.9050 | 73 | 0.0672 | | |
| Site B | 63 | −0.0113 | 0.2489 | −0.2355 | 0.1643 | −0.1805 | 0.0509 | 1 | 0.0509 | 2.05 | N.S. |
| | | | | | | | 1.5100 | 61 | 0.0248 | | |
| Site E | 79 | 0.6925 | 0.2966 | −0.7648 | 0.1915 | −0.4142 | 0.9139 | 1 | 0.9139 | 15.95 | *** |
| | | | | | | | 4.4118 | 77 | 0.0573 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | −2.2210 | 1.4275 | 0.7490 | 0.9392 | 0.1800 | 0.0755 | 1 | 0.0755 | 0.64 | N.S. |
| | | | | | | | 2.2569 | 19 | 0.1188 | | |
| Site B | 35 | 0.5402 | 0.6165 | −0.6058 | 0.3696 | −0.2744 | 0.2510 | 1 | 0.2510 | 2.69 | N.S. |
| | | | | | | | 3.0830 | 33 | 0.0934 | | |
| Site E | 25 | 1.4632 | 1.0148 | −1.4465 | 0.6274 | −0.4333 | 1.0659 | 1 | 1.0659 | 5.32 | * |
| | | | | | | | 4.6120 | 23 | 0.2005 | | |
| Regression model: logFSCd = a + b logFDW | | | | | | | | | | | |
| Non−parasitised Stickleback | | | | | | | | | | | |
| Site A | 75 | −0.9003 | 0.0748 | 0.0089 | 0.0646 | 0.0161 | 0.0013 | 1 | 0.0013 | 0.02 | N.S. |
| | | | | | | | 4.9096 | 73 | 0.0673 | | |
| Site B | 63 | −0.4438 | 0.0557 | −0.0692 | 0.0467 | −0.1861 | 0.0541 | 1 | 0.0541 | 2.19 | N.S. |
| | | | | | | | 1.5068 | 61 | 0.0247 | | |
| Site E | 79 | −0.7226 | 0.0647 | −0.2251 | 0.0563 | −0.4149 | 0.9167 | 1 | 0.9167 | 16.01 | *** |
| | | | | | | | 4.4090 | 77 | 0.0573 | | |
| Parasitised Stickleback | | | | | | | | | | | |
| Site A | 21 | −0.7594 | 0.3742 | 0.2804 | 0.3166 | 0.1991 | 0.0925 | 1 | 0.0925 | 0.78 | N.S. |
| | | | | | | | 2.2399 | 19 | 0.1179 | | |
| Site B | 35 | −0.5881 | 0.0919 | −0.1750 | 0.1094 | −0.2682 | 0.2398 | 1 | 0.2398 | 2.56 | N.S. |
| | | | | | | | 3.0942 | 33 | 0.0938 | | |
| Site E | 25 | −1.1671 | 0.1810 | −0.3583 | 0.1862 | −0.3724 | 0.7875 | 1 | 0.7875 | 3.70 | + |
| | | | | | | | 4.8905 | 23 | 0.2126 | | |

(for significance abbreviations see Table 4.2)

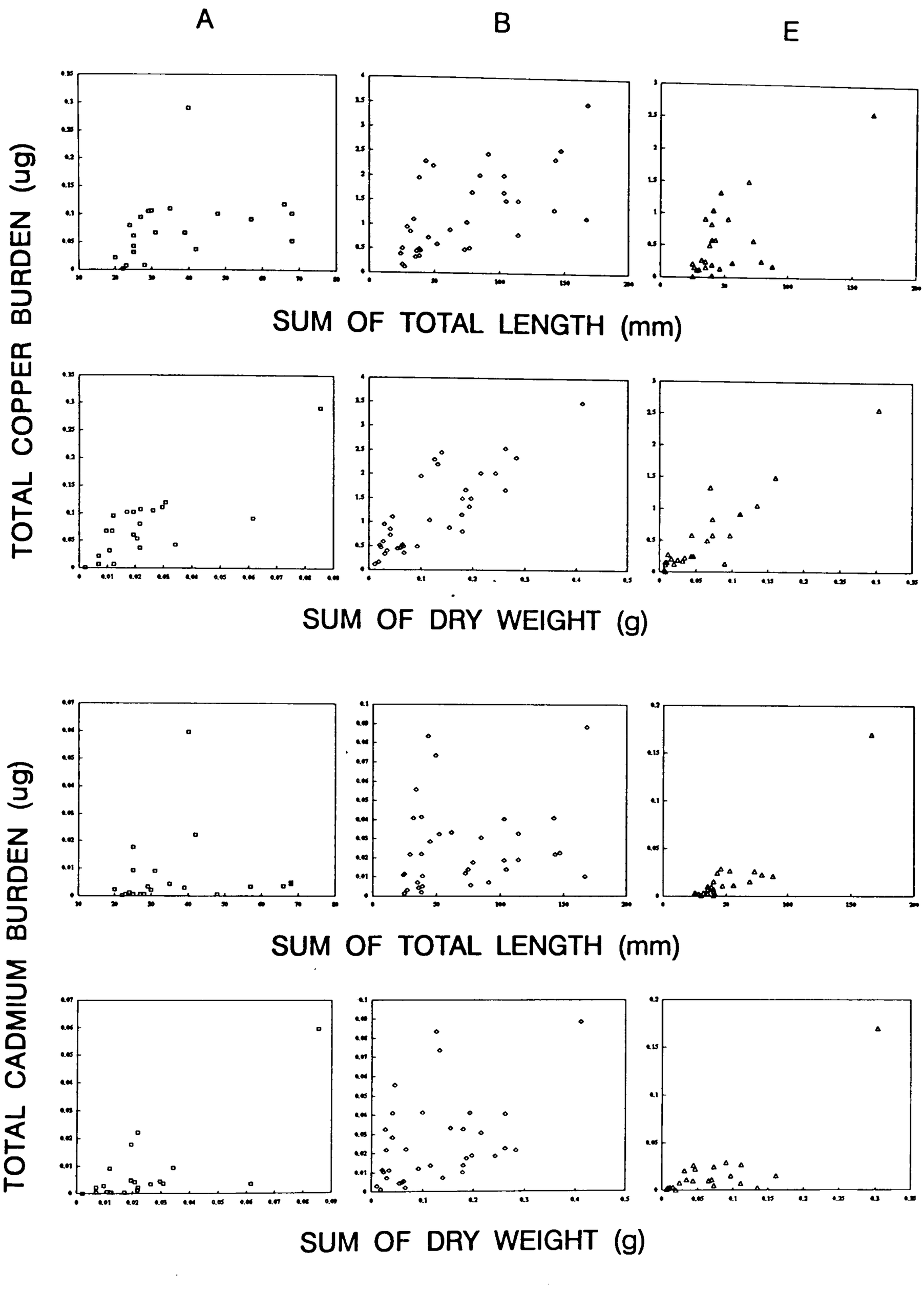



**Figure 4.17** The relationships between log total burdens of Cu or Cd, and log total lengths, or log dry weights in cestode parasites in the parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.2).

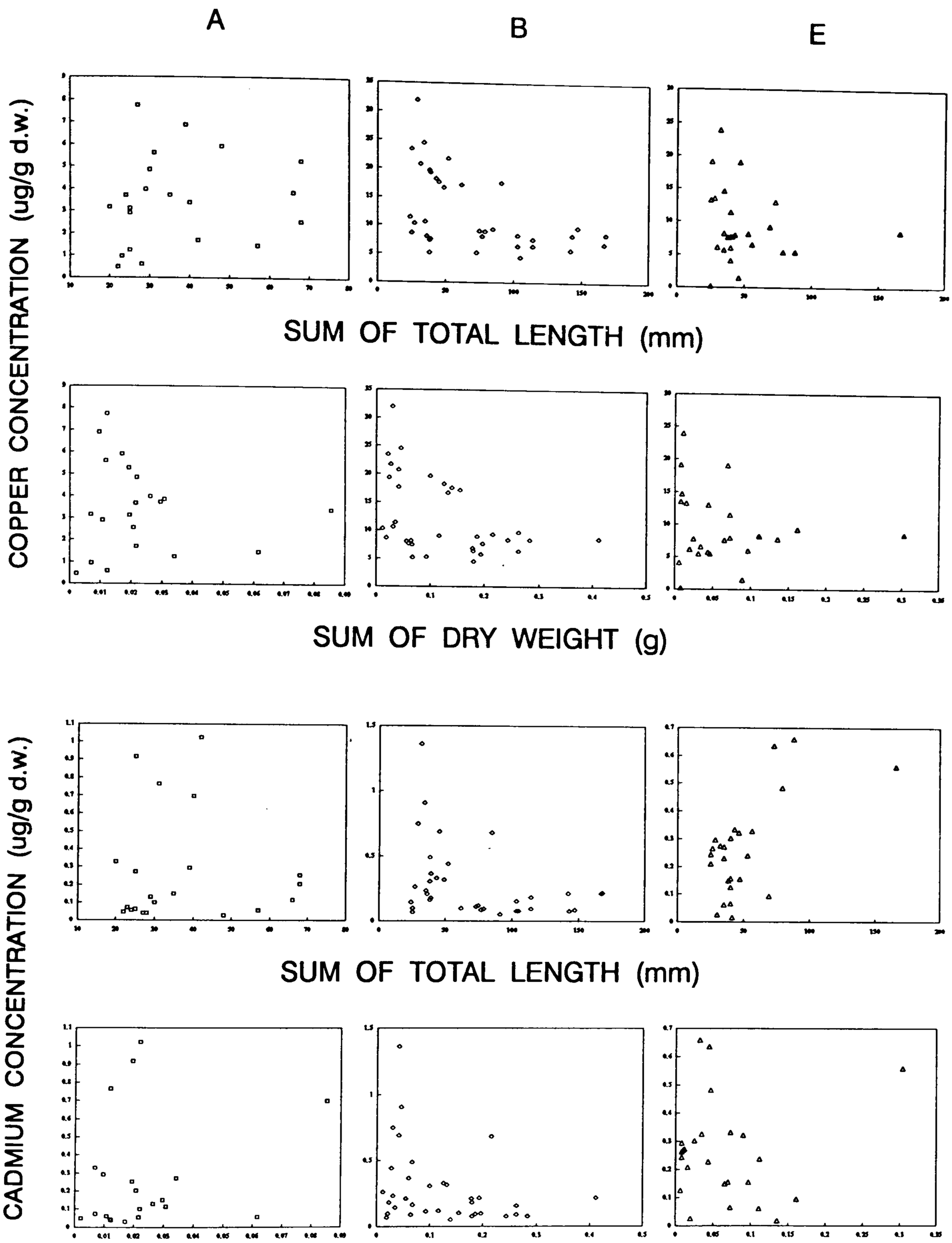



**Figure 4.18** The relationships between log concentrations of Cu or Cd, and log total lengths, or log dry weights in cestode parasites in parasitised sticklebacks at the three sites (A, B, & E) in the River Wandle. (The original data are listed in Appendix C.2).

**Table 4.17** Results of linear regressions between total copper burdens (PTCu) or total cadmium burdens (PTCd) and total lengths (PTL) or dry weights (PDW), and the linearity tests for cestode parasites in parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: PTCu = a + b PTL | | | | | | | | | | | |
| Site A | 21 | 0.02621 | 0.03421 | 0.00133 | 0.00086 | 0.335491 | 0.00882 | 1 | 0.00882 | 2.41 | n.s. |
| | | | | | | | 0.06953 | 19 | 0.00366 | | |
| Site B | 35 | 0.42274 | 0.24746 | 0.00941 | 0.00297 | 0.483400 | 5.80501 | 1 | 5.80501 | 10.06 | ** |
| | | | | | | | 19.03720 | 33 | 0.57689 | | |
| Site E | 25 | 0.01904 | 0.23922 | 0.01266 | 0.00419 | 0.533168 | 3.37474 | 1 | 3.37474 | 9.13 | ** |
| | | | | | | | 8.49695 | 23 | 0.36943 | | |
| Regression model: PTCu = a + b PDW | | | | | | | | | | | |
| Site A | 21 | 0.01644 | 0.01308 | 2.58535 | 0.44173 | 0.802014 | 0.04896 | 1 | 0.04896 | 34.25 | **** |
| | | | | | | | 0.02716 | 19 | 0.00143 | | |
| Site B | 35 | 0.30928 | 0.13163 | 7.19404 | 0.85055 | 0.827244 | 16.20626 | 1 | 16.20626 | 71.54 | **** |
| | | | | | | | 7.47561 | 33 | 0.22653 | | |
| Site E | 25 | 0.01477 | 0.06424 | 8.22168 | 0.70449 | 0.924947 | 7.13678 | 1 | 7.13678 | 136.20 | **** |
| | | | | | | | 1.20518 | 23 | 0.05240 | | |
| Regression model: PTCd = a + b PTL | | | | | | | | | | | |
| Site A | 21 | 0.01272 | 0.00943 | −0.00008 | 0.00024 | −0.080415 | 0.00003 | 1 | 0.00003 | 0.12 | n.s. |
| | | | | | | | 0.00528 | 19 | 0.00028 | | |
| Site B | 35 | 0.08177 | 0.04058 | −0.00025 | 0.00049 | −0.089661 | 0.00415 | 1 | 0.00415 | 0.27 | n.s. |
| | | | | | | | 0.51203 | 33 | 0.01552 | | |
| Site E | 25 | −0.06752 | 0.01334 | 0.00197 | 0.00023 | 0.869107 | 0.08152 | 1 | 0.08152 | 71.01 | **** |
| | | | | | | | 0.02641 | 23 | 0.00115 | | |
| Regression model: PTCd = a + b PDW | | | | | | | | | | | |
| Site A | 21 | −0.00430 | 0.00324 | 0.50441 | 0.10955 | 0.726199 | 0.00186 | 1 | 0.00186 | 21.2 | **** |
| | | | | | | | 0.00167 | 19 | 0.00009 | | |
| Site B | 35 | 0.01320 | 0.00565 | 0.09761 | 0.03654 | 0.421677 | 0.00298 | 1 | 0.00298 | 7.14 | * |
| | | | | | | | 0.01380 | 33 | 0.00042 | | |
| Site E | 25 | −0.00837 | 0.00570 | 0.39809 | 0.06253 | 0.798715 | 0.01673 | 1 | 0.01673 | 40.53 | **** |
| | | | | | | | 0.00950 | 23 | 0.00041 | | |

(for significance abbreviations see Table 4.2)

**Table 4.18** Results of linear regressions between concentrations of copper (PSCu) or cadmium (PSCd) and total lengths (PTL) or dry weights (PDW), and the linearity tests for cestode parasites in parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Regression model: PSCu = a + b PTL | | | | | | | | | | | |
| Site A | 21 | 2.73834 | 1.16837 | 0.02024 | 0.02932 | 0.156386 | 2.0334 | 1 | 2.0334 | 0.48 | N.S. |
| | | | | | | | 81.1110 | 19 | 4.2690 | | |
| Site B | 35 | 17.81550 | 1.94980 | −0.07742 | 0.02338 | −0.499461 | 392.8441 | 1 | 392.8441 | 10.97 | ** |
| | | | | | | | 1181.9225 | 33 | 35.8158 | | |
| Site E | 25 | 10.66200 | 2.20932 | −0.02702 | 0.03867 | −0.144133 | 15.3762 | 1 | 15.3762 | 0.49 | N.S. |
| | | | | | | | 724.7746 | 23 | 31.5119 | | |
| Regression model: PSCu = a + b PDW | | | | | | | | | | | |
| Site A | 21 | 3.75653 | 0.71916 | −11.93780 | 24.28850 | −0.112048 | 1.0438 | 1 | 1.0438 | 0.24 | N.S. |
| | | | | | | | 82.1005 | 19 | 4.3211 | | |
| Site B | 35 | 16.11000 | 1.71638 | −31.14420 | 11.09050 | −0.439176 | 303.7335 | 1 | 303.7335 | 7.89 | ** |
| | | | | | | | 1271.0331 | 33 | 38.5162 | | |
| Site E | 25 | 10.18550 | 1.57158 | −13.33080 | 17.23570 | −0.159216 | 18.7626 | 1 | 18.7626 | 0.60 | N.S. |
| | | | | | | | 721.3882 | 23 | 31.3647 | | |
| Regression model: PSCd = a + b PTL | | | | | | | | | | | |
| Site A | 21 | 0.27803 | 0.18117 | 0.00091 | 0.00460 | 0.004663 | 0.00004 | 1 | 0.00004 | 0.0004 | N.S. |
| | | | | | | | 1.8398 | 19 | 0.1022 | | |
| Site B | 35 | 0.45005 | 0.08806 | −0.00234 | 0.00106 | −0.359134 | 0.3570 | 1 | 0.3570 | 4.89 | * |
| | | | | | | | 2.4107 | 33 | 0.0731 | | |
| Site E | 25 | 0.07797 | 0.05479 | 0.00369 | 0.00096 | 0.625515 | 0.2865 | 1 | 0.2865 | 14.78 | *** |
| | | | | | | | 0.4457 | 23 | 0.0194 | | |
| Regression model: PSCd = a + b PDW | | | | | | | | | | | |
| Site A | 21 | 0.21171 | 0.11070 | 2.99169 | 3.67788 | 0.188298 | 0.0652 | 1 | 0.0652 | 0.662 | N.S. |
| | | | | | | | 1.7746 | 19 | 0.0986 | | |
| Site B | 35 | 0.39841 | 0.07601 | −0.93699 | 0.49115 | −0.315170 | 0.2749 | 1 | 0.2749 | 3.64 | + |
| | | | | | | | 2.4927 | 33 | 0.0755 | | |
| Site E | 25 | 0.25042 | 0.05000 | 0.14032 | 0.54834 | 0.053281 | 0.0021 | 1 | 0.0021 | 0.07 | N.S. |
| | | | | | | | 0.7302 | 23 | 0.0317 | | |

(for significance abbreviations see Table 4.2)

In the following sections various statistical comparisons are made that evaluate differences between age groups and surveys. As excellent regressions have been established between metal burdens and the dry weight of fish, these have been used a basis for the comparisons using analysis of covariance to assess the degree of difference between regression equations. However because there is a lack of significant regression models relating metal concentrations to fish size, further comparisons have been done by ANOVA (see section 4.3.5 for site differences and section 4.3.8 for other comparisons). As a cross check the data for length, weight and body burden of metal have also been compared by ANOVA and the trends conform closely to ANOCOVA analyses described below.

### 4.3.7 Evaluation of the Metal Body Burden - Fish Dry Weight Relationships Between (A) Sites, (B) Age, and (C) Seasonal Surveys

(A) Site Differences

A comparison of metal burdens in non-parasitised and parasitised sticklebacks and cestode parasites between sites was carried out by ANOCOVA. It was assumed that the differences of the slopes would represent the difference of the rate of accumulations in the life time between the regression equations and the differences of the intercepts meant the difference of metal loading at the time fish were born. The basis of the model for each comparison was the log metal burdens versus log dry weight of the fish or parasite. The analysis included all age groups.

A comparison of the slopes and intercepts of the above regression equations in Table 4.19 reveals that there are not only site differences but also further differences can be seen between non-parasitised and parasitised sticklebacks; a) in non-parasitised fish for both Cu and Cd the slopes ($P<0.05$) and intercepts are significantly different between sites. That is, the initial body burdens in conjunction with the rate of metal accumulation in the life time of the fish were different at each site. b) in parasitised fish for both metals the slopes are not significantly different between sites but there is a significant difference in the intercepts and therefore in the initial body burdens. c) in the

**Table 4.19** The ANOCOVA tables of the site differences on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weights (logFDW) in non-parasitised and parasitised sticklebacks, and between total burdens of Cu (PCu) or Cd (PCd) and dry weight (PDW) in the cestode parasites at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Regression Model: logFTCu = a + b logFDW | | | | | | |
| Non–Parasitised | Equality of Slopes | 2 | 0.3486 | 0.1743 | 3.262 | * |
| Stickleback | Residual Error | 211 | 11.2741 | 0.0534 | | |
| | Equality of Adj. Means | 2 | 24.0093 | 12.0046 | 220.000 | **** |
| | Residual Error | 213 | 11.6227 | 0.0546 | | |
| Parasitised | Equality of Slopes | 2 | 0.1874 | 0.0937 | 0.810 | n.s. |
| Stickleback | Residual Error | 75 | 8.6813 | 0.1158 | | |
| | Equality of Adj. Means | 2 | 8.8964 | 4.4482 | 38.620 | **** |
| | Residual Error | 77 | 8.8687 | 0.1152 | | |
| Regression Model: logFTCd = a + b logFDW | | | | | | |
| Non–Parasitised | Equality of Slopes | 2 | 0.4845 | 0.2422 | 4.721 | ** |
| Stickleback | Residual Error | 211 | 10.8253 | 0.0513 | | |
| | Equality of Adj. Means | 2 | 11.4884 | 5.7442 | 108.182 | **** |
| | Residual Error | 213 | 11.3098 | 0.0531 | | |
| Parasitised | Equality of Slopes | 2 | 0.3500 | 0.1750 | 1.263 | n.s. |
| Stickleback | Residual Error | 75 | 10.3939 | 0.1386 | | |
| | Equality of Adj. Means | 2 | 5.9473 | 2.9736 | 21.312 | **** |
| | Residual Error | 77 | 10.7439 | 0.1395 | | |
| Regression Model: PTCu = a + b PDW | | | | | | |
| Cestode | Equality of Slopes | 2 | 0.2540 | 0.1270 | 1.094 | n.s. |
| Parasites | Residual Error | 75 | 8.7079 | 0.1161 | | |
| | Equality of Adj. Means | 2 | 1.5164 | 0.7582 | 6.514 | ** |
| | Residual Error | 77 | 8.9619 | 0.1164 | | |
| Regression Model: PTCd = a + b PDW | | | | | | |
| Cestode | Equality of Slopes | 2 | 0.0079 | 0.0040 | 11.893 | ** |
| Parasites | Residual Error | 75 | 0.0250 | 0.0003 | | |
| | Equality of Adj. Means | 2 | 0.0001 | 0.0001 | 0.118 | n.s |
| | Residual Error | 77 | 0.0329 | 0.0004 | | |

(for significance abbreviations see Table 4.2)

cestode parasite, the results of the ANOCOVA between site are different for Cu and Cd. For Cd the intercepts are not significantly different and therefore the initial body burden is the same at each site, but the slopes are significantly different, showing different rates of accumulation at each site. For Cu the slopes are not significantly different but the intercepts are significantly different, which indicates that the initial body burden is different between sites but the rate of accumulation is the same.

(B) Age Groups

The basis of the comparison was to examine the regression models for the different age groups of non-parasitised sticklebacks at the same site. The comparison is restricted because there are few fish in some of the groups, and this particularly applies to the parasitised fish (Tables 4.20 and 4.21). For the logFTCu-logFDW relationship there were no significant differences between the slopes or intercepts at site A, but at the contaminated sites of B and E, the regression equations were significantly different ($P<0.05$). For the logFTCd-logFDW relationship there were significant differences ($P<0.05$) in the slopes of regression equations at sites A and E. At site B only the intercepts were significantly different ($P<0.01$; Table 4.22).

(C) Seasonal Surveys

For this comparison only the $0^+$ age groups of non-parasitised fish were used. The regression equations relating logFTCu to logFDW and logFTCd to logFDW for each survey were significant at sites B and E but not at site A where only the first (summer) and the third (winter) surveys showed any degree of significance (Tables 4.23 and 4.24). Consequently ANOCOVA analysis was only carried out on the regressions at sites B and E (Table 4.25).

For the copper regressions, at site B the equations were significantly different ($P<0.01$) but at site E the slopes were not significantly

**Table 4.20** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic dry weights (logFDW), and the linearity tests in the different age groups of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Site | Age | Sample Size | LINEAR REGRESSION Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Correlation Coefficient | LINEARITY TEST Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Non−parasitised Stickleback | | | | | | | | | | | | |
| Site A | 0+ | 43 | 0.5191 | 0.0912 | 0.8202 | 0.0692 | 0.87981 | 6.6033 | 1 | 6.6033 | 140.5 | **** |
| | | | | | | | | 1.9273 | 41 | 0.0470 | | |
| | 1+ | 17 | 0.3068 | 0.1407 | 0.5291 | 0.1622 | 0.64412 | 0.5329 | 1 | 0.5329 | 10.6 | ** |
| | | | | | | | | 0.7515 | 15 | 0.0501 | | |
| | 2+ | 13 | 0.3706 | 0.1836 | 0.7458 | 0.2139 | 0.72462 | 0.5518 | 1 | 0.5518 | 12.2 | ** |
| | | | | | | | | 0.4991 | 11 | 0.0453 | | |
| Site B | 0+ | 39 | 1.1964 | 0.1425 | 0.7554 | 0.1118 | 0.74317 | 2.7124 | 1 | 2.7124 | 45.6 | **** |
| | | | | | | | | 2.1988 | 37 | 0.0594 | | |
| | 1+ | 22 | 0.8352 | 0.1156 | 0.3285 | 0.1384 | 0.46880 | 0.1209 | 1 | 0.1209 | 5.6 | * |
| | | | | | | | | 0.4292 | 20 | 0.0215 | | |
| Site E | 0+ | 43 | 1.21407 | 0.12407 | 0.92576 | 0.09446 | 0.83576 | 4.921507 | 1 | 4.92151 | 94.99 | **** |
| | | | | | | | | 2.124341 | 41 | 0.05181 | | |
| | 1+ | 16 | 0.892095 | 0.1259 | 0.26106 | 0.14238 | 0.44003 | 0.043291 | 1 | 0.04329 | 3.362 | + |
| | | | | | | | | 0.180289 | 14 | 0.01288 | | |
| | 2+ | 10 | 1.01962 | 0.10596 | 0.32097 | 0.18971 | 0.51334 | 0.033018 | 1 | 0.03302 | 2.863 | n.s. |
| | | | | | | | | 0.092278 | 8 | 0.01153 | | |
| | 3+ | 6 | 1.20703 | 0.08057 | 0.83217 | 0.19242 | 0.90764 | 0.250335 | 1 | 0.25036 | 18.7 | * |
| | | | | | | | | 0.053538 | 4 | 0.01338 | | |
| Parasitised Stickleback | | | | | | | | | | | | |
| Site A | 0+ | 11 | −0.74097 | 0.3475 | −0.2112 | 0.26371 | −0.25791 | 0.016067 | 1 | 0.01607 | 0.641 | n.s. |
| | | | | | | | | 0.22547 | 9 | 0.02505 | | |
| | 1+ | 8 | 0.832015 | 0.19613 | 1.08294 | 0.16323 | 0.93810 | 1.595749 | 1 | 1.59575 | 44.01 | *** |
| | | | | | | | | 0.217531 | 6 | 0.03626 | | |
| Site B | 0+ | 5 | −1.2906 | 0.5198 | −1.6908 | 0.4364 | −0.91294 | 0.4961 | 1 | 0.4961 | 15.0 | * |
| | | | | | | | | 0.0991 | 3 | 0.0330 | | |
| | 1+ | 13 | 1.8917 | 0.3332 | 1.2600 | 0.3024 | 0.78239 | 3.8377 | 1 | 3.8377 | 17.4 | ** |
| | | | | | | | | 2.4317 | 11 | 0.2211 | | |
| | 2+ | 12 | 1.4312 | 0.1475 | 0.9616 | 0.4296 | 0.57773 | 0.1272 | 1 | 0.1272 | 5.01 | * |
| | | | | | | | | 0.2538 | 10 | 0.0254 | | |
| | 3+ | 5 | 1.1691 | 0.2664 | 0.0736 | 0.9294 | 0.04568 | 0.0003 | 1 | 0.0003 | 0.006 | n.s. |
| | | | | | | | | 0.1611 | 3 | 0.0537 | | |
| Site E | 0+ | 16 | 1.3668 | 0.4962 | 1.1590 | 0.3651 | 0.69148 | 1.5921 | 1 | 1.5921 | 10.08 | ** |
| | | | | | | | | 1.7376 | 11 | 0.1580 | | |
| | 2+ | 8 | 0.9762 | 0.7864 | 0.8687 | 1.5955 | 0.21699 | 0.0361 | 1 | 0.0361 | 0.297 | n.s. |
| | | | | | | | | 0.7311 | 6 | 0.1218 | | |

(for significance abbreviations see Table 4.2)

**Table 4.21** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic dry weights (logFDW), and the linearity tests in the different age groups of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| | | Sample | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Age | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Non−parasitised Stickleback | | | | | | | | | | | | |
| Site A | 0+ | 43 | −0.8485 | 0.1079 | 0.9908 | 0.0819 | 0.88384 | 9.6364 | 1 | 9.6364 | 146.4 | **** |
| | | | | | | | | 2.6995 | 41 | 0.0658 | | |
| | 1+ | 17 | −0.5153 | 0.1005 | 1.4834 | 0.1158 | 0.95719 | 4.1890 | 1 | 4.1890 | 164.0 | **** |
| | | | | | | | | 0.3831 | 15 | 0.0255 | | |
| | 2+ | 13 | −0.7968 | 0.1211 | 1.4242 | 0.1410 | 0.95008 | 2.0122 | 1 | 2.0122 | 102.0 | **** |
| | | | | | | | | 0.2170 | 11 | 0.0197 | | |
| Site B | 0+ | 39 | −0.4835 | 0.0565 | 0.8581 | 0.0443 | 0.95399 | 3.4997 | 1 | 3.4997 | 374.5 | **** |
| | | | | | | | | 0.3457 | 37 | 0.0093 | | |
| | 1+ | 22 | −0.4387 | 0.0928 | 1.0023 | 0.1111 | 0.89589 | 1.1252 | 1 | 1.1252 | 81.3 | **** |
| | | | | | | | | 0.2767 | 20 | 0.0138 | | |
| Site E | 0+ | 43 | −0.8606 | 0.1260 | 0.6875 | 0.0959 | 0.74583 | 2.7449 | 1 | 2.7449 | 51.4 | **** |
| | | | | | | | | 2.1895 | 41 | 0.0534 | | |
| | 1+ | 16 | 0.020229 | 0.3010 | 1.5925 | 0.3405 | 0.78089 | 1.6109 | 1 | 1.6109 | 21.9 | * |
| | | | | | | | | 1.0308 | 14 | 0.0736 | | |
| | 2+ | 10 | −0.84038 | 0.1079 | 0.6218 | 0.1931 | 0.75127 | 0.1239 | 1 | 0.1239 | 10.4 | * |
| | | | | | | | | 0.095646 | 8 | 0.01196 | | |
| | 3+ | 6 | −0.70735 | 0.1595 | 0.6809 | 0.3808 | 0.66647 | 0.1676 | 1 | 0.1676 | 3.20 | n.s. |
| | | | | | | | | 0.2097 | 4 | 0.0524 | | |
| Parasitised Stickleback | | | | | | | | | | | | |
| Site A | 0+ | 11 | −0.97708 | 0.9332 | 1.1592 | 0.7082 | 0.47897 | 0.4841 | 1 | 0.4841 | 2.68 | n.s. |
| | | | | | | | | 1.6261 | 9 | 0.1807 | | |
| | 1+ | 8 | −0.69521 | 0.2024 | 1.2226 | 0.1685 | 0.94749 | 2.0340 | 1 | 2.0340 | 52.7 | *** |
| | | | | | | | | 0.2317 | 6 | 0.0386 | | |
| Site B | 0+ | 5 | −1.33809 | 0.2830 | −0.0470 | 0.2376 | −0.11342 | 0.0004 | 1 | 0.0004 | 0.039 | n.s. |
| | | | | | | | | 0.0294 | 3 | 0.0098 | | |
| | 1+ | 13 | −0.0375 | 0.2066 | 1.3781 | 0.1875 | 0.91147 | 4.5907 | 1 | 4.5907 | 54.0 | **** |
| | | | | | | | | 0.9351 | 11 | 0.0850 | | |
| | 2+ | 12 | −0.6386 | 0.1925 | 0.9209 | 0.5606 | 0.46099 | 0.1166 | 1 | 0.1166 | 2.70 | n.s. |
| | | | | | | | | 0.4322 | 10 | 0.0432 | | |
| | 3+ | 5 | −1.0594 | 0.2912 | −0.7566 | 1.0161 | −0.39494 | 0.0356 | 1 | 0.0356 | 0.55 | n.s. |
| | | | | | | | | 0.1925 | 3 | 0.0642 | | |
| Site E | 0+ | 16 | −0.2730 | 0.5049 | 1.3119 | 0.3715 | 0.72895 | 2.0398 | 1 | 2.0398 | 12.5 | ** |
| | | | | | | | | 1.7989 | 11 | 0.1635 | | |
| | 2+ | 8 | −1.1088 | 0.8228 | 0.6341 | 1.6695 | 0.15324 | 0.0192 | 1 | 0.0192 | 0.144 | n.s. |
| | | | | | | | | 0.8005 | 6 | 0.1334 | | |

(for significance abbreviations see Table 4.2)

**Table 4.22** The ANOCOVA tables of the age effects on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weight (logFDW) in the non-parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Regession Model : logFTCu = a + b logFDW | | | | | | |
| Different age at site A | Equality of Slopes | 2 | 0.1357 | 0.0679 | 1.4315 | n.s. |
| | Residual Error | 67 | 3.1779 | 0.0474 | | |
| | Equality of Adj. Means | 2 | 0.0886 | 0.0443 | 0.9220 | n.s. |
| | Residual Error | 69 | 3.3137 | 0.0480 | | |
| Different age at site B | Equality of Slopes | 1 | 0.1652 | 0.1652 | 3.5835 | * |
| | Residual Error | 57 | 2.6279 | 0.0461 | | |
| | Equality of Adj. Means | 1 | 0.0027 | 0.0027 | 0.0550 | n.s. |
| | Residual Error | 58 | 2.7931 | 0.0482 | | |
| Different age at site E | Equality of Slopes | 4 | 0.4215 | 0.1054 | 2.8876 | * |
| | Residual Error | 69 | 2.5180 | 0.0365 | | |
| | Equality of Adj. Means | 4 | 0.7387 | 0.1897 | 4.7100 | *** |
| | Residual Error | 73 | 2.9395 | 0.0403 | | |
| Regession Model : logFTCd = a + b logFDW | | | | | | |
| Different age at site A | Equality of Slopes | 2 | 0.5011 | 0.2506 | 5.0877 | ** |
| | Residual Error | 67 | 3.2996 | 0.0492 | | |
| | Equality of Adj. Means | 2 | 1.0485 | 0.5242 | 9.5170 | *** |
| | Residual Error | 69 | 3.8007 | 0.0551 | | |
| Different age at site B | Equality of Slopes | 1 | 0.0188 | 0.0188 | 1.7255 | n.s. |
| | Residual Error | 57 | 0.6224 | 0.0109 | | |
| | Equality of Adj. Means | 1 | 0.0676 | 0.0676 | 6.1110 | ** |
| | Residual Error | 58 | 0.6413 | 0.0111 | | |
| Different age at site E | Equality of Slopes | 4 | 0.6493 | 0.1623 | 3.1429 | * |
| | Residual Error | 69 | 3.5636 | 0.0516 | | |
| | Equality of Adj. Means | 4 | 0.1961 | 0.0490 | 0.8490 | n.s. |
| | Residual Error | 73 | 4.2129 | 0.0577 | | |

(for significance abbreviations see Table 4.2)

**Table 4.23** Results of linear regressions between logarithmic total copper burdens (logFTCu) and logarithmic dry weights (logFDW), and the linearity tests in the seasonal batches of $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle.

| Sample | | LINEAR REGRESSION | | | | Correlation | LINEARITY TEST | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Coefficient | Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
| Site A | | | | | | | | | | | |
| July | 12 | 1.08208 | 0.54136 | 1.20736 | 0.30025 | 0.786054 | 0.72774 | 1 | 0.72774 | 16.17 | ** |
| | | | | | | | 0.45006 | 10 | 0.04501 | | |
| October | 10 | 0.73005 | 0.63438 | 0.77505 | 0.45343 | 0.517215 | 0.07187 | 1 | 0.07187 | 2.92 | n.s. |
| | | | | | | | 0.19680 | 8 | 0.02460 | | |
| January | 10 | 0.67565 | 0.36156 | 0.98781 | 0.32365 | 0.733469 | 0.13822 | 1 | 0.13822 | 9.32 | * |
| | | | | | | | 0.11870 | 8 | 0.01484 | | |
| April | 11 | 0.11058 | 0.16816 | 0.24154 | 0.29399 | 0.264145 | 0.00259 | 1 | 0.00259 | 0.68 | n.s. |
| | | | | | | | 0.03457 | 9 | 0.00384 | | |
| Site B | | | | | | | | | | | |
| July | 6 | 2.74150 | 0.68720 | 1.67873 | 0.37736 | 0.912065 | 1.27479 | 1 | 1.27479 | 19.79 | * |
| | | | | | | | 0.25766 | 4 | 0.06442 | | |
| October | 15 | 1.96994 | 0.32310 | 1.20476 | 0.24492 | 0.806543 | 0.47851 | 1 | 0.47851 | 24.20 | *** |
| | | | | | | | 0.25708 | 13 | 0.01978 | | |
| January | 17 | 0.84771 | 0.22693 | 0.50839 | 0.22627 | 0.501812 | 0.11468 | 1 | 0.11468 | 5.05 | * |
| | | | | | | | 0.34072 | 15 | 0.02272 | | |
| Site E | | | | | | | | | | | |
| July | 10 | 1.94015 | 0.20347 | 1.39159 | 0.11633 | 0.973169 | 1.81629 | 1 | 1.81629 | 143.11 | **** |
| | | | | | | | 0.10153 | 8 | 0.01269 | | |
| October | 11 | 1.79930 | 0.14248 | 1.19903 | 0.10741 | 0.965733 | 0.62023 | 1 | 0.52023 | 124.6 | **** |
| | | | | | | | 0.04480 | 9 | 0.00498 | | |
| January | 10 | 1.31549 | 0.37894 | 0.90057 | 0.31850 | 0.706992 | 0.15815 | 1 | 0.15815 | 7.99 | * |
| | | | | | | | 0.15825 | 8 | 0.01978 | | |
| April | 12 | 1.18529 | 0.15936 | 1.15054 | 0.17382 | 0.902316 | 0.57478 | 1 | 0.57478 | 43.81 | **** |
| | | | | | | | 0.13119 | 10 | 0.01312 | | |

(for significance abbreviations see Table 4.2)

**Table 4.24** Results of linear regressions between logarithmic total cadmium burdens (logFTCd) and logarithmic dry weights (logFDW), and the linearity tests in the seasonal batches of $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle.

| Sample | Size | LINEAR REGRESSION Intercept(a) | S.E. of a | Slope(b) | S.E. of b | Correlation Coefficient | LINEARITY TEST Sum of Sq. | d.f. | Mean Sq. | F. value | Sig. level |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Site A | | | | | | | | | | | |
| July | 12 | −0.44791 | 0.47748 | 1.29414 | 0.26482 | 0.839559 | 0.83612 | 1 | 0.83612 | 23.88 | *** |
| | | | | | | | 0.35010 | 10 | 0.03501 | | |
| October | 10 | −1.29309 | 0.55760 | 0.50524 | 0.39855 | 0.408997 | 0.03054 | 1 | 0.03054 | 1.61 | n.s. |
| | | | | | | | 0.15205 | 8 | 0.01901 | | |
| January | 10 | 0.60587 | 0.42229 | 2.22313 | 0.37801 | 0.901194 | 0.70008 | 1 | 0.70008 | 34.59 | *** |
| | | | | | | | 0.16193 | 8 | 0.02024 | | |
| April | 11 | −0.67057 | 0.74408 | 1.53904 | 1.30087 | 0.366866 | 0.10525 | 1 | 0.10525 | 1.40 | n.s |
| | | | | | | | 0.67678 | 9 | 0.07520 | | |
| Site B | | | | | | | | | | | |
| July | 6 | −0.89878 | 0.27160 | 0.64807 | 0.14915 | 0.908396 | 0.18998 | 1 | 0.18998 | 18.88 | * |
| | | | | | | | 0.04025 | 4 | 0.01006 | | |
| October | 15 | −0.85574 | 0.12648 | 0.60154 | 0.09587 | 0.867035 | 0.11930 | 1 | 0.11930 | 39.37 | **** |
| | | | | | | | 0.03940 | 13 | 0.00303 | | |
| January | 17 | −0.68887 | 0.12548 | 0.59777 | 0.12511 | 0.776840 | 0.15854 | 1 | 0.15854 | 22.83 | *** |
| | | | | | | | 0.10417 | 15 | 0.00695 | | |
| Site E | | | | | | | | | | | |
| July | 10 | −1.05278 | 0.09732 | 0.68303 | 0.05564 | 0.974469 | 0.43757 | 1 | 0.43757 | 150.70 | **** |
| | | | | | | | 0.02323 | 8 | 0.00290 | | |
| October | 11 | −1.33471 | 0.14581 | 0.24222 | 0.10992 | 0.591986 | 0.02531 | 1 | 0.02531 | 4.856 | * |
| | | | | | | | 0.04692 | 9 | 0.00521 | | |
| January | 10 | −0.31412 | 0.27087 | 0.90245 | 0.22767 | 0.814017 | 0.15881 | 1 | 0.15881 | 15.71 | ** |
| | | | | | | | 0.08086 | 8 | 0.01011 | | |
| April | 12 | −1.12195 | 0.18457 | 0.60875 | 0.20132 | 0.691098 | 0.16090 | 1 | 0.16090 | 0.14 | * |
| | | | | | | | 0.17599 | 10 | 0.01760 | | |

(for significance abbreviations see Table 4.2)

**Table 4.25** The ANOCOVA tables of the seasonal effects on the relationships between logarithmic body burdens of Cu (logFTCu) or Cd (logFTCd) and logarithmic dry weight (logFDW) in the $O^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Regession Model : logFTCu = a + b logFDW | | | | | | |
| Seasonal samples | Equality of Slopes | 2 | 0.3095 | 0.1547 | 5.79 | ** |
| of 0+ age group | Residual Error | 32 | 0.8555 | 0.0267 | | |
| at site B | Equality of Adj. Means | 2 | 1.0338 | 0.5169 | 15.09 | *** |
| | Residual Error | 34 | 1.1649 | 0.0343 | | |
| Seasonal samples | Equality of Slopes | 3 | 0.0480 | 0.0160 | 1.32 | n.s. |
| of 0+ age group | Residual Error | 36 | 0.4358 | 0.0121 | | |
| at site E | Equality of Adj. Means | 3 | 1.6405 | 0.5468 | 42.95 | **** |
| | Residual Error | 38 | 0.4838 | 0.0127 | | |
| Regession Model : logFTCd = a + b logFDW | | | | | | |
| Seasonal samples | Equality of Slopes | 2 | 0.0007 | 0.0003 | 0.06 | n.s. |
| of 0+ age group | Residual Error | 32 | 0.1838 | 0.0057 | | |
| at site B | Equality of Adj. Means | 2 | 0.1612 | 0.0806 | 14.86 | **** |
| | Residual Error | 34 | 0.1845 | 0.0054 | | |
| Seasonal samples | Equality of Slopes | 3 | 0.0795 | 0.0265 | 2.92 | * |
| of 0+ age group | Residual Error | 36 | 0.3270 | 0.0091 | | |
| at site E | Equality of Adj. Means | 3 | 1.7831 | 0.5944 | 55.57 | **** |
| | Residual Error | 38 | 0.4065 | 0.0107 | | |

(for significance abbreviations see Table 4.2)

different but the intercepts were significantly different (P<0.0001). In the cadmium equations at site E there were significant differences in slopes (P<0.05), but at site B the slopes were not significantly different and the intercepts were significantly different (P<0.0001) (Table 4.25).

### 4.3.8 Evaluation of the Concentrations of Cu and Cd in Sticklebacks at the three Sites

(A) Age Differences

The metal concentrations in the different age groups are compared by ANOVA in Table 4.26. In non-parasitised fish no significant difference was found for the concentrations of Cu in fish of different ages at site A, but for Cu at sites B and E, and Cd at all sites, the concentrations in different age groups were significantly different (P<0.05). The multiple range test reveals that there is a tendency for the middle ages (medium sized) fish to show the greatest difference and have higher concentrations than the younger or the older ones. Parasitised fish showed very little trend with age, only Cd concentration at site B were significantly different (P<0.01) with age. The final column of Table 4.26 shows that there is no consistent trend in the t-tests between metal concentrations in different age groups of non-parasitised and parasitised fish.

Metal concentrations in the cestode parasite were not significantly different between the different age groups of host fish (Table 4.27).

(B) Seasonal Surveys

ANOVA analysis was carried out on the metal concentrations in fish found in the four seasonal surveys. This analysis was restricted to the $0^+$ age group of the non-parasitised fish.

It can be seen from Table 4.28 that the concentrations of Cu and Cd in fish from the four surveys are significantly different (P<0.1). The multiple range test reveals that the highest concentrations are generally in fish collected in the October and January surveys.

**Table 4.26** Assessment of the differences between age groups by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in non-parasitised and parasitised sticklebacks at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.), as well as t-tests on the parasitic effects (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.6).

| | | NON-PARASITISED STICKLEBACK | | | | | | PARASITISED STICKLEBACK | | | | | | t-statistic of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Age | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Site Effect Sig. Level | Sample Size | Mean | S.D. | Lower C. Int. | Upper C. Int. | Age Effect Sig. Level | Parasitism Ho:NP-P=0 |
| Total Length (AM) | | | | | | | | | | | | | | |
| A | -0 | 2 | 29.50 | | 14.57 | 44.43 | ** a | 1 | 24.00 | | 15.54 | 32.46 | *** a | |
| | 0+ | 43 | 32.73 | 10.91 | 29.51 | 35.95 | ab | 11 | 30.55 | 4.49 | 27.99 | 33.10 | a | 0.65 n.s. |
| | 1+ | 17 | 42.47 | 10.56 | 37.35 | 47.59 | abc | 8 | 37.06 | 3.21 | 34.07 | 40.05 | b | 1.40 n.s. |
| | 2+ | 13 | 41.46 | 9.63 | 35.61 | 47.32 | a c | 1 | 47.00 | | 38.54 | 55.46 | b | |
| B | -0 | 2 | 13.50 | | 3.62 | 23.38 | **** a | | | | | | | |
| | 0+ | 39 | 30.96 | 7.04 | 28.72 | 33.20 | b | 5 | 33.00 | 4.64 | 27.81 | 38.19 | **** a | -0.63 n.s. |
| | 1+ | 22 | 39.73 | 7.06 | 36.75 | 42.71 | c | 13 | 39.08 | 7.77 | 35.86 | 42.30 | a | 0.254 n.s. |
| | 2+ | | | | | | | 12 | 57.58 | 3.37 | 54.23 | 60.94 | b | |
| | 3+ | | | | | | | 5 | 66.60 | 4.16 | 61.41 | 71.79 | c | |
| E | -0 | 4 | 20.88 | 3.90 | 14.02 | 27.73 | **** a | | | | | | | |
| | 0+ | 43 | 31.34 | 7.82 | 29.25 | 33.43 | b | 13 | 31.23 | 5.27 | 28.62 | 33.84 | **** a | 0.046 n.s. |
| | 1+ | 16 | 38.84 | 5.23 | 35.42 | 42.27 | c | | | | | | | |
| | 2+ | 10 | 49.00 | 5.98 | 44.67 | 53.33 | d | 8 | 51.13 | 3.76 | 47.80 | 54.45 | b | -0.87 n.s. |
| | 3+ | 6 | 59.00 | 5.48 | 53.40 | 64.60 | d | 4 | 65.50 | 2.52 | 60.80 | 70.20 | c | -2.19 n.s. |
| Dry Weight (GM) | | | | | | | | | | | | | | |
| A | -0 | 2 | 0.0498 | | 0.0125 | 0.1984 | ** a | 1 | 0.0408 | | 0.0191 | 0.0873 | *** a | |
| | 0+ | 43 | 0.0591 | 3.0439 | 0.0439 | 0.0797 | ab | 11 | 0.0495 | 1.5481 | 0.0394 | 0.0623 | ab | 0.517 n.s. |
| | 1+ | 17 | 0.1583 | 2.2076 | 0.0986 | 0.2543 | a c | 8 | 0.1028 | 1.2284 | 0.0786 | 0.1345 | a c | 1.498 n.s. |
| | 2+ | 13 | 0.1538 | 1.9387 | 0.0894 | 0.2645 | a c | 1 | 0.2131 | | 0.0996 | 0.4558 | c | |
| B | -0 | 2 | 0.0050 | | 0.0018 | 0.0139 | **** a | | | | | | | |
| | 0+ | 39 | 0.0594 | 2.2577 | 0.0472 | 0.0749 | b | 5 | 0.0666 | 1.6155 | 0.0355 | 0.1251 | **** a | -0.30 n.s. |
| | 1+ | 22 | 0.1570 | 1.7020 | 0.1154 | 0.2136 | c | 13 | 0.0968 | 2.8108 | 0.0655 | 0.1432 | a | 1.83 n.s. |
| | 2+ | | | | | | | 12 | 0.4718 | 1.2936 | 0.3141 | 0.7087 | b | |
| | 3+ | | | | | | | 5 | 0.5445 | 1.3325 | 0.2899 | 1.0226 | b | |
| E | -0 | 4 | 0.0146 | 1.9885 | 0.0071 | 0.0301 | **** a | | | | | | | |
| | 0+ | 43 | 0.0548 | 2.3542 | 0.0439 | 0.0684 | b | 13 | 0.0530 | 1.5556 | 0.0433 | 0.0649 | **** a | 0.14 n.s. |
| | 1+ | 16 | 0.1376 | 1.6061 | 0.0957 | 0.1977 | c | | | | | | | |
| | 2+ | 10 | 0.2958 | 1.5442 | 0.1870 | 0.4679 | cd | 8 | 0.3260 | 1.2097 | 0.2518 | 0.4221 | b | -0.59 n.s. |
| | 3+ | 6 | 0.4579 | 1.8573 | 0.2533 | 0.8278 | d | 4 | 0.7821 | 1.2346 | 0.5427 | 1.1270 | c | -1.64 n.s. |
| Total Copper Burden (AM) | | | | | | | | | | | | | | |
| A | -0 | 2 | 0.2640 | 0.1485 | -0.302 | 0.8300 | ** a | 1 | 0.0890 | | -0.381 | 0.5594 | * a | |
| | 0+ | 43 | 0.4789 | 0.3487 | 0.3568 | 0.6009 | ab | 11 | 0.3640 | 0.1401 | 0.2222 | 0.5058 | a | 1.06 n.s. |
| | 1+ | 17 | 0.8971 | 0.4462 | 0.7030 | 1.0913 | abc | 8 | 0.6661 | 0.3043 | 0.4998 | 0.8324 | ab | 1.32 n.s. |
| | 2+ | 13 | 0.7195 | 0.5083 | 0.4975 | 0.9415 | a c | 1 | 0.5935 | | 0.1232 | 1.0638 | b | |
| B | -0 | 2 | 0.058 | | -2.122 | 2.237 | *** a | | | | | | | |
| | 0+ | 39 | 2.362 | 1.570 | 1.869 | 2.856 | a | 5 | 6.521 | 4.379 | 1.197 | 11.846 | ** a | -4.35 n.s. |
| | 1+ | 22 | 3.983 | 1.524 | 3.326 | 4.641 | b | 13 | 7.228 | 4.307 | 3.935 | 10.530 | a | -3.23 n.s. |
| | 2+ | | | | | | | 12 | 14.393 | 7.272 | 10.956 | 17.830 | b | |
| | 3+ | | | | | | | 5 | 15.305 | 6.614 | 9.981 | 20.630 | b | |
| E | -0 | 4 | 0.429 | 0.289 | -1.288 | 2.146 | **** a | | | | | | | |
| | 0+ | 43 | 1.530 | 0.998 | 1.007 | 2.054 | a | 13 | 1.109 | 0.747 | 0.025 | 2.193 | **** a | 1.40 n.s. |
| | 1+ | 16 | 4.813 | 1.260 | 3.954 | 5.671 | b | | | | | | | |
| | 2+ | 10 | 7.326 | 2.151 | 6.240 | 8.412 | c | 8 | 4.376 | 2.678 | 2.993 | 5.758 | b | 2.60 n.s |
| | 3+ | 6 | 9.499 | 4.737 | 8.097 | 10.901 | c | 4 | 11.861 | 2.663 | 9.906 | 13.816 | c | -0.90 n.s |

(continued)

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total Cadmium Burden (GM) | | | | | | | | | | | | | | | | |
| A | −0 | 2 | 0.0047 | | 0.0009 | 0.0260 | + | | 1 | 0.0063 | | 0.0009 | 0.0439 | + | | |
| | 0+ | 43 | 0.0086 | 3.4830 | 0.0060 | 0.0124 | | | 11 | 0.0032 | 2.8799 | 0.0018 | 0.0058 | | 2.39 | * |
| | 1+ | 17 | 0.0198 | 3.4242 | 0.0111 | 0.0355 | | | 8 | 0.0115 | 1.9406 | 0.0058 | 0.0228 | | 1.17 | n.s. |
| | 2+ | 13 | 0.0111 | 2.6978 | 0.0057 | 0.0216 | | | 1 | 0.0103 | | 0.0015 | 0.0711 | | | |
| B | −0 | 2 | 0.0011 | | 0.0004 | 0.0028 | **** | a | | | | | | | | |
| | 0+ | 39 | 0.0291 | 2.0802 | 0.0233 | 0.0364 | | b | 5 | 0.0521 | 1.2198 | 0.0202 | 0.1347 | * ab | −1.75 | n.s. |
| | 1+ | 22 | 0.0569 | 1.8129 | 0.0423 | 0.0766 | | c | 13 | 0.0368 | 4.7708 | 0.0204 | 0.0662 | a | 1.19 | n.s. |
| | 2+ | | | | | | | | 12 | 0.1151 | 1.6725 | 0.0624 | 0.2124 | b | | |
| | 3+ | | | | | | | | 5 | 0.1381 | 1.7330 | 0.0535 | 0.3570 | b | | |
| E | −0 | 4 | 0.0105 | 3.0496 | 0.0047 | 0.0223 | **** | a | | | | | | | | |
| | 0+ | 43 | 0.0187 | 2.2017 | 0.0147 | 0.0239 | | a | 13 | 0.0107 | 2.9592 | 0.0055 | 0.0209 | * a | 2.03 | * |
| | 1+ | 16 | 0.0445 | 2.6282 | 0.0299 | 0.0662 | | b | | | | | | | | |
| | 2+ | 10 | 0.0677 | 1.4328 | 0.0410 | 0.1119 | | b | 8 | 0.0382 | 2.1989 | 0.0164 | 0.0890 | b | 2.05 | n.s. |
| | 3+ | 6 | 0.1153 | 1.8823 | 0.0602 | 0.2205 | | b | 4 | 0.0385 | 6.6408 | 0.0117 | 0.1273 | ab | 1.35 | n.s. |
| Copper Concentration (GM) | | | | | | | | | | | | | | | | |
| A | −0 | 2 | 4.3808 | | 2.0305 | 9.4517 | n.s. | | 1 | 2.1821 | | 0.4373 | 10.888 | n.s. | | |
| | 0+ | 43 | 5.4942 | 1.7029 | 4.6546 | 6.4852 | | | 11 | 5.6108 | 2.5243 | 3.4557 | 9.110 | | −0.10 | n.s. |
| | 1+ | 17 | 4.8274 | 1.8657 | 3.7082 | 6.2843 | | | 8 | 5.9650 | 1.5358 | 3.3791 | 10.530 | | −0.86 | n.s. |
| | 2+ | 13 | 3.7772 | 1.6468 | 2.7943 | 5.1080 | | | 1 | 3.7909 | | 0.5581 | 13.897 | | | |
| B | −0 | 2 | 8.505 | | 3.834 | 18.870 | ** | a | | | | | | | | |
| | 0+ | 39 | 31.352 | 1.801 | 26.176 | 37.553 | | b | 5 | 75.060 | 3.821 | 33.959 | 165.91 | n.s. | −2.64 | * |
| | 1+ | 22 | 23.724 | 1.625 | 18.657 | 30.167 | | ab | 13 | 42.475 | 2.918 | 25.972 | 69.463 | | −2.21 | * |
| | 2+ | | | | | | | | 12 | 27.779 | 1.419 | 16.648 | 46.352 | | | |
| | 3+ | | | | | | | | 5 | 25.925 | 1.704 | 11.729 | 57.304 | | | |
| E | −0 | 4 | 21.888 | 1.559 | 13.682 | 35.017 | ** | abc | | | | | | | | |
| | 0+ | 43 | 20.617 | 1.686 | 17.864 | 23.794 | | ab | 13 | 16.917 | 2.320 | 10.934 | 26.176 | n.s. | 1.03 | n.s. |
| | 1+ | 16 | 33.409 | 1.540 | 26.707 | 42.727 | | a | | | | | | | | |
| | 2+ | 10 | 23.925 | 1.457 | 17.774 | 32.205 | | abc | 8 | 10.968 | 2.105 | 6.288 | 19.133 | | 2.90 | * |
| | 3+ | 6 | 18.364 | 1.297 | 12.512 | 26.951 | | a c | 4 | 14.872 | 1.360 | 6.770 | 32.668 | | 1.17 | n.s. |
| Cadmium Concentration (GM) | | | | | | | | | | | | | | | | |
| A | −0 | 2 | 0.0952 | | 0.0442 | 0.2050 | ** | ab | 1 | 0.1556 | | 0.0300 | 0.8065 | n.s. | | |
| | 0+ | 43 | 0.1455 | 1.7929 | 0.1233 | 0.1716 | | b | 11 | 0.0653 | 2.5375 | 0.0398 | 0.1073 | | 3.56 | *** |
| | 1+ | 17 | 0.1252 | 1.6884 | 0.0963 | 0.1629 | | ab | 8 | 0.1119 | 1.6284 | 0.0625 | 0.2002 | | 0.51 | n.s. |
| | 2+ | 13 | 0.0722 | 1.5190 | 0.0534 | 0.097 | | a | 1 | 0.0483 | | 0.0093 | 0.2502 | | | |
| B | −0 | 2 | 0.2111 | | 0.1352 | 0.3303 | *** | a | | | | | | | | |
| | 0+ | 39 | 0.4903 | 1.2817 | 0.4433 | 0.5424 | | b | 5 | 0.7830 | 1.7152 | 0.4409 | 1.3907 | ** b | −0.34 | ** |
| | 1+ | 22 | 0.3626 | 1.3025 | 0.3170 | 0.4148 | | a | 13 | 0.3795 | 2.1217 | 0.2658 | 0.5419 | a | −0.26 | n.s. |
| | 2+ | | | | | | | | 12 | 0.2439 | 1.5791 | 0.1683 | 0.3534 | a | | |
| | 3+ | | | | | | | | 5 | 0.2537 | 2.0416 | 0.1429 | 0.4506 | a | | |
| E | −0 | 4 | 0.7183 | 1.6064 | 0.4072 | 1.2668 | * | b | | | | | | | | |
| | 0+ | 43 | 0.3416 | 1.8038 | 0.2873 | 0.4061 | | ab | 13 | 0.2028 | 2.5583 | 0.1116 | 0.3686 | + | 2.41 | * |
| | 1+ | 16 | 0.3235 | 1.9459 | 0.2436 | 0.4296 | | ab | | | | | | | | |
| | 2+ | 10 | 0.2289 | 1.3347 | 0.1599 | 0.3278 | | a | 8 | 0.1173 | 2.1853 | 0.0546 | 0.2512 | | 2.51 | * |
| | 3+ | 6 | 0.2517 | 1.6674 | 0.1584 | 0.4001 | | a | 4 | 0.0492 | 5.5760 | 0.0168 | 0.1446 | | 2.24 | + |

(end)

**Table 4.27** Assessment of the differences of the effects of host's age by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in cestode parasites at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.) (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.7).

| | | CESTODE PARASITE | | | | | Effect of |
|---|---|---|---|---|---|---|---|
| Site | Age | Sample Size | Mean | S.D. | Lower Con. Int. | Upper Con. Int. | Host's Age Sig. Level |
| Total Length (AM) | | | | | | | |
| Site A | −0 | 1 | 40.00 | | 4.89 | 75.11 | n.s. |
| | 0+ | 11 | 35.18 | 17.26 | 24.60 | 45.77 | |
| | 1+ | 8 | 40.00 | 15.71 | 27.59 | 52.41 | |
| | 2+ | 1 | 25.00 | | −10.11 | 60.11 | |
| Site B | 0+ | 5 | 47.60 | 19.20 | 21.37 | 73.83 | **** a |
| | 1+ | 13 | 36.35 | 12.43 | 20.08 | 52.61 | a |
| | 2+ | 12 | 111.83 | 32.54 | 94.90 | 128.77 | b |
| | 3+ | 5 | 88.60 | 51.61 | 62.37 | 114.83 | b |
| Site E | 0+ | 13 | 44.38 | 22.17 | 27.89 | 60.88 | n.s. |
| | 2+ | 8 | 44.50 | 11.58 | 23.48 | 65.52 | |
| | 3+ | 4 | 74.25 | 61.22 | 44.52 | 103.98 | |
| Dry Weight (AM) | | | | | | | |
| Site A | −0 | 1 | 0.0854 | | 0.0578 | 0.1130 | ** b |
| | 0+ | 11 | 0.0171 | 0.0085 | 0.0088 | 0.0255 | a |
| | 1+ | 8 | 0.0218 | 0.0177 | 0.0120 | 0.0316 | a |
| | 2+ | 1 | 0.0342 | | 0.0066 | 0.0618 | a |
| Site B | 0+ | 5 | 0.0354 | 0.0175 | −0.0164 | 0.0872 | **** a |
| | 1+ | 13 | 0.0521 | 0.0313 | 0.0199 | 0.0842 | a |
| | 2+ | 12 | 0.1928 | 0.0489 | 0.1594 | 0.2262 | b |
| | 3+ | 5 | 0.2240 | 0.1231 | 0.1722 | 0.2758 | b |
| Site E | 0+ | 13 | 0.0205 | 0.0142 | −0.0062 | 0.0473 | *** a |
| | 2+ | 8 | 0.0939 | 0.0360 | 0.0598 | 0.1280 | b |
| | 3+ | 4 | 0.1452 | 0.1095 | 0.0970 | 0.1934 | b |
| Total Copper Burden (AM) | | | | | | | |
| Site A | −0 | 1 | 0.2900 | | 0.2073 | 0.3728 | *** b |
| | 0+ | 11 | 0.0733 | 0.0422 | 0.0484 | 0.0983 | a |
| | 1+ | 8 | 0.0567 | 0.0344 | 0.0275 | 0.0859 | a |
| | 2+ | 1 | 0.0424 | | −0.0403 | 0.1251 | a |
| Site B | 0+ | 5 | 0.5577 | 0.1040 | 0.0370 | 1.0784 | **** a |
| | 1+ | 13 | 0.6362 | 0.5735 | 0.3233 | 0.9691 | a |
| | 2+ | 12 | 1.5970 | 0.6096 | 1.2609 | 1.9331 | b |
| | 3+ | 5 | 2.2627 | 0.7107 | 1.7420 | 2.7834 | b |
| Site E | 0+ | 13 | 0.1869 | 0.1399 | −0.4964 | 0.4234 | *** a |
| | 2+ | 8 | 0.6970 | 0.4330 | 0.3955 | 0.9986 | ab |
| | 3+ | 4 | 1.3774 | 0.8507 | 0.9510 | 1.8038 | b |

(continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Total Cadmium Burden (AM)** | | | | | | | |
| Site A | −0 | 1 | 0.05959 | | 0.04707 | 0.07211 | **** b |
| | 0+ | 11 | 0.00321 | 0.00509 | −0.00057 | 0.00699 | a |
| | 1+ | 8 | 0.00613 | 0.00696 | 0.00170 | 0.01056 | a |
| | 2+ | 1 | 0.00931 | | 0.00321 | 0.02183 | a |
| Site B | 0+ | 5 | 0.01761 | 0.01194 | −0.00005 | 0.03528 | ** a |
| | 1+ | 13 | 0.02070 | 0.02494 | 0.00974 | 0.03165 | a |
| | 2+ | 12 | 0.02079 | 0.01008 | 0.00938 | 0.03219 | a |
| | 3+ | 5 | 0.05477 | 0.02483 | 0.03711 | 0.07244 | b |
| Site E | 0+ | 13 | 0.00802 | 0.00906 | −0.00946 | 0.02549 | + |
| | 2+ | 8 | 0.01457 | 0.00891 | −0.00771 | 0.03685 | |
| | 3+ | 4 | 0.05171 | 0.07908 | 0.02020 | 0.08322 | |
| **Copper Concentration (AM)** | | | | | | | |
| Site A | −0 | 1 | 3.3963 | | −1.0576 | 7.8503 | n.s. |
| | 0+ | 11 | 3.8884 | 2.0891 | 2.5455 | 5.2313 | |
| | 1+ | 8 | 3.2148 | 2.1409 | 1.6400 | 4.7895 | |
| | 2+ | 1 | 1.2406 | | −3.2134 | 5.6946 | |
| Site B | 0+ | 5 | 18.0374 | 5.9928 | 12.1677 | 23.9071 | + |
| | 1+ | 13 | 13.0653 | 8.2744 | 9.4251 | 16.7056 | |
| | 2+ | 12 | 9.1492 | 4.1139 | 5.3603 | 12.9381 | |
| | 3+ | 5 | 12.1003 | 5.7411 | 6.2306 | 17.9699 | |
| Site E | 0+ | 13 | 10.2020 | 6.6737 | 6.9902 | 13.4137 | n.s. |
| | 2+ | 8 | 7.1994 | 2.9396 | 3.1053 | 11.2936 | |
| | 3+ | 4 | 10.7748 | 5.4972 | 4.9848 | 16.5648 | |
| **Cadmium Concentration (AM)** | | | | | | | |
| Site A | −0 | 1 | 0.6978 | | 0.0451 | 1.3504 | n.s. |
| | 0+ | 11 | 0.1794 | 0.2571 | −0.0270 | 0.3858 | |
| | 1+ | 8 | 0.3578 | 0.3536 | 0.1270 | 0.5885 | |
| | 2+ | 1 | 0.2722 | | −0.3804 | 0.9248 | |
| Site B | 0+ | 5 | 0.2984 | 0.2611 | 0.0560 | 0.5408 | n.s. |
| | 1+ | 13 | 0.4144 | 0.3778 | 0.2641 | 0.5647 | |
| | 2+ | 12 | 0.1146 | 0.0556 | −0.0419 | 0.2710 | |
| | 3+ | 5 | 0.3346 | 0.2053 | 0.0922 | 0.5770 | |
| Site E | 0+ | 13 | 0.3161 | 0.1807 | 0.2198 | 0.4125 | n.s. |
| | 2+ | 8 | 0.1639 | 0.0930 | 0.0410 | 0.2867 | |
| | 3+ | 4 | 0.2660 | 0.2346 | 0.0923 | 0.4397 | |

**(end)**

**Table 4.28** Assessment of the differences between different seasonal batches by one-way analysis of variance (ANOVA) on total burdens of Cu and Cd, and concentrations of Cu and Cd in the $0^+$ age groups of non-parasitised at the three sites in the River Wandle, with the arithmetic (AM) or geometric (GM) means, their standard deviations and 95% confidence limits (C.Int.) (for the significance abbreviations see Table 4.2 and the ANOVA tables see Appendix C.8).

| Site | Season | Sample Size | Mean | S.D. | Lower Con. Int. | Upper Con. Int. | Seasonal Effect Sig. Level | |
|---|---|---|---|---|---|---|---|---|
| Total Length (AM) | | | | | | | | |
| Site A | July | 12 | 21.92 | 2.72 | 20.40 | 23.43 | **** | a |
| | October | 10 | 26.60 | 2.30 | 24.94 | 28.26 | | b |
| | January | 10 | 33.85 | 3.10 | 32.19 | 35.51 | | c |
| | April | 11 | 49.09 | 2.16 | 47.51 | 50.68 | | d |
| Site B | July | 6 | 21.25 | 3.67 | 18.38 | 24.12 | **** | a |
| | October | 15 | 27.73 | 2.63 | 25.92 | 29.55 | | b |
| | January | 17 | 36.29 | 3.98 | 34.59 | 38.00 | | c |
| | April | 1 | 47.00 | | | | | d |
| Site E | July | 10 | 21.90 | 3.75 | 19.58 | 24.22 | **** | a |
| | October | 11 | 28.73 | 3.47 | 26.51 | 30.94 | | b |
| | January | 10 | 32.25 | 2.64 | 29.93 | 34.57 | | b |
| | April | 12 | 40.83 | 3.32 | 38.71 | 42.96 | | c |
| Dry Weight (AM) | | | | | | | | |
| Site A | July | 12 | 0:0162 | 1.6332 | 0.0133 | 0.0196 | **** | a |
| | October | 10 | 0.0403 | 1.3041 | 0.0326 | 0.0498 | | b |
| | January | 10 | 0.0775 | 1.3349 | 0.0627 | 0.0958 | | c |
| | April | 11 | 0.2701 | 1.1659 | 0.2208 | 0.3305 | | d |
| Site B | July | 6 | 0.0158 | 1.9989 | 0.0111 | 0.0226 | **** | a |
| | October | 15 | 0.0489 | 1.4238 | 0.0390 | 0.0613 | | b |
| | January | 17 | 0.1024 | 1.4673 | 0.0828 | 0.1266 | | c |
| | April | 1 | 0.2991 | | | | | d |
| Site E | July | 10 | 0.0190 | 2.1029 | 0.0136 | 0.0265 | **** | a |
| | October | 11 | 0.0488 | 1.6133 | 0.0355 | 0.0671 | | b |
| | January | 10 | 0.0658 | 1.4035 | 0.0472 | 0.0919 | | b |
| | April | 12 | 0.1268 | 1.5801 | 0.0935 | 0.1719 | | c |
| Total Copper Burden (AM) | | | | | | | | |
| Site A | July | 12 | 0.1064 | 0.0780 | 0.0149 | 0.1979 | **** | a |
| | October | 10 | 0.4820 | 0.2267 | 0.3818 | 0.5822 | | b |
| | January | 10 | 0.4055 | 0.1591 | 0.3052 | 0.5057 | | b |
| | April | 11 | 0.9491 | 0.1413 | 0.8535 | 1.0447 | | c |
| Site B | July | 6 | 0.8884 | 0.8162 | −0.3353 | 2.1121 | + | |
| | October | 15 | 2.8556 | 1.9115 | 2.0817 | 3.6295 | | |
| | January | 17 | 2.3978 | 1.1668 | 1.6708 | 3.1247 | | |
| | April | 1 | 3.2078 | | | | | |
| Site E | July | 10 | 0.5199 | 0.4383 | −0.0209 | 1.0588 | ** | a |
| | October | 11 | 1.9610 | 1.0951 | 1.4463 | 2.4757 | | b |
| | January | 10 | 1.9298 | 0.8069 | 1.3900 | 2.4697 | | b |
| | April | 12 | 1.6451 | 0.8627 | 1.1523 | 2.1379 | | b |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Total Cadmium Burden (AM)** | | | | | | | | |
| Site A | July | 12 | 0.00171 | 2.13004 | 0.00118 | 0.00249 | **** | a |
| | October | 10 | 0.01005 | 1.38815 | 0.00667 | 0.01514 | | b |
| | January | 10 | 0.01369 | 2.03929 | 0.00909 | 0.02062 | | b c |
| | April | 11 | 0.02848 | 1.90392 | 0.01928 | 0.04208 | | c |
| Site B | July | 6 | 0.00860 | 1.63902 | 0.00663 | 0.01116 | **** | a |
| | October | 15 | 0.02268 | 1.27781 | 0.01924 | 0.02675 | | b |
| | January | 17 | 0.05241 | 1.34319 | 0.04490 | 0.06118 | | c |
| | April | 1 | 0.08712 | | | | | c |
| Site E | July | 10 | 0.00590 | 1.68374 | 0.00460 | 0.00757 | **** | a |
| | October | 11 | 0.02226 | 1.21615 | 0.01757 | 0.02822 | | b |
| | January | 10 | 0.04165 | 1.45609 | 0.03248 | 0.05340 | | c |
| | April | 12 | 0.02148 | 1.49625 | 0.01712 | 0.02694 | | b |
| **Copper Concentration (AM)** | | | | | | | | |
| Site A | July | 12 | 5.1358 | 1.6108 | 4.2094 | 6.2661 | **** | a |
| | October | 10 | 11.0601 | 1.4130 | 8.8947 | 13.7529 | | b |
| | January | 10 | 4.8886 | 1.3027 | 3.9315 | 6.0788 | | a |
| | April | 11 | 3.4811 | 1.1955 | 2.8281 | 4.2850 | | a |
| Site B | July | 6 | 33.0834 | 2.0198 | 23.2854 | 47.0047 | **** | a b |
| | October | 15 | 50.2956 | 1.3775 | 40.2775 | 62.8055 | | b |
| | January | 17 | 21.5933 | 1.4700 | 17.5269 | 26.6033 | | a |
| | April | 1 | 3.2078 | | | | | a |
| Site E | July | 10 | 18.4412 | 1.4626 | 15.3418 | 22.1666 | **** | b |
| | October | 11 | 34.5342 | 1.1986 | 28.9774 | 41.1571 | | c |
| | January | 10 | 27.1000 | 1.3596 | 22.5456 | 32.5751 | | c |
| | April | 12 | 11.2274 | 1.2979 | 9.4915 | 13.2810 | | a |
| **Cadmium Concentration (AM)** | | | | | | | | |
| Site A | July | 12 | 0.1060 | 1.5456 | 0.0804 | 0.1396 | *** | a |
| | October | 10 | 0.2495 | 1.3866 | 0.1844 | 0.3374 | | b |
| | January | 10 | 0.1767 | 1.5989 | 0.1307 | 0.2390 | | a b |
| | April | 11 | 0.1054 | 1.8307 | 0.0791 | 0.1406 | | a |
| Site B | July | 6 | 0.5430 | 1.3765 | 0.4469 | 0.6597 | + | |
| | October | 15 | 0.4641 | 1.2049 | 0.4103 | 0.5249 | | |
| | January | 17 | 0.5120 | 1.2731 | 0.4561 | 0.5748 | | |
| | April | 1 | 0.2913 | | | | | |
| Site E | July | 10 | 0.3112 | 1.3009 | 0.2542 | 0.3811 | **** | b |
| | October | 11 | 0.4562 | 1.4847 | 0.3761 | 0.5534 | | b c |
| | January | 10 | 0.6326 | 1.2470 | 0.5166 | 0.7746 | | c |
| | April | 12 | 0.1694 | 1.4075 | 0.1408 | 0.2038 | | a |

(end)

## 4.4 DISCUSSION

### 4.4.1 The Incidence and Intensity of *Schistocephalus solidus* in the Wandle Sticklebacks.

The levels of *Schistocephalus* infection of sticklebacks in the Wandle were fairly low compared to other reports of the same species. Chappell (1969) reported on populations were usually infected with only one worm per fish and an incidence of 1-55%, but elsewhere, Hopkins & Smyth (1951) found 100% infection and an intensity of nearly 20 worms per fish. Moreover, Arme & Owen (1967) showed that stickleback populations at Farnley pond, Leeds, had an incidence of infection from 36% to 100%. The intensity of infection varied from 1.3 to 46.5 parasites per fish, and the maximum number of parasites found in one fish was 130. Also, sticklebacks in the Priddy pool, Somerset, were reported to have very high levels of infection of *Schistocephalus solidus* with an incidence of 88%, an intensity of 4.4 worms and a maximum number of 106 worms in one infected fish (Pennycuick 1971a & b).

The Parasite Index (PI) of Wandle fish varied between 5.7 and 67.7, with a mean value of 25.3 which is similar to published values, such as Farnley stickleback where the mean range of PI was 14.8-47.4 and the maximum value was 68.5 (Arme & Owen 1967); the mean PI for sticklebacks in Priddy stickleback was 26.6 (Pennycuick 1971a,b); and the mean PI reported by Hopkins & Smyth (1951) was 33.1.

The relationship between the size of the stickleback host and the size of the cestode parasite has been examined by Arme & Owen (1967) and Pennycuick (1971c). They both found that the parasite index (PI) and the intensity of parasitism were inversely related to the size of the host. This is at odds with results found in this study, except at site A. It is suggested that the lack of an overall inverse relationship could be a consequence of the low degree of infection of the fish in the Wandle. One aspect that agrees with published trends (Pennycuick 1971c) is that the weight of *S. solidus* was positively related with the age of the host. Undoubtedly food supply plays an important role in the level of infection and particularly the abundance of *Schistocephalus* infected copepods in the habitat.

### 4.4.2 The Age Composition and Growth of the Stickleback

In this study determination of the age groups of sticklebacks was based on the Cassie method and involved dividing the populations into a series of length frequency distributions by plotting the Cassie curves. As sampling was done on a seasonal basis the modal length of each group of fish increased from one survey to the next. The smallest yearling fish occurred in July at the three sites (18 mm, 13 mm and 15 mm) which implied that the spawnings took place at least until the end of June, since reportedly newly hatched larvae at 3 to 5 mm would grow up to 17 mm within 30 days of hatching and taking on the adult form (Wootton 1976). This pattern of spawning agrees well with those found in other populations (Table 4.29).

Although quantitative information is not available on the reproductive success of the sticklebacks at the sites in the Wandle, the fact that a sizeable number of healthy looking fish are produced at the appropriate time, suggested that the populations do not suffer from any overt physical or chemical stress.

The $0^+$ age group was the major one at each site and in all the surveys undertaken. Also the $0^+$ age group becomes more dominant during the year as the other age groups, particularly the $1^+$ group, decrease in frequency so that by the following April, just before spawning the $0^+$ group is predominant. This indicates that the fish spawn at the age of one and then suffer a high mortality rate after spawning. This deduction is in agreement with Allen and Wootton (1982) who studied *G. aculeatus* at Lake Frongoch, mid-Wales and Mann (1971) who studied those in Devil's Brook and Bere stream, S.W. England.

The maximum life span of Wandle sticklebacks was estimated between 3 to 4 years. This agrees with stickleback populations from Birket stream, U.K. (Jones & Hynes 1950) and from Priddy Pool, S.W. England (Pennycuik 1971c). The shorter life span of sticklebacks at the control site may be as a result of predation by fish-eating birds, for example moorhens and herons. The population of parasitised stickleback at site B tended to be older and may be as a result of the low predation rate.

**Table 4.29** Summary the age structure and the mean length of age groups in various populations of *Gasterosteus aculeatus*.

| Population | Morph | Mean length (mm) of age group 1+ | 2+ | 3+ | 4+ | Max. Length | Age of Spawning | Max. Age | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Germany | Trachura | 30 | 50 | 70 | | ? | >1+ | 4 | Wunder 1928, 1930 in (1) |
| Germany | Trachura | | | | | 78 | >1+ | | Leiner 1930, 1931, 1934 in (1) |
| Esdale island Scotland | Trachura | 50 | ? | 85 | | | | | (1) Jones & Hynes 1950 |
| Baltic sea | ? | | | | | | 2 | 4 | Aneer 1973 in (2) Wootton 1976 |
| Belgium | ? | | | | | | | 1 | Heuts 1947a & b in (1) |
| Priddy Pool S.W. England | Semiarmatus | | L=41.7 t ^0.288 | | | | 1st June | 3.5 | Pennycuik 1971a |
| Island of Tholen Netherland | ? | 48 | 60 | | | | | 1.25 | Van Mullen & Van der Vlugt 1964 in (1) |
| Germany | Gymnura* | 35–40 | 45 | 50–55 | | 60 | ? | 3–5 | Bock 1928 in (1) |
| Britain | Gymnura* | | | | | 55 | ? | 3 | Craig–Bennett 1931 in (1) |
| Birket stream in U.K. | Leiurus | 42.7 | 45.6 | 51.4 | | | second summer | 3.5 | (1) |
| Bare lake Alaska | Leiurus | | 52 | 54–73 | | | 1 (May–Jun.) | 2.25 | Coad & Power 1973b in (2) |
| Karluk lake Alaska | ? | | 60 | 72–79 | | | 1 (May–Jun.) | | Coad & Power 1973b in (2) |
| Bere stream S.W. England | ? | 44 | | | | | 1 May | 2 | Mann 1971 |
| Devil's brook S.W. England | ? | 45 | | | | | | 2 | Mann 1971 |
| Lake Frongoch Mid–Wales, U.K. | Leiurus | 34.5 | | | | | 1 | 2 | Allen & Wootton 1982 |
| River Wandle, A | Leiurus | 49 | 54 | 62 | | | 1 (May–Jun.) | 3 | This study |
| River Wandle, B | Leiurus | 47 | >49 | >58 | >58 | | 1 (May–Jun.) | 4 | This study |
| River Wandle, E | Leiurus | 40 | >43 | >62 | >63 | | 1 (May–Jun.) | 4 | This study |

Gymnura is the old name of Leiurus.

(B) Cu and Cd Body Burdens and Concentrations in the Sticklebacks Between Sites

The literature contains many examples of Cu and Cd concentrations in a number of different fish species collected from various water bodies. A few examples were introduced at the beginning of the chapter. In some instances fish were obtained from grossly polluted waters which receive metal rich effluents, others come from water bodies which were less heavily polluted and more generally contaminated.

One example of Cu and Cd in sticklebacks is that of Nordin et al. (1984 from Roch et al. 1985). The fish were collected from a river in Vancouver Island, Canada which was contaminated with effluent from a base metal mine and the sticklebacks had concentrations of 23 ug/g Cu and 2 ug/g Cd. Some of the highest Cu and Cd concentrations in fish have been found in the Severn estuary, U.K., where values are commonly in the range 3-8 ug Cd/g and 17-25 ug Cu/g. Other published concentrations that are considered to be more in the generally contaminated category are in the range 0.1-0.3 ug/g Cd and 1-6 ug/g Cu.

It can be seen from Figure 4.12 that Cu concentrations in sticklebacks in this study from site A are in the low contamination category but the concentrations at sites B and E indicate gross contamination. For Cd concentrations site A can be classed as background levels and values at sites B and E are in the low contamination category.

(C) Concentrations of Cu and Cd in the Sticklebacks

A number of studies have reported direct correlations between metal concentration and the size and therefore age of fish. Badha & Goldspink (1982) showed that whole body concentration of Pb and Cd increased with increasing age in roach (*Rutilus rutilus*) from NW England. Barak and Mason (1990 a&b) also found that in larger eel (*Anguilla anguilla*), roach (*Rutilus rutilus*), dace (*Leuciscus leuciscus*), chub (*L. cephalus*), tench (*Tinca tinca*), perch (*Perca fluviatilis*), pike (*Esox lucius*) from rivers in Eastern England, concentrations of Hg, Cd, and Pb were greater. Examples of studies which related metal concentration in specific tissues to the age of the fish include Law &

### 4.4.2 The Age Composition and Growth of the Stickleback

In this study determination of the age groups of sticklebacks was based on the Cassie method and involved dividing the populations into a series of length frequency distributions by plotting the Cassie curves. As sampling was done on a seasonal basis the modal length of each group of fish increased from one survey to the next. The smallest yearling fish occurred in July at the three sites (18 mm, 13 mm and 15 mm) which implied that the spawnings took place at least until the end of June, since reportedly newly hatched larvae at 3 to 5 mm would grow up to 17 mm within 30 days of hatching and taking on the adult form (Wootton 1976). This pattern of spawning agrees well with those found in other populations (Table 4.29).

Although quantitative information is not available on the reproductive success of the sticklebacks at the sites in the Wandle, the fact that a sizeable number of healthy looking fish are produced at the appropriate time, suggested that the populations do not suffer from any overt physical or chemical stress.

The $0^+$ age group was the major one at each site and in all the surveys undertaken. Also the $0^+$ age group becomes more dominant during the year as the other age groups, particularly the $1^+$ group, decrease in frequency so that by the following April, just before spawning the $0^+$ group is predominant. This indicates that the fish spawn at the age of one and then suffer a high mortality rate after spawning. This deduction is in agreement with Allen and Wootton (1982) who studied *G. aculeatus* at Lake Frongoch, mid-Wales and Mann (1971) who studied those in Devil's Brook and Bere stream, S.W. England.

The maximum life span of Wandle sticklebacks was estimated between 3 to 4 years. This agrees with stickleback populations from Birket stream, U.K. (Jones & Hynes 1950) and from Priddy Pool, S.W. England (Pennycuik 1971c). The shorter life span of sticklebacks at the control site may be as a result of predation by fish-eating birds, for example moorhens and herons. The population of parasitised stickleback at site B tended to be older and may be as a result of the low predation rate.

**Table 4.29** Summary the age structure and the mean length of age groups in various populations of *Gasterosteus aculeatus*.

| Population | Morph | Mean length (mm) of age group 1+ | 2+ | 3+ | 4+ | Max. Length | Age of Spawning | Max. Age | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Germany | Trachura | 30 | 50 | 70 | | ? | >1+ | 4 | Wunder 1928, 1930 in (1) |
| Germany | Trachura | | | | | 78 | >1+ | | Leiner 1930, 1931, 1934 in (1) |
| Esdale island Scotland | Trachura | 50 | ? | 85 | | | | | (1) Jones & Hynes 1950 |
| Baltic sea | ? | | | | | | 2 | 4 | Aneer 1973 in (2) Wootton 1976 |
| Belgium | ? | | | | | | | 1 | Heuts 1947a & b in (1) |
| Priddy Pool S.W. England | Semiarmatus | | L=41.7 t ^0.288 | | | | 1st June | 3.5 | Pennycuik 1971a |
| Island of Tholen Netherland | ? | 48 | 60 | | | | | 1.25 | Van Mullen & Van der Vlugt 1964 in (1) |
| Germany | Gymnura* | 35–40 | 45 | 50–55 | | 60 | ? | 3–5 | Bock 1928 in (1) |
| Britain | Gymnura* | | | | | 55 | ? | 3 | Craig–Bennett 1931 in (1) |
| Birket stream in U.K. | Leiurus | 42.7 | 45.6 | 51.4 | | | second summer | 3.5 | (1) |
| Bare lake Alaska | Leiurus | | 52 | 54–73 | | | 1 (May–Jun.) | 2.25 | Coad & Power 1973b in (2) |
| Karluk lake Alaska | ? | | 60 | 72–79 | | | 1 (May–Jun.) | | Coad & Power 1973b in (2) |
| Bere stream S.W. England | ? | 44 | | | | | 1 May | 2 | Mann 1971 |
| Devil's brook S.W. England | ? | 45 | | | | | | 2 | Mann 1971 |
| Lake Frongoch Mid–Wales, U.K. | Leiurus | 34.5 | | | | | 1 | 2 | Allen & Wootton 1982 |
| River Wandle, A | Leiurus | 49 | 54 | 62 | | | 1 (May–Jun.) | 3 | This study |
| River Wandle, B | Leiurus | 47 | >49 | >58 | >58 | | 1 (May–Jun.) | 4 | This study |
| River Wandle, E | Leiurus | 40 | >43 | >62 | >63 | | 1 (May–Jun.) | 4 | This study |

Gymnura is the old name of Leiurus.

The growth of $0^+$age groups of the sticklebacks increased sharply prior to spawning in spring and declined to low values in winter. The growth rate of the older fish (over 1 year old at site A and over 2 year old at site B and E) were very slow. These agrees with Allen and Wootton (1982) in their study of the fish in Lake Frongoch.

Comparison of the size differences between the same age groups of parasitised and non-parasitised stickleback at each site were not significantly different. However, there is a significant difference of length-weight relationship between the two groups of fish. This result agrees with that Pennycuick (1971b) stated that the weight of an infected fish was less than that of an uninfected one of the same length, the difference increasing with increasing weight of *Schistocephalus*. This supports the general statement that parasite infestation reduces the growth of sticklebacks (Wootton 1976).

### 4.4.3 Copper and Cadmium in the Sticklebacks

(A) Frequency Distribution of Cu and Cd in Stickleback

The frequency distribution of copper and cadmium burdens and concentrations in sticklebacks was found to have either a normal or lognormal distribution; Cu concentration conformed to a normal distribution, whereas Cu and Cd body burdens and Cd concentration followed lognormal distributions. Giesy and Wiener (1977) also found that the frequency distribution of whole body concentrations of trace elements may be fitted to either normal or lognormal distributions. There was a tendency for essential elements (e.g. Cu, Fe, and Zn) to have a normal distribution and for non-essential elements (i.e. Cd and Pb) to be lognormally distributed. Murphy et al. (1978) also showed that the cadmium and zinc distributions in several fishes, i.e. bluegill (*Lepomis macrochirus*), redear sunfish (*L. microlophus*), and largemouth bass (*Micropterus salmoides*) in Palestine Lake, north-central Indiana, had a lognormal distribution. Very few studies have examined total body metals in fish and no examples could be found where the frequency distribution of metal concentrations in fish had been considered.

(B) Cu and Cd Body Burdens and Concentrations in the Sticklebacks Between Sites

The literature contains many examples of Cu and Cd concentrations in a number of different fish species collected from various water bodies. A few examples were introduced at the beginning of the chapter. In some instances fish were obtained from grossly polluted waters which receive metal rich effluents, others come from water bodies which were less heavily polluted and more generally contaminated.

One example of Cu and Cd in sticklebacks is that of Nordin et al. (1984 from Roch et al. 1985). The fish were collected from a river in Vancouver Island, Canada which was contaminated with effluent from a base metal mine and the sticklebacks had concentrations of 23 ug/g Cu and 2 ug/g Cd. Some of the highest Cu and Cd concentrations in fish have been found in the Severn estuary, U.K., where values are commonly in the range 3-8 ug Cd/g and 17-25 ug Cu/g. Other published concentrations that are considered to be more in the generally contaminated category are in the range 0.1-0.3 ug/g Cd and 1-6 ug/g Cu.

It can be seen from Figure 4.12 that Cu concentrations in sticklebacks in this study from site A are in the low contamination category but the concentrations at sites B and E indicate gross contamination. For Cd concentrations site A can be classed as background levels and values at sites B and E are in the low contamination category.

(C) Concentrations of Cu and Cd in the Sticklebacks

A number of studies have reported direct correlations between metal concentration and the size and therefore age of fish. Badha & Goldspink (1982) showed that whole body concentration of Pb and Cd increased with increasing age in roach (*Rutilus rutilus*) from NW England. Barak and Mason (1990 a&b) also found that in larger eel (*Anguilla anguilla*), roach (*Rutilus rutilus*), dace (*Leuciscus leuciscus*), chub (*L. cephalus*), tench (*Tinca tinca*), perch (*Perca fluviatilis*), pike (*Esox lucius*) from rivers in Eastern England, concentrations of Hg, Cd, and Pb were greater. Examples of studies which related metal concentration in specific tissues to the age of the fish include Law &

Singh (1991) who found that concentrations of Cu, Zn, Pb, and Hg in the edible muscle of *Arius thalassinus* and *Plotosus anguillaris* in estuary of Kelang River, Malaysia, were positively correlated to the body weight of the fish. McFarlane & Franzin (1980) in a study of the northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) demonstrated that the concentration of Cd in livers increased with increasing age, and in northern pike from five lakes near a Base Metal Smelter at Flin Flon, Manitoba, concentration of Cu and Hg in livers increased with age of the fish.

On the other hand, there are also studies which have demonstrated that metal concentrations and age of fish are either not correlated or that an inverse relationship exists showing that metal concentration decreases with age. For instance, Singh et al. (1991) found that Cu, Fe & Zn concentrations in muscle tissues were not always positively correlated to the fish length for *Lutjanus synagris*, *Micropogon furnieri*, *Cynoscion leiarchus*, *Caranx hippos*, and *Scomberomorus brasiliensis* from Trinidad, Caribbean. Also Law & Singh (1991) did not find any relationships between the Cu and Hg concentration in the edible muscle and the length of *Dasyatis zugeri*.

The results of this study show that there is a tendency for an inverse relationship between Cu and Cd concentrations and the size of sticklebacks, but in some cases it is not significant. At site B and to a lesser extent at site E, the correlations were distinctly negative, but at site A the relationship was not significant. Furthermore Cu and Cd concentrations in different age groups have been shown to be significantly different and a trend was noted whereby Cu and Cd concentrations increased in fish up to 30 - 40 mm in length but thereafter concentrations tended to decrease.

It is very difficult to interpret and reconcile the above trends. On the one hand, the body burden of Cu and Cd accumulate with age of fish, whilst on the other hand concentration tends to decrease with age. Some simple models that could explain these trends at least in part are as follows:

1. It is possible that the amount of metal absorbed by the fish (difference between uptake and excretion) is relatively constant over

the life time of the fish and the that growth of the fish exerts a dilution effect on the metal concentration.

2. Alternatively, the accumulations of Cu and Cd in the tissues may initially increase with age until a steady state is achieved. Thereafter, continued growth of the fish would result in a diluting effect on the concentration, while at the same time the overall amount of Cu and Cd would increase in the fish.

3. The above possibilities do not take account of different metal kinetics in different tissues. Liver is known to be the major organ of accumulation (this is considered further in Chapter 7). The concentration and total amount of Cu and Cd in the liver may increase with age but the growth of the remainder of the stickleback (with lower concentrations) may have a diluting effect on the overall concentration but the body burden would show an increase. A slow down in the rate of accumulation or a steady state of Cu and Cd in the liver could explain the tendency for maximum body concentrations in medium sized fish.

(D) Cu and Cd Body Burden in the Sticklebacks

The study has examined two aspects of the metal content of the fish; one was to consider the total amount or body burden of metal in each fish; and the other was the weight of metal per unit weight of fish or concentration of metal. These two ways of assessing metal content reveal contrasting relationships when they are related to the weight and length of fish. The body burden of Cu and Cd increase in proportion to the size and therefore the age of the fish. On the other hand the concentrations of Cu and Cd tend to be inversely correlated with the size of the fish, i.e. more metal with age but less concentrated.

The significant regression models relating metal body burden and weight (or length) of sticklebacks provided a basis for the analysis of covariance to assess site, age and survey differences of these models. There are significant differences in the slopes and intercepts or in either slopes or intercepts in between the regression equations at each site. In non-parasitised fish the regression equations are significantly

different between sites, although for parasitised fish only the intercepts are significantly different. At each site in non-parasitised fish further differences were found for slopes and/or intercepts between age groups and seasonal surveys. Thus these results emphasise not only site differences, but also other influences such as age, changes in metal exposure with time and other factors such as parasitic infection (see section 4.4.4), physiological condition of fish.

### 4.4.4 Assessment of the Influence of Infection by *Schistocephalus solidus* on the Cu and Cd Content of the Sticklebacks

An unique and important feature of the study has been the comparison of metal levels between non-parasitised fish and between the host and the actual cestode parasite. It is unique in the sense that no other published work could found which examined these relationships in field populations. It is important because such a comparison takes into account the possible interaction of pollutant stress with natural stress. Pascoe and Cram (1977) in a series of laboratory experiments, found that parasitised sticklebacks were more vulnerable than non-parasitised fish to pollutants such as Cd. What has not been considered before is the influence of parasite infection on metal uptake.

Before discussing specific aspects of the metal content of the fish and the parasites, it is important to consider some general points in relation to the growth and intensity of parasitic infection. At the beginning of the discussion to this chapter it was concluded that there were some atypical features about the nature of the parasite infection. In reported cases fish were more often found to be infected with large number of parasitic worms. In this study although the overall biomass of parasite is similar to the other cases, most fish had only one or two worms. Fish infected with cestode parasites were usually lighter than non-infected fish same as in other studies. The total length and dry weight of the parasitised and non-parasitised sticklebacks at site A are not significantly different, but at site B the parasitised sticklebacks are larger and heavier than non-parasitised fish. At site

E the parasitised group are significantly longer, although there is no difference in the dry body weight between the two groups of fish. Similar trends can seen for the actual cestode parasite; the greatest total lengths and body weights of parasite are found at site B, site E is intermediate and site A has the smallest lengths and weights of parasite. It was mentioned in the introduction that infected fish have a higher food and oxygen consumption (Lester 1970, Godin & Sproul 1988) and where food supply is limited the infected fish may die from starvation. However it is feasible that were food supply is not limiting which could be the case at site B, growth of fish and parasite may be both maintained. Alternatively as pointed out earlier the difference may be more a consequence of different age structures between the non-parasitised and parasitised fish particularly at site B.

Figure 4.12 presented the mean values for body burden and concentration of Cu and Cd in non-parasitised and parasitised sticklebacks, and the cestode parasite. It will be recalled that the metal contents of parasitised fish are generally significantly greater than non-parasitised fish at site B, but at site E the opposite is the case. It appears therefore that the largest parasitised fish found at site B have the highest metal contents, although these figures do not take account of possible differences in the age structures of the various groups of fish.

In order to eliminate the size effect the significant regression equations which relate size of fish (length) to body burden of metal were used to construct Table 4.30. The fish have been standardised to a common total length of 40 mm which corresponds to an approximate age $1^{+}$ year. It can be seen that this has resulted in a number changes. Comparing the size adjusted body burdens of Cu and Cd between non-parasitised and parasitised fish, the values at A are essentially the same. With exception of Cu at site B, non-

**Table 4.30** Comparison of the body burdens of Cu and Cd of non-parasitised and parasitised sticklebacks adjusted to a standard size of 40 mm TL, at each site.

| Site | Body burden of copper (ug) | | Body burden of cadmium (ug) | |
|---|---|---|---|---|
| | Non-parasitised | Parasitied | Non-parasitised | Parasitied |
| A | 0.62 | 0.61 | 0.005 | 0.012 |
| B | 3.89 | 5.25 | 0.066 | 0.047 |
| E | 3.21 | 1.77 | 0.042 | 0.018 |

parasitised fish have a higher body burden than the parasitised fish. For Cu at site B, the age adjustment has reduced the body burden in parasitised fish although it is still higher than in the non-parasitised fish.

It seems that the dynamics of Cu and Cd accumulations in non-parasitised and parasitised fish are different. Referring back to the results of the analysis of covariance for body burdens of Cu and Cd versus dry weight. It will be recalled that between sites difference were not the same for parasitised fish and non-parasitised fish. On the assumption that the intake and absorption of metals is the same in the two groups of fish, the trends suggest that in parasitised fish there is a diversion of metal from the fish to the parasite.

For Cd the parasite accumulates the diverted metal load and this is shown by the fact that the concentration of Cd in the cestode is greater than in the fish. For Cu the parasite appears to regulate the body burden of Cu and this is supported by the fact that the concentrations of Cu is lower in the cestode parasite than it is in the host fish. It would appear therefore that there is some degree of metabolic control regulating the transfer of Cu from the host to the parasite, but for Cd there is tendency for the metal to accumulate in the parasite.

The overall conclusion is that sticklebacks from contaminated sites in the river Wandle have elevated body burdens and concentrations of Cu and Cd, and also their cestode parasites. Moreover, in broad agreement with Moriarty et al. (1984) and Douben (1989), body burden rather than concentration should be used as a basis of comparison. However in this study it has been shown that a single regression model is too simplistic and future studies should take more account of various factors that influence body burden of metals.

Bearing in mind that the water of river Wandle is relatively hard and as a consequence the concentrations of Cu and Cd in the water would be expected to be quite low, it was considered important to assess the route and rate of supply of the metals to the fish in order to ascertain if food makes a significant contribution to the body burden of metal in the fish. This theme is explored in the following chapters. The next chapter is concerned with the composition of the food of sticklebacks. This is followed by examination of the distribution of Cu and Cd in the habitat and the major food items of the sticklebacks.

# CHAPTER FIVE

# THE DIET OF THREE-SPINED STICKLEBACK IN THE RIVER WANDLE

## 5.1 INTRODUCTION

The analysis of the stomach contents of fish is an important way of understanding the composition of their diets and from this an assessment may be made of their trophic level in a given aquatic ecosystem. The three-spined stickleback, *Gasterosteus aculeatus*, is known to be a voracious, visual predator. From the trophic standpoint, it is essentially a secondary consumer although quantities of vegetable material may be ingested.

The diet of sticklebacks has been studied extensively. From reviews by Wootton (1976, 1984), Hynes (1950) it is clear that a broadly similar spectrum of food items is consumed.

Planktonic crustaceans (mainly copepods and cladocerans) and insect larvae (mainly chironomid larvae and pupae) are the predominant prey of the partially-plated, 'leiurus' form of this species. Molluscs, oligochaetes, algae and plant tissue make up a smaller part of the diet (Allen and Wootton 1984, Manzer 1976, Hynes 1950). However, Ukegbu and Hungtingford (1988) found an urban population of 'leiurus' which had a quite different composition of diet, that included an unusually high proportion of the isopod *Asellus*. In the diet of the fully-plated, 'trachurus,' larger crustaceans, such as *Asellus* and amphipods, e.g. *Gammarus*, form an important part (Hynes 1950).

Feeding activities vary with season being highest in spring and summer when the principal food items are copepods and chironomid larvae. Conversely, in winter, feeding activity decreases and the larger crustaceans, such as *Asellus*, and plant material become more important (Ukegbu and Hungtingford 1988, Allen and Wootton 1984, Manzer 1976, Mann and Orr 1969, Hynes 1950).

Dietary differences related to sexual maturation have also been reported. In the breeding season male sticklebacks tend to eat less (Hynes 1950) but consume more benthic and epibenthic foods than

females (Manzer 1976). Breeding females consumed more ephemeropterans and molluscs (Ukegbu and Hungtingford 1988).

Moreover, a positive relationship between food size and stickleback size exists (Manzer 1976). The diet of small fish typically consists of small copepods and less seldom larger items of prey such as *Asellus*, *Gammarus*. It seems that as sticklebacks grow a wider range of organisms become potential prey (Wootton 1976).

Thus a broad picture of the diet of sticklebacks can be drawn, but it seems that there are differences to some extent in the diet of populations from different environments. In the progress towards establishing the main routes of entry of metals to stickleback populations in the Wandle, it is essential to discover the composition of the food. This has involved looking for differences of diet composition between size classes and between different populations.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Fish Sampling and Handling

Four seasonal samples of sticklebacks were collected from each of the three sampling sites (A, B, and E) along River Wandle (Fig. 3.1). These samples were taken at the same time as those which were analysed for copper and cadmium, and were reported in the previous chapter. When possible samples of 20 to 30 fish were obtained from each site. Fish were caught by seine net (Windell and Bowen 1978), and transported live to the laboratory, where they were killed by striking them on the bench, and frozen at -20$^{o}$C for later study.

Subsequently the fish were thawed, and the total length of each one were measured and then assigned into one of the eleven size categories (i.e. 11-16 mm, 17-22 mm, 23-28 mm, 29-34 mm, 35-40 mm, 41-46 mm, 47-52 mm, 53-58 mm, 59-64 mm, 65-70 mm, and 71-76 mm). They were divided into males and females and examined for evidence of parasite infection by *Schistocephalus* and the number of plerocercoids was counted where present. The stomachs of three-spined sticklebacks were easily distinguished from

the rest of the alimentary tract. These were removed and their contents were examined.

### 5.2.2 Methods of Diet Analysis

There are several methods for diet analysis of fish. They include determining the frequency of occurrence of food items, the total numbers, the volume and the weight of food items. There are also methods, such as the 'point method', which assess the abundance of food items in the stomach of fish. Each method has advantages and disadvantages, and it is generally recommended that more than one method should been used in any dietary research (Hynes 1950, Windell and Bowen 1978, Hyslop 1980). Typically an investigator should adopt or modify a method to suit their purpose.

Sticklebacks are known to eat a broad range of food items, including small zooplankton, relatively large *Asellus*, *Gammarus*, and snails, and broken vegetable and floculent materials. Consequently, a numerical or counting method is impractical and would in any case devalue the importance of the large food items. Similarly, since the analysis will include fish down to 15 mm in total length and 0.018 g in fresh weight, gravimetric and volumetric methods would be very difficult to apply.

As the aim is to discover the different items of food and to relate these to the quantity of metal taken in by the fish, it was decided to measure the frequency of occurrence and the relative abundance of items in the stomachs of sticklebacks. This enabled a comparison of the diet between the three different localities, the different seasons, and to see if the diet also varied with size and sex of the fish. The diet of parasitised and non-parasitised fish were also examined.

### 5.2.3 The Diet Analysis of 3-spined Stickleback

For the analysis of data by site, season, fish size, sex and for parasitism the following procedures and calculations have been made.

(1) The percentage of empty stomach (%EMP) and the average fullness score:

Before opening the guts, the degree of stomach fullness was scored visually according to one of the following four categories; 0= empty stomach, 1= quarter full, 2= half full, and 5= full stomach. Then, the percentage of fish with empty stomach was calculated. The average fullness score was calculated from the individual scores of stomach fullness, excluding the empty stomachs.

(2) The frequency of occurrence (O%):

The stomach contents were scraped out on a slide and examined under a binocular microscope and identified, normally to the level of class or family, but occasionally to the level of species. The frequency of occurrence of each food item was calculated according the following equation and expressed as percentage:

The frequency of occurrence (O%)= the number of stomach containing a specific food item / the number of examined stomach containing food x %.

(3) The percentage relative abundance (P%):

The relative abundance of different food items in individual guts was assessed in bulk and scored visually; 5 points was given to the most abundant food item, 2 points was given to the second most abundant food item, and 1 point was given to all other food items.

The percentage relative abundance of different foods was calculated from the sum of the points awarded to a food and expressed as a percentage of the total sum of points for all food items ingested:

The relative abundance (P%)= the sum of the points awarded to a specific food in a sub-population of fish examined / the total sum of the points for all food items ingested in the sub-population of fish x %.

(4) The percentage of parasitised fish (% PAR):

The level of parasitisation was calculated as the number of fish found to contain *Schistocephalus* as a percentage of the number of fish examined.

## 5.3 RESULTS

### 5.3.1 The Diet and Feeding of Three-spined Stickleback in the River Wandle

A total of 255 stomachs were examined. The food items which were identified are listed in full in Tables 5.1 - 5.7. They include small and large crustaceans (the amphipods *Gammarus* and *Crangonyx* were not separated), a variety of molluscs, insects (chiefly chironomid larvae and pupae), miscellaneous invertebrate materials, filamentous algae, plant fragments, fine detritus and items of terrestrial origin (mostly insects).

The percentages of empty stomachs and average stomach fullness values are also shown in Tables 5.2 - 5.7. At the flowing water sites B and E, with a single exception, all stomachs contained food on all sample dates. By contrast, at Waddon Pond, site A, a small number of empty stomachs was always found. Average fullness values ranged between 2.8 and 4.3, and their seasonal pattern was generally similar at all sites. Lower values occurred in winter and mid-summer, and higher values in spring and autumn.

### 5.3.2 Comparison of the Diet Between Sites

(A) Percentage relative abundance

Table 5.1 shows the mean percentage relative abundance of food in stomachs at each site. The differences in the diet between site are clearly shown in Figure 5.1.

At site A small crustaceans (37.3%) were predominant with cladocera and copepoda as the main groups. Abundance of chironomids was less than half that of crustaceans (16.7%) with plant materials and 'floc' contributing most of the remainder of the diet. By contrast, at

**Table 5.1** The percentage relative abundance (P%) and frequency of occurrence (O%) of different foods in stomachs of the 3-spined sticklebacks at the three sampling sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990. ( )* indicates number of fish examined.

| SITE | A | B | E | A | B | E |
|---|---|---|---|---|---|---|
| FOOD ITEMS | | | | | | |
| Sample size | (102)* | (65)* | (88)* | (102)* | (65)* | (88)* |
| %EMP | 5.76 | 0.00 | 0.83 | 5.76 | 0.00 | 0.83 |
| %PAR | 7.09 | 16.44 | 14.28 | 7.09 | 16.44 | 14.28 |
| Mean fullness | 3.51 | 3.63 | 3.89 | 3.51 | 3.63 | 3.89 |
| Method | P% | P% | P% | O% | O% | O% |
| Crustacea | | | | | | |
| Asellus sp. | 1.3 | 0.4 | 9.8 | 3.5 | 2.6 | 25.1 |
| Amphipoda | 4.3 | 0.4 | 9.6 | 9.9 | 3.4 | 32.2 |
| Cladocera | 13.3 | 0.4 | 1.9 | 67.0 | 2.6 | 17.1 |
| Copepoda | 19.8 | 15.3 | 7.5 | 55.8 | 51.2 | 49.8 |
| Ostracoda | 4.3 | – | – | 24.2 | – | – |
| Mollusca | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | – | 17.1 | – | – | 38.1 | – |
| Lymnaea sp. | 1.1 | 3.1 | 4.0 | 3.5 | 13.5 | 17.6 |
| Physa sp. | – | 2.5 | 0.1 | – | 11.4 | 0.9 |
| Planorbis sp. | 0.2 | 0.2 | 1.1 | 1.5 | 1.5 | 4.3 |
| Snail unidentified | – | 4.8 | 2.2 | – | 17.5 | 8.0 |
| Ancylus sp. | – | – | 0.4 | – | – | 3.6 |
| Sphaerium sp. | 0.3 | 0.2 | 0.7 | 1.0 | 1.5 | 5.1 |
| Insecta | | | | | | |
| Chironomid larvae | 9.6 | 29.4 | 21.8 | 29.0 | 71.2 | 68.4 |
| Chironomid pupae | 7.2 | 5.8 | 7.2 | 21.2 | 19.3 | 26.7 |
| Insect unidentified | 2.2 | 3.3 | 4.2 | 8.3 | 13.5 | 13.9 |
| Polyzoan | | | | | | |
| Statoblasts | 2.0 | 0.2 | 0.1 | 13.0 | 1.7 | 0.9 |
| Hydracarina | – | 1.8 | 0.6 | – | 8.4 | 3.8 |
| Nematoda | 0.1 | 1.3 | 2.7 | 0.6 | 6.7 | 8.8 |
| Invertebrate eggs | 3.7 | 1.9 | 2.1 | 17.8 | 9.9 | 7.7 |
| Filamentous algae | 11.5 | – | 10.7 | 22.1 | – | 39.5 |
| Vegetable matter | 10.3 | 2.6 | 3.2 | 37.6 | 16.9 | 17.7 |
| 'Floc'(fine detritus) | 8.0 | 7.9 | 9.6 | 44.1 | 27.3 | 54.7 |
| Others (unident. terrestrial) | 1.0 | 1.4 | 0.6 | 5.1 | 7.6 | 3.5 |

**Table 5.2** The percentage relative abundance of different foods in stomachs of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

| FOOD ITEMS | SITE / MONTH | A JUL | A OCT | A JAN | A APR | B JUL | B OCT | B JAN | B APR | E JUL | E OCT | E JAN | E APR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample size | | 43 | 26 | 18 | 15 | 29 | 18 | 15 | 3 | 30 | 28 | 10 | 20 |
| %EMP | | 6.98 | 3.85 | 5.56 | 6.66 | 0.00 | 0.00 | 0.00 | 0.00 | 3.30 | 0.00 | 0.00 | 0.00 |
| %PAR | | 4.65 | 11.50 | 5.56 | 6.66 | 13.80 | 5.56 | 13.10 | 33.30 | 30.00 | 7.10 | 0.00 | 20.00 |
| Mean fullness | | 3.02 | 3.92 | 2.88 | 4.21 | 3.45 | 4.27 | 3.80 | 3.00 | 3.93 | 4.32 | 3.10 | 4.20 |
| Crustacea | | | | | | | | | | | | | |
| Asellus sp. | | 4.1 | 1.1 | – | – | 1.4 | – | – | – | 10.9 | 10.3 | 14.7 | 3.3 |
| Amphipoda | | 15.2 | 2.2 | – | – | 0.9 | – | 0.8 | – | 3.6 | 10.3 | 16.2 | 8.3 |
| Cladocera | | 8.8 | 12.9 | 18.6 | 12.8 | 1.4 | – | – | – | 0.5 | 5.1 | 1.5 | 0.6 |
| Copepoda | | 22.6 | 12.4 | 38.6 | 5.5 | 22.3 | 16.0 | 1.5 | 21.4 | 6.4 | 8.2 | 5.9 | 9.4 |
| Ostracoda | | 3.4 | 4.3 | 5.7 | 3.7 | – | – | – | – | – | – | – | – |
| Mollusca | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – | – | – | – | 3.8 | 49.6 | 15.0 | – | – | – | – | – |
| Lymnaea sp. | | 1.7 | 2.7 | – | – | 10.9 | 0.8 | 0.8 | – | 4.1 | 3.4 | – | 8.3 |
| Physa sp. | | – | – | – | – | 3.3 | 6.9 | – | – | – | 0.3 | – | – |
| Planorbis sp. | | – | – | 0.7 | – | – | 0.8 | – | – | – | 0.7 | – | 3.9 |
| Snail unidentified | | – | – | – | – | 0.5 | – | 18.8 | – | 3.2 | 5.5 | – | – |
| Ancylus sp. | | – | – | – | – | – | – | – | – | – | 1.7 | – | – |
| Sphaerium sp. | | – | 1.1 | – | – | – | 0.8 | – | – | 0.5 | 0.7 | 1.5 | – |
| Insecta | | | | | | | | | | | | | |
| Chironomid larvae | | 16.9 | 14.5 | 5.0 | 1.8 | 39.8 | 10.7 | 9.8 | 57.1 | 14.5 | 28.8 | 20.6 | 23.3 |
| Chironomid pupae | | 2.4 | 13.4 | – | 12.8 | 1.9 | – | – | 21.4 | 3.2 | 7.2 | 7.4 | 11.1 |
| Insect unidentified | | 2.7 | 0.5 | – | 5.5 | 4.3 | 6.9 | 2.3 | – | 0.9 | 2.1 | 11.8 | 2.2 |
| Polyzoan | | | | | | | | | | | | | |
| Statoblasts | | 1.4 | 5.9 | 0.7 | – | – | – | 0.8 | – | – | 0.3 | – | – |
| Hydracarina | | – | – | – | – | 1.9 | – | 5.3 | – | – | – | – | 2.2 |
| Nematoda | | 0.3 | – | – | – | – | – | 5.3 | – | 3.6 | – | 5.9 | 1.1 |
| Invertebrate eggs | | 4.7 | 5.9 | 4.3 | – | 0.9 | 3.8 | 3.0 | – | 6.4 | 0.3 | – | 1.7 |
| Filamentous algae | | 3.0 | – | – | 43.1 | – | – | – | – | 26.4 | 5.5 | – | 11.1 |
| Vegetable matter | | 7.1 | 16.7 | 16.4 | 0.9 | 0.5 | 2.3 | 7.5 | – | 3.6 | 0.7 | 7.4 | 1.1 |
| 'Floc'(fine detritus) | | 4.1 | 6.5 | 8.6 | 12.8 | 2.8 | 1.5 | 27.1 | – | 10.9 | 7.9 | 7.4 | 12.2 |
| Others (unident. terrestrial) | | 1.7 | – | 1.4 | 0.9 | 3.3 | – | 2.3 | – | 1.4 | 1.0 | – | – |

**Table 5.3** The percentage occurrence of different foods in stomachs of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

| FOOD ITEMS | SITE | A | A | A | A | B | B | B | B | E | E | E | E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MONTH | JUL | OCT | JAN | APR | JUL | OCT | JAN | APR | JUL | OCT | JAN | APR |
| Sample size | | 43 | 26 | 18 | 15 | 29 | 18 | 15 | 3 | 30 | 28 | 10 | 20 |
| %EMP | | 6.98 | 3.85 | 5.56 | 6.66 | 0.00 | 0.00 | 0.00 | 0.00 | 3.30 | 0.00 | 0.00 | 0.00 |
| %PAR | | 4.65 | 11.50 | 5.56 | 6.66 | 13.80 | 5.56 | 13.10 | 33.30 | 30.00 | 7.10 | 0.00 | 20.00 |
| Mean fullness | | 3.02 | 3.92 | 2.88 | 4.21 | 3.45 | 4.27 | 3.80 | 3.00 | 3.93 | 4.32 | 3.10 | 4.20 |
| Crustacea | | | | | | | | | | | | | |
| Asellus sp. | | 10.0 | 4.0 | – | – | 10.3 | – | – | – | 31.0 | 39.3 | 20.0 | 10.0 |
| Amphipoda | | 27.5 | 12.0 | – | – | 6.9 | – | 6.7 | – | 17.2 | 46.4 | 40.0 | 25.0 |
| Cladocera | | 52.5 | 56.0 | 88.2 | 71.4 | 10.3 | – | – | – | 3.4 | 50.0 | 10.0 | 5.0 |
| Copepoda | | 50.0 | 48.0 | 82.4 | 42.9 | 48.3 | 76.5 | 13.3 | 66.7 | 37.9 | 71.4 | 40.0 | 50.0 |
| Ostracoda | | 12.5 | 32.0 | 23.5 | 28.6 | – | – | – | – | – | – | – | – |
| Mollusca | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – | – | – | – | 24.1 | 88.2 | 40.0 | – | – | – | – | – |
| Lymnaea sp. | | 10.0 | 4.0 | – | – | 41.4 | 5.9 | 6.7 | – | 10.3 | 25.0 | – | 35.0 |
| Physa sp. | | – | – | – | – | 10.3 | 35.3 | – | – | – | 3.6 | – | – |
| Planorbis sp. | | – | – | 5.9 | – | – | 5.9 | – | – | – | 7.1 | – | 10.0 |
| Snail unidentified | | – | – | – | – | 3.4 | – | 66.7 | – | 6.9 | 25.0 | – | – |
| Ancylus sp. | | – | – | – | – | – | – | – | – | – | 14.3 | – | – |
| Sphaerium sp. | | – | 4.0 | – | – | – | 5.9 | – | – | 3.4 | 7.1 | 10.0 | – |
| Insecta | | | | | | | | | | | | | |
| Chironomid larvae | | 50.0 | 40.0 | 11.8 | 14.3 | 89.7 | 35.3 | 60.0 | 100 | 37.9 | 85.7 | 70.0 | 80.0 |
| Chironomid pupae | | 7.5 | 20.0 | – | 57.1 | 10.3 | – | – | 66.7 | 6.9 | 50.0 | 10.0 | 40.0 |
| Insect unidentified | | 15.0 | 4.0 | – | 14.3 | 17.2 | 23.5 | 13.3 | – | 3.4 | 7.1 | 30.0 | 15.0 |
| Polyzoan | | | | | | | | | | | | | |
| Statoblasts | | 10.0 | 36.0 | 5.9 | – | – | – | 6.7 | – | – | 3.6 | – | – |
| Hydracarina | | – | – | – | – | 13.8 | – | 20.0 | – | – | – | – | 15.0 |
| Nematoda | | 2.5 | – | – | – | – | – | 26.7 | – | 10.3 | – | 20.0 | 5.0 |
| Invertebrate eggs | | 20.0 | 16.0 | 35.3 | – | 6.9 | 5.9 | 26.7 | – | 17.2 | 3.6 | – | 10.0 |
| Filamentous algae | | 10.0 | – | – | 78.6 | – | – | – | – | 55.2 | 42.9 | – | 60.0 |
| Vegetable matter | | 32.5 | 40.0 | 70.6 | 7.1 | 3.4 | 17.6 | 46.7 | – | 17.2 | 3.6 | 40.0 | 10.0 |
| 'Floc'(fine detritus) | | 17.5 | 24.0 | 70.6 | 64.3 | 17.2 | 11.8 | 80.0 | – | 48.3 | 60.7 | 50.0 | 60.0 |
| Others (unident. terrestrial) | | 7.5 | – | 5.9 | 7.1 | 10.3 | – | 20.0 | – | 6.9 | 7.1 | – | – |

**Table 5.4** The percentage relative abundance of different foods in the 5 size classes (mm TL) of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

| FOOD ITEMS | SITE | A | A | A | A | A | B | B | B | B | B | E | E | E | E | E | E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CLASS | <22 | 23–28 | 29–34 | 35–40 | >41 | <22 | 23–28 | 29–34 | 35–40 | >41 | <22 | 23–28 | 29–34 | 35–40 | 41–46 | >47 |
| Sample size | | 22 | 27 | 26 | 11 | 16 | 12 | 11 | 20 | 12 | 10 | 13 | 24 | 11 | 11 | 19 | 10 |
| %EMP | | 9.1 | 7.4 | 3.8 | 0.0 | 6.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| %PAR | | 4.5 | 14.8 | 3.8 | 9.0 | 0.0 | 0.0 | 0.0 | 10.0 | 8.3 | 50.0 | 0.0 | 8.3 | 18.2 | 9.0 | 31.6 | 40.0 |
| Mean fullness | | 3.4 | 3.4 | 2.8 | 3.6 | 4.2 | 3.1 | 4.1 | 3.5 | 4.4 | 3.6 | 4.2 | 4.3 | 3.5 | 3.8 | 4.3 | 3.0 |
| Crustacea | | | | | | | | | | | | | | | | | |
| Asellus sp. | | – | 1.1 | 3.0 | 6.9 | – | 1.2 | – | 1.4 | – | – | 5.0 | 10.6 | 15.1 | 6.2 | 8.3 | 9.0 |
| Amphipoda | | 4.4 | 11.5 | 9.1 | 5.9 | – | – | – | 0.7 | 0.9 | 1.5 | 3.0 | 8.5 | 12.9 | 3.1 | 5.8 | 23.9 |
| Cladocera | | 10.7 | 13.7 | 12.2 | 16.7 | 8.9 | 2.4 | 1.2 | – | – | – | 1.0 | 4.9 | 3.2 | 2.1 | – | – |
| Copepoda | | 28.9 | 17.6 | 18.9 | 28.4 | 9.7 | 45.9 | 11.6 | 9.7 | 3.7 | 9.1 | 11.9 | 8.9 | 5.4 | 10.3 | 3.2 | 7.5 |
| Ostracoda | | 5.7 | 2.2 | 4.9 | 3.9 | 4.0 | – | – | – | – | – | – | – | – | – | – | – |
| Mollusca | | | | | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – | – | – | – | – | 1.2 | 22.1 | 22.2 | 28.7 | 15.2 | – | – | – | – | – | – |
| Lymnaea sp. | | 2.5 | 0.5 | 3.0 | – | – | 2.4 | 2.3 | 9.0 | 0.9 | 10.6 | 7.9 | 3.7 | – | 2.1 | 8.3 | 3.0 |
| Physa sp. | | – | – | – | – | – | 5.9 | 2.3 | 2.1 | 4.6 | 1.5 | – | 0.4 | – | – | – | – |
| Planorbis sp. | | – | – | – | 1.0 | – | – | – | – | 0.9 | – | – | 0.8 | – | – | 4.5 | – |
| Snail unidentified | | – | – | – | – | – | – | 1.2 | 4.2 | 13.0 | 7.6 | – | 1.6 | 3.2 | 6.2 | 6.4 | – |
| Ancylus sp. | | – | – | – | – | – | – | – | – | – | – | – | 1.6 | – | 1.0 | – | – |
| Sphaerium sp. | | – | – | 1.2 | – | – | – | – | – | 0.9 | – | – | 0.8 | 1.1 | – | 0.6 | – |
| Insecta | | | | | | | | | | | | | | | | | |
| Chironomid larvae | | 18.2 | 14.3 | 13.4 | 6.9 | 1.6 | 31.8 | 27.9 | 28.5 | 11.1 | 22.7 | 26.7 | 26.8 | 29.0 | 20.6 | 17.3 | 7.5 |
| Chironomid pupae | | 1.3 | 2.7 | 9.1 | 9.8 | 11.3 | 1.2 | 2.3 | 0.7 | – | 4.5 | 9.9 | 5.3 | 6.5 | 6.2 | 7.7 | 9.0 |
| Insect unidentified | | 1.9 | 2.2 | 1.2 | – | 4.8 | 3.5 | 8.1 | 6.3 | 0.9 | 1.5 | – | 2.8 | – | 7.2 | 1.9 | 4.5 |
| Polyzoan | | | | | | | | | | | | | | | | | |
| Statoblasts | | 1.9 | 4.4 | 1.8 | 2.0 | – | – | – | – | – | 1.5 | – | 0.4 | – | – | – | – |
| Hydracarina | | – | – | – | – | – | – | – | 1.4 | 6.5 | 3.0 | – | – | – | 3.1 | 0.6 | – |
| Nematoda | | 0.6 | – | – | – | – | – | 1.2 | 1.4 | 1.9 | 3.0 | 1.0 | 1.6 | 2.2 | – | 4.5 | – |
| Invertebrate eggs | | 11.9 | 2.7 | 1.2 | 2.9 | 1.6 | 1.2 | 8.1 | 1.4 | 0.9 | – | 1.0 | 2.4 | – | 4.1 | – | 10.4 |
| Filamentous algae | | 5.0 | 0.5 | – | – | 37.9 | – | – | – | – | – | 22.8 | 9.3 | 14.0 | 8.2 | 12.2 | 11.9 |
| Vegetable matter | | 1.3 | 22.5 | 9.8 | 8.8 | 6.5 | – | – | 1.4 | 7.4 | 6.1 | – | – | 1.1 | 4.1 | 3.8 | 9.0 |
| 'Floc'(fine detritus) | | 3.1 | 3.3 | 11.0 | 4.9 | 12.9 | 3.5 | 10.5 | 8.3 | 17.6 | 1.5 | 8.9 | 8.5 | 6.5 | 14.4 | 14.7 | 1.5 |
| Others (unident. terrestrial) | | 2.5 | 0.5 | – | 2.0 | 0.8 | – | 1.2 | 1.4 | – | 10.6 | 1.0 | 0.8 | – | 1.0 | – | 3.0 |

**Table 5.5** The percentage occurrence of different foods in the 5 size classes (mm TL) of 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

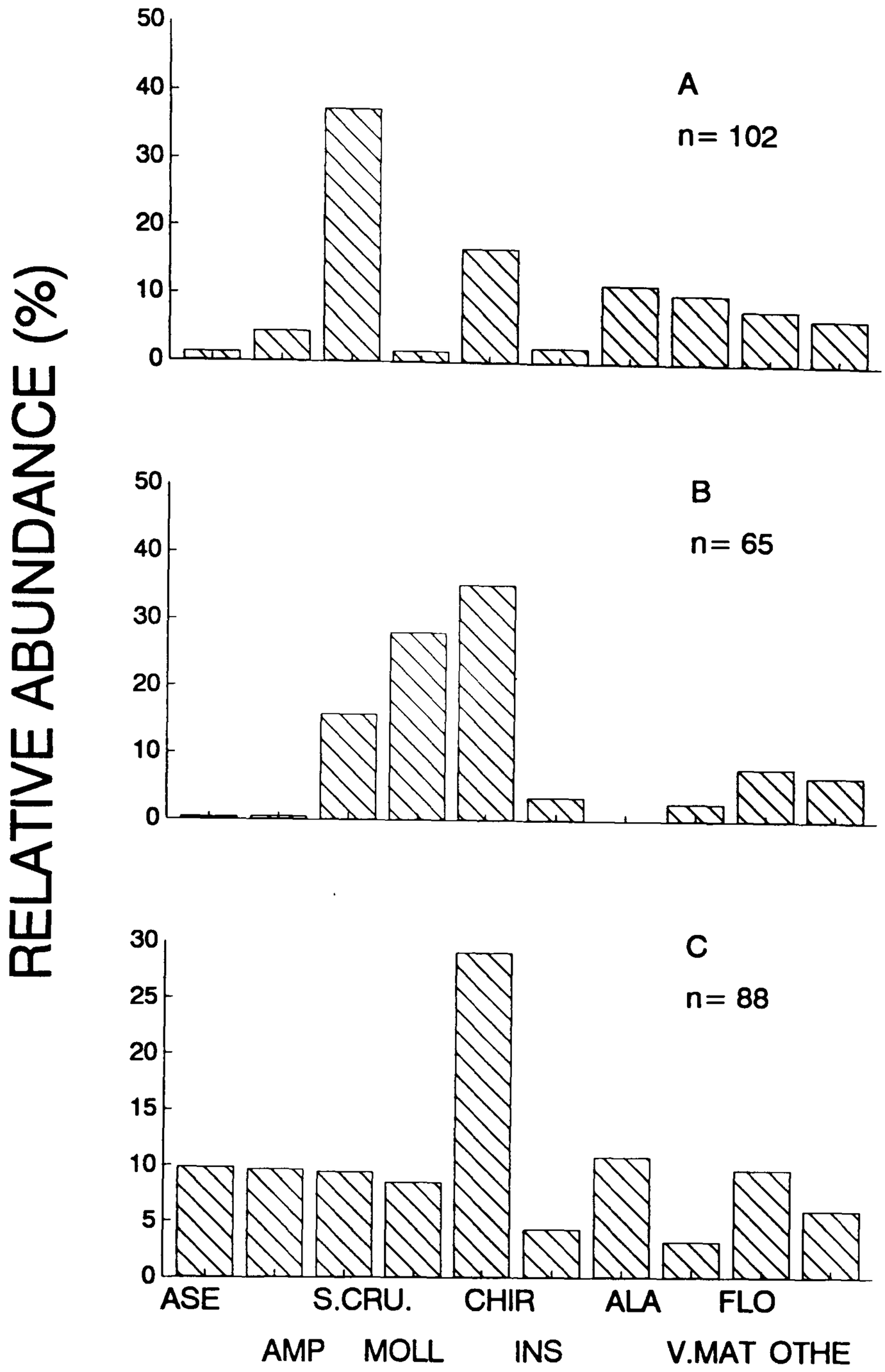
| FOOD ITEMS | SITE | A | A | A | A | A | B | B | B | B | B | E | E | E | E | E | E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CLASS | <22 | 23–28 | 29–34 | 35–40 | >41 | <22 | 23–28 | 29–34 | 35–40 | >41 | <22 | 23–28 | 29–34 | 35–40 | 41–46 | >47 |
| Sample size | | 38 | 27 | 26 | 11 | 16 | 12 | 11 | 20 | 12 | 10 | 13 | 24 | 11 | 11 | 19 | 10 |
| %EMP | | 2.6 | 7.4 | 3.8 | 0.0 | 6.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| %PAR | | 2.6 | 14.8 | 3.8 | 9.0 | 0.0 | 0.0 | 0.0 | 10.0 | 8.3 | 50.0 | 0.0 | 8.3 | 18.2 | 9.0 | 31.6 | 40.0 |
| Mean fullness | | 3.4 | 3.4 | 2.8 | 3.6 | 4.2 | 3.1 | 4.1 | 3.5 | 4.4 | 3.6 | 4.2 | 4.3 | 3.5 | 3.8 | 4.3 | 3.0 |
| Crustacea | | | | | | | | | | | | | | | | | |
| Asellus sp. | | – | 8.0 | 4.0 | 18.2 | – | 8.3 | – | 10.0 | – | – | 8.3 | 33.3 | 54.5 | 27.3 | 16.7 | 20.0 |
| Amphipoda | | 10.0 | 20.0 | 20.0 | 18.2 | – | – | – | 5.0 | 8.3 | 10.0 | 16.7 | 41.7 | 36.4 | 18.2 | 16.7 | 50.0 |
| Cladocera | | 70.0 | 56.0 | 48.0 | 81.8 | 73.3 | 16.7 | 9.1 | – | – | – | 8.3 | 45.8 | 27.3 | 18.2 | – | – |
| Copepoda | | 75.0 | 44.0 | 44.0 | 72.7 | 46.7 | 83.3 | 45.5 | 40.0 | 33.3 | 40.0 | 75.0 | 75.0 | 45.5 | 45.5 | 16.7 | 40.0 |
| Ostracoda | | 20.0 | 16.0 | 16.0 | 36.4 | 33.3 | – | – | – | – | – | – | – | – | – | – | – |
| Mollusca | | | | | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – 15.0 | – | – | – | – | 8.3 | 54.5 | 50.0 | 58.3 | 40.0 | – | – | – | – | – | – |
| Lymnaea sp. | | – | 4.0 | 4.0 | – | – | 16.7 | 18.2 | 35.0 | 8.3 | 20.0 | 25.0 | 25.0 | – | 9.1 | 25.0 | 10.0 |
| Physa sp. | | – | – | – | – | – | 8.3 | 18.2 | 10.0 | 25.0 | 10.0 | – | 4.2 | – | – | – | – |
| Planorbis sp. | | – | – | – | 9.1 | – | – | – | – | 8.3 | – | – | 8.3 | – | – | 8.3 | – |
| Snail unidentified | | – | – | – | – | – | – | 9.1 | 20.0 | 41.7 | 10.0 | – | 12.5 | 18.2 | 18.2 | 8.3 | – |
| Ancylus sp. | | – | – | – | – | – | – | – | – | – | – | – | 12.5 | – | 9.1 | – | – |
| Sphaerium sp. | | – | – | 4.0 | – | – | – | – | – | 8.3 | – | – | 8.3 | 9.1 | – | 4.2 | – |
| Insecta | | | | | | | | | | | | | | | | | |
| Chironomid larvae | | 55.0 | 36.0 | 36.0 | 27.3 | 13.3 | 91.7 | 54.5 | 65.0 | 66.7 | 60.0 | 66.7 | 83.3 | 90.9 | 63.6 | 37.5 | 40.0 |
| Chironomid pupae | | 10.0 | 4.0 | 12.0 | 18.2 | 53.3 | 8.3 | 9.1 | 5.0 | – | 20.0 | 33.3 | 33.3 | 18.2 | 36.4 | 20.8 | 20.0 |
| Insect unidentified | | 10.0 | 12.0 | 8.0 | – | 13.3 | 25.0 | 18.2 | 20.0 | 8.3 | 10.0 | – | 12.5 | – | 18.2 | 8.3 | 20.0 |
| Polyzoan | | | | | | | | | | | | | | | | | |
| Statoblasts | | 15.0 | 24.0 | 12.0 | 18.2 | – | – | – | – | – | 10.0 | – | 4.2 | – | – | – | – |
| Hydracarina | | – | – | – | – | – | – | – | 10.0 | 25.0 | 20.0 | – | – | – | 18.2 | 4.2 | – |
| Nematoda | | 5.0 | – | – | – | – | – | 9.1 | 5.0 | 8.3 | 10.0 | 8.3 | 8.3 | 9.1 | – | 8.3 | – |
| Invertebrate eggs | | 40.0 | 12.0 | 8.0 | 27.3 | 13.3 | 8.3 | 27.3 | 10.0 | 8.3 | – | 8.3 | 8.3 | – | 27.3 | – | 20.0 |
| Filamentous algae | | 15.0 | 4.0 | – | – | 73.3 | – | – | – | – | – | 50.0 | 45.8 | 45.5 | 36.4 | 29.2 | 70.0 |
| Vegetable matter | | 10.0 | 52.0 | 48.0 | 54.5 | 20.0 | – | – | 10.0 | 50.0 | 30.0 | – | – | 9.1 | 27.3 | 12.5 | 50.0 |
| 'Floc'(fine detritus) | | 5.0 | 20.0 | 48.0 | 45.5 | 73.3 | 25.0 | 36.4 | 20.0 | 58.3 | 10.0 | 66.7 | 62.5 | 45.5 | 54.5 | 54.2 | 10.0 |
| Others (unident. terrestrial) | | 10.0 | 4.0 | – | 9.1 | 6.7 | – | 9.1 | 10.0 | – | 30.0 | 8.3 | 4.2 | – | 9.1 | – | 10.0 |

**Table 5.6** The percentage relative abundance (P%) and occurrence (O%) of different foods in male and female 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

| FOOD ITEMS | SITE / SEX | A FEMALE | A MALE | B FEMALE | B MALE | E FEMALE | E MALE | A FEMALE | A MALE | B FEMALE | B MALE | E FEMALE | E MALE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample size | 38 | 15 | 27 | 15 | 28 | 23 | 38 | 15 | 27 | 15 | 28 | 23 |
| | %EMP | 2.6 | 6.7 | 0.0 | 0.0 | 0.0 | 0.0 | 2.6 | 6.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| | %PAR | 2.6 | 6.7 | 18.5 | 20.0 | 28.6 | 21.7 | 2.6 | 6.7 | 18.5 | 20.0 | 28.6 | 21.7 |
| | Mean fullness | 3.4 | 3.2 | 4.2 | 3.0 | 3.8 | 3.6 | 3.4 | 3.2 | 4.2 | 3.0 | 3.8 | 3.6 |
| | Method | P% | P% | P% | P% | P% | P% | O% | O% | O% | O% | O% | O% |
| Crustacea | | | | | | | | | | | | | |
| Asellus sp. | | 2.4 | 5.2 | 0.9 | – | 9.5 | 9.3 | 5.4 | 7.1 | 7.4 | – | 25.0 | 34.8 |
| Amphipoda | | 4.8 | 7.2 | 0.4 | 2.1 | 9,1 | 10.4 | 13.5 | 14.3 | 3.7 | 13.3 | 21.4 | 39.1 |
| Cladocera | | 14.0 | 7.2 | – | – | 1.4 | 1.0 | 70.3 | 42.9 | – | – | 10.7 | 8.7 |
| Copepoda | | 18.8 | 17.5 | 7.1 | 8.5 | 6.4 | 5.7 | 54.1 | 42.9 | 44.4 | 26.7 | 32.1 | 39.1 |
| Ostracoda | | 3.4 | 7.2 | – | – | – | – | 27.0 | 21.4 | – | – | – | – |
| Mollusca | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – | – | 27.7 | 11.7 | – | – | – | – | 59.3 | 33.3 | – | – |
| Lymnaea sp. | | 1.7 | – | 6.7 | 6.4 | 3.6 | 4.7 | 2.7 | – | 18.5 | 33.3 | 17.9 | 13.0 |
| Physa sp. | | – | – | 3.1 | 2.1 | – | – | – | – | 18.5 | 6.7 | – | – |
| Planorbis sp. | | 0.3 | – | 0.4 | – | – | 3.6 | 2.7 | – | 3.7 | – | – | 8.7 |
| Snail unidentified | | – | – | 9.8 | 3.2 | 6.8 | 2.1 | – | – | 29.6 | 13.3 | 10.7 | 13.0 |
| Ancylus sp. | | – | – | – | – | – | 0.5 | – | – | – | – | – | 4.3 |
| Sphaerium sp. | | 0.7 | – | 0.4 | 0.0 | 0.5 | 0.5 | 2.7 | – | 3.7 | – | 3.6 | 4.3 |
| Insecta | | | | | | | | | | | | | |
| Chironomid larvae | | 7.2 | 10.3 | 15.6 | 35.1 | 19.5 | 18.7 | 24.3 | 35.7 | 59.3 | 73.3 | 60.7 | 56.5 |
| Chironomid pupae | | 8.5 | 14.4 | 0.4 | 3.2 | 8.2 | 6.2 | 24.3 | 28.6 | 3.7 | 13.3 | 25.0 | 26.1 |
| Insect unidentified | | 2.4 | 1.0 | 1.8 | 7.4 | 3.6 | 2.6 | 8.1 | 7.1 | 11.1 | 20.0 | 10.7 | 13.0 |
| Polyzoan | | | | | | | | | | | | | |
| Statoblasts | | 1.4 | 1.0 | 0.4 | – | – | – | 10.8 | 7.1 | 3.7 | – | – | – |
| Hydracarina | | – | – | 1.8 | 7.4 | 0.9 | 1.0 | – | – | 14.8 | 20.0 | 7.1 | 4.3 |
| Nematoda | | – | – | 1.8 | 2.1 | 3.2 | 1.0 | – | – | 7.4 | 6.7 | 7.1 | 4.3 |
| Invertebrate eggs | | 1.4 | 3.1 | 0.9 | 1.1 | 2.3 | 3.1 | 10.8 | 21.4 | 7.4 | 6.7 | 3.6 | 17.4 |
| Filamentous algae | | 14.0 | 6.2 | – | – | 8.2 | 15.5 | 24.3 | 14.3 | – | – | 32.1 | 60.9 |
| Vegetable matter | | 7.2 | 12.4 | 5.8 | 1.1 | 5.5 | 2.6 | 35.1 | 57.1 | 37.0 | 6.7 | 28.6 | 17.4 |
| 'Floc'(fine detritus) | | 10.9 | 7.2 | 11.2 | 7.4 | 11.4 | 9.8 | 56.8 | 50.0 | 33.3 | 20.0 | 53.6 | 43.5 |
| Others (unident. terrestrial) | | 1.0 | – | 3.6 | 1.1 | – | 1.6 | 5.4 | – | 14.8 | 6.7 | – | 8.7 |

**Table 5.7** The percentage relative abundance (P%) and occurrence (O%) of different foods in non-parasitised (N-PAR) and parasitised (PAR) 3-spined sticklebacks at the three sites in the River Wandle for each survey from Jul. 1989 to Apr. 1990.

| FOOD ITEMS | SITE PARASITISM | A N-PAR | A PAR | B N-PAR | B PAR | E N-PAR | E PAR | A N-PAR | A PAR | B N-PAR | B PAR | E N-PAR | E PAR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample size | 82 | 7 | 34 | 8 | 60 | 15 | 82 | 7 | 34 | 8 | 60 | 15 |
| | %EMP | 4.9 | 14.3 | 0 | 0 | 0 | 0 | 4.9 | 14.3 | 0 | 0 | 0 | 0 |
| | Mean fullness | 3.32 | 3.16 | 3.79 | 3.75 | 4.1 | 3.4 | 3.32 | 3.16 | 3.79 | 3.75 | 4.1 | 3.4 |
| | Method | P% | P% | P% | P% | P% | P% | O% | O% | O% | O% | O% | O% |
| Crustacea | | | | | | | | | | | | | |
| Asellus sp. | | 2.4 | – | 0.4 | 1.7 | 10.4 | 7.6 | 6.4 | – | 2.7 | 12.5 | 33.3 | 20.0 |
| Amphipoda | | 8.2 | – | 0.4 | 3.4 | 9.1 | 10.1 | 17.9 | – | 2.7 | 25.0 | 36.7 | 20.0 |
| Cladocera | | 11.8 | 30.6 | – | – | 2.6 | 2.5 | 61.5 | 50.0 | – | – | 21.7 | 20.0 |
| Copepoda | | 21.5 | 47.2 | 8.5 | 3.4 | 7.6 | 5.0 | 52.6 | 100 | 37.8 | 25.0 | 53.3 | 26.7 |
| Ostracoda | | 4.2 | 2.8 | – | – | – | – | 20.5 | 16.7 | – | – | – | – |
| Mollusca | | | | | | | | | | | | | |
| Potamopyrgus sp. & Bythinia sp. | | – | – | 25.1 | 13.6 | – | – | – | – | 43.2 | 62.5 | – | – |
| Lymnaea sp. | | 1.7 | – | 4.6 | 15.3 | 4.4 | 1.7 | 6.4 | – | 18.9 | 37.5 | 21.7 | 6.7 |
| Physa sp. | | – | – | 3.1 | 1.7 | 0.2 | – | – | – | 13.5 | 12.5 | 1.7 | – |
| Planorbis sp. | | 0.2 | – | 0.4 | – | 1.7 | – | 1.3 | – | 2.7 | – | 6.7 | – |
| Snail unidentified | | – | – | 9.7 | – | 3.3 | 4.2 | – | – | 27.0 | – | 13.3 | 6.7 |
| Ancylus sp. | | – | – | – | – | 0.9 | – | – | – | – | – | 6.7 | – |
| Sphaerium sp. | | 0.3 | – | 0.4 | – | 0.7 | – | 1.3 | – | 2.7 | – | 6.7 | – |
| Insecta | | | – | | | | | | – | | | | |
| Chironomid larvae | | 14.1 | – | 20.5 | 25.4 | 22.4 | 20.2 | 41.0 | – | 56.8 | 75.0 | 73.3 | 40.0 |
| Chironomid pupae | | 5.7 | – | 0.8 | 3.4 | 7.0 | 4.2 | 11.5 | – | 5.4 | 12.5 | 30.0 | 20.0 |
| Insect unidentified | | 1.5 | – | 4.2 | – | 3.1 | 2.5 | 9.0 | – | 16.2 | – | 11.7 | 13.3 |
| Polyzoan | | | | | | | | | | | | | |
| Statoblasts | | 2.5 | 2.8 | – | 1.7 | – | 0.8 | 16.7 | 16.7 | – | 12.5 | – | 6.7 |
| Hydracarina | | – | – | 1.5 | 11.9 | 0.7 | – | – | – | 10.8 | 37.5 | 5.0 | – |
| Nematoda | | 0.2 | – | 1.5 | 3.4 | 1.5 | 4.2 | 1.3 | – | 5.4 | 12.5 | 6.7 | 6.7 |
| Invertebrate eggs | | 5.2 | – | 1.2 | – | 1.9 | 5.9 | 23.1 | – | 8.1 | – | 6.7 | 20.0 |
| Filamentous algae | | 1.5 | – | – | – | 10.9 | 10.1 | 5.1 | – | – | – | 45.0 | 46.7 |
| Vegetable matter | | 11.8 | 13.9 | 4.6 | 3.4 | 2.0 | 5.0 | 43.6 | 16.7 | 24.3 | 25.0 | 13.3 | 26.7 |
| 'Floc'(fine detritus) | | 6.1 | – | 12.0 | 1.7 | 8.9 | 14.3 | 32.1 | – | 29.7 | 12.5 | 55.0 | 46.7 |
| Others (unident. terrestrial) | | 1.0 | 2.8 | 1.2 | 10.2 | 0.6 | 1.7 | 3.8 | 16.7 | 8.1 | 25.0 | 3.3 | 6.7 |




ASE= Asellus, AMP= Amphipods (Gammarus & Crangonyx)
S.CRU.= Small crustaceans (Cladocera, Copepoda & Ostracoda)
MOLL= Molluscs, CHIR= Chironomid larvae & pupae
INS= Insects, ALA= Filamentous algae, V.MAT.= Vegetable material
FLO= Fine detritus, OTHE= Others food items

**Figure 5.1** Comparison of the percentage relative abundances of individual food items in the stomachs of 3-spined sticklebacks at the three sites (A, B and E) in the River Wandle.

site B and E chironomids were predominant food with 35.2% and 29%, respectively. At B molluscs (28%), and small crustaceans (15.7%) were predominant, whereas at E a wide range of foods were found, including *Asellus*, amphipods, small crustaceans, molluscs, algae and 'floc' which each had average relative abundances of about 10%.

(B) Frequency of occurrence

Table 5.1 shows the frequency of occurrence of foods in stomachs at each site. The occurrence of copepoda appeared equally at each site, with a frequency of about 50%. The occurrences of chironomid larvae were very high at B and E, with a frequency of 71% and 68% respectively, but only 29% at site A. Relatively, the occurrence of vegetable matter at A (38%) was higher than B (17%) and E (18%) and cladocera at A (67%) was higher than at B (3%) and E (17%). However, the occurrence of 'floc' fine detritus at B (27%) was lower than at A (44%) and E 55%).

In addition to these differences in the frequency of occurrence of items of food between sites, certain organisms appeared to be absent some of the sites; for example there were absences of ostracods at B and E, operculate snails at A and E, and filamentous algae at B.

Considering the above results in summary, at A small crustaceans were the most frequent and abundant components of the diet. Chironomid larvae and pupae, and vegetable matter made up most of the remainder of the diet. At B chironomid larvae and pupae were the most frequent and abundant items of the diet, followed by molluscs and small crustaceans. Site E was similar to site B in that chironomid larvae and pupae were the most important, but large crustaceans, small crustaceans, molluscs, vegetable matter and 'floc' were also significant components.

### 5.3.3 Seasonal Variations in the Diet Within Sites

(A) Percentage relative abundance

Seasonal variation in the percentage relative abundance is detailed in Table 5.2. Seasonal shifts in relative abundances of food items were found and these are highlighted for each site in Figure 5.2.

At site A small crustaceans were one of the most abundant food items in the summer, autumn, and winter survey periods. In January 1990, they made up over 75 % of the diet. However in spring, filamentous algae took over as the main food item. Larger crustaceans were significant in July and chironomids in July and October.

There are no data for site B in spring because very few fish were available for examination, but in July the diet consisted of chironomids and small crustaceans, which shifted to molluscs and small crustaceans in October, and detritus and small crustaceans in January.

At site E the diet was more of a mixture and all major food items were present, except in January when filamentous algae were absent. The larger crustaceans, (*Asellus* and amphipods) and chironomids together, were always significant in the diet but their proportion varied from one season to another.

(B) Frequency of occurrence

Seasonal variation in the frequency of occurrence is detailed in Table 5.3. At the three sites, in general the occurrence results closely matched the trend in relative abundance but there were exceptions. Cladocera were only present in fish stomachs in July at site B, and at site A they were present with very high occurrence in January and April, but the amount of Cladocera taken in by the fish was small. Moreover, at site A, the occurrence of vegetable matter and 'floc' in stomachs of fish were very high (70.6%) but the amount taken in by the fish were quite small.

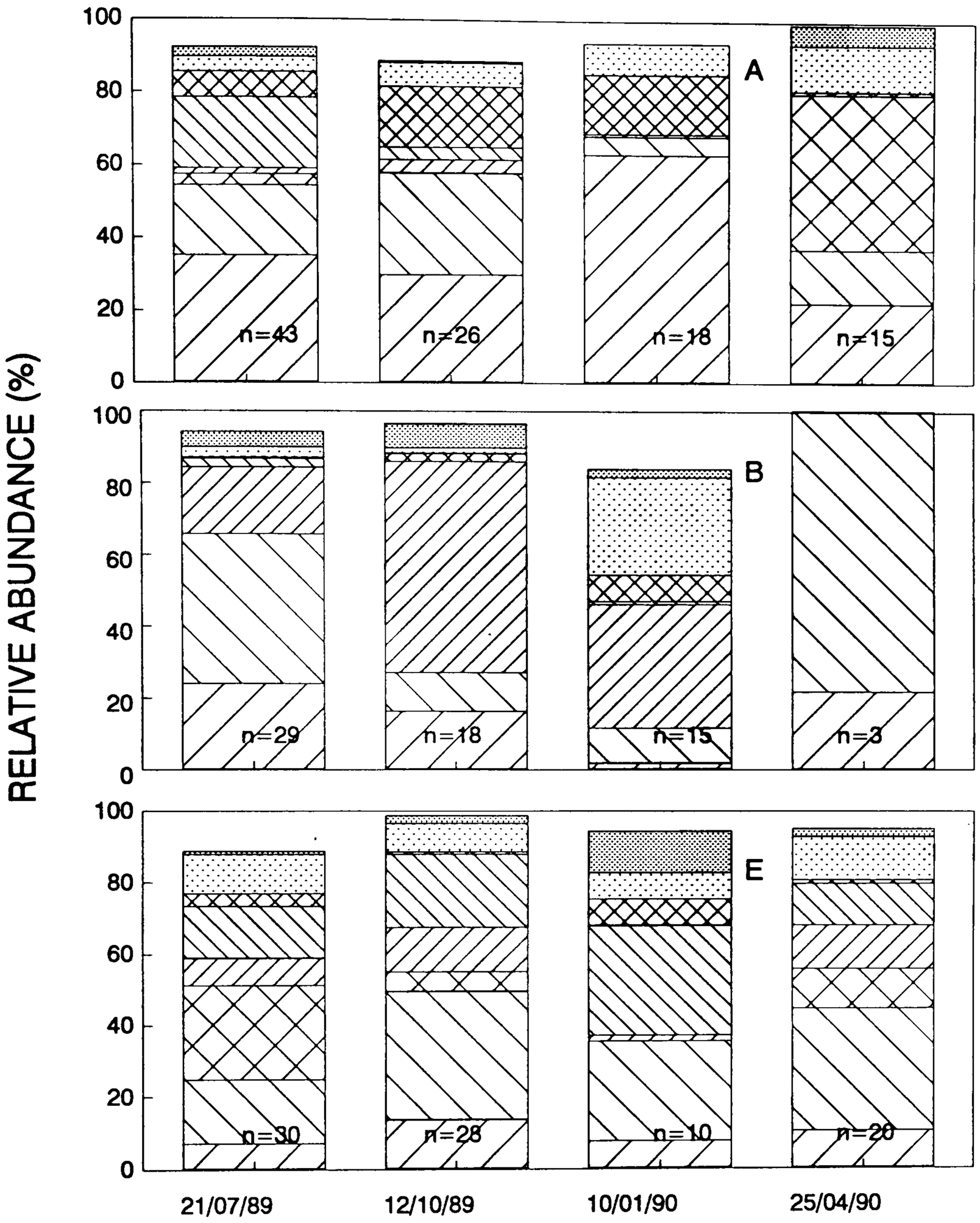



**Figure 5.2** Seasonal changes in the percentage relative abundances of major food groups in the stomachs of 3-spined sticklebacks at the three site (A, B and E) in the River Wandle.

### 5.3.4 Comparison of the Diet in Different Size Classes at Each Site

(A) Percentage relative abundance

The percentage relative abundances of food items in fish of different total lengths at each site are detailed in Table 5.3. The principal changes in the relative abundance of food ingested with size groups are illustrated in Figure 5.3.

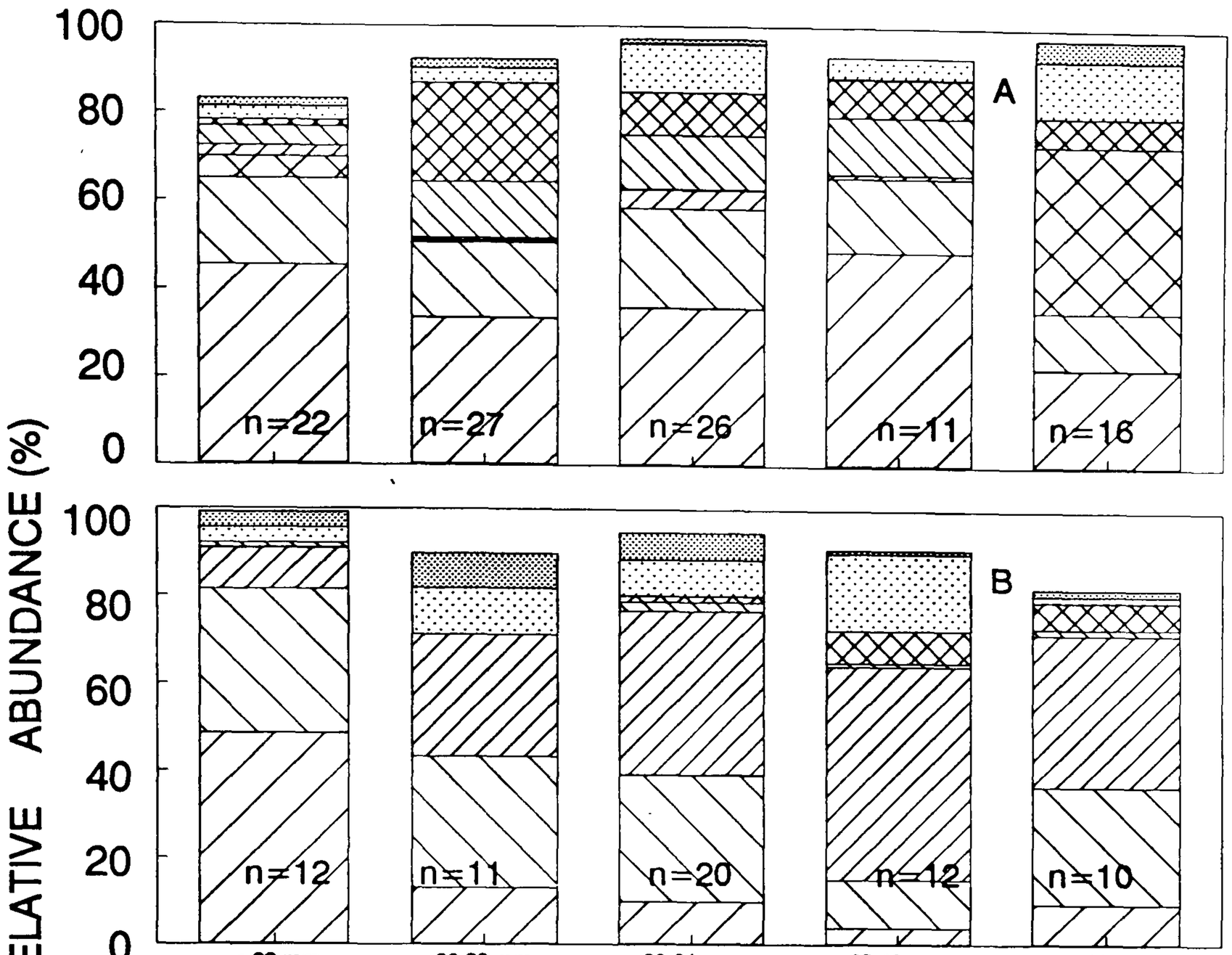
At the pond site A, it is interesting that small crustaceans and chironomids were important in all size groups, although algae in spring were predominant in the larger fish. This may reflect the co-occurrence of spring and summer algae growth with sticklebacks achieving their maximum sizes, and possibly the nest-building habit of adults when algae may become ingested.

At site B as the size of fish increases the main pattern to emerge, is of a decrease in the proportion of small crustaceans with a corresponding rise in abundance of molluscs.

Site E is distinguished by the fact that the full range of major food items (including the larger invertebrate, - molluscs, chironomids, amphipods and *Asellus*) are consumed by all size groups of fish with a trend that as the fish size increases there is a decreasing consumption of small crustaceans and chironomid larvae and pupae, relative to the other larger food items.

(B) Frequency of occurrence

The frequency of occurrence in fish of different total lengths at each site are detailed in Table 5.4. At site E the result of occurrence reveals the same as that of relative abundance. However, at A and B there are some exceptions. At A, Cladocera and floc were found with as high a frequency as that of filamentous algae in the fish group larger than 41 mm fish, but the amount of taken in was much less. At B the occurrence of small crustaceans and chironomid larvae dropped sharply in the groups larger than 23 mm, because of an increase frequency of molluscs in the diet.




S.CRU.= Small crustaceans (Cladocera, Copepoda & Ostracoda)
L.CRU.= Larger crustaceans (Asellus, Gammarus & Crangonyx)
CHIR= Chironomid larvae & pupae, ALA= Filamentous algae
MOLL= Molluscs, V.MAT.= Vegetable material, INS= Insects

**Figure 5.3** Comparison of the percentage relative abundance of major food items in the stomachs of 3-spined sticklebacks according to different size classes and sites (A, B and E).

### 5.3.5 Comparison of the Diet Between Males and Females

At each site the sex of most of the fish larger than 29 mm could be identified. The diets of males and females are shown in Table 5.5 and the percentage relative abundances of major food groups are shown in Figure 5.4, neither disclose any significant difference between males and females at each site.

### 5.3.6 Comparison of the Diet Between Non-parasitised and Parasitised Fish.

Plerocercoid larvae of *Schistocephalus* in the abdominal cavities of sticklebacks are very large so that parasitised fish were easily recognised.

All fish in those size groups which contained parasites were used in the analysis. The diets of non-parasitised and parasitised fish are detailed in Table 5.6 and the percentage relative abundances of major food groups are show in Figure 5.5.

There was very little differences in the average stomach fullness between the groups of fish. At each site the proportion of parasitised fish was 20% or less, and because of the small number of parasitised fish it was difficult to make comparisons. However, the data indicated that parasitised fish have a less varied diet and this is seen particularly at site A (Figure 5.5) where the diet consisted only of small crustaceans, vegetable matter and floc. So that it is not surprising that an overall greater range of food items was identified in the non-parasitised fish (Table 5.6).

## 5.4 DISCUSSION

The analysis of 255 stomach contents was sufficient to give a clear picture of the diet of the sticklebacks at a 'still-water' an two running water sites in the Wandle. The range of prey items found in this study is in broad agreement with published information reviewed in the introduction on the feeding habits of stickleback.

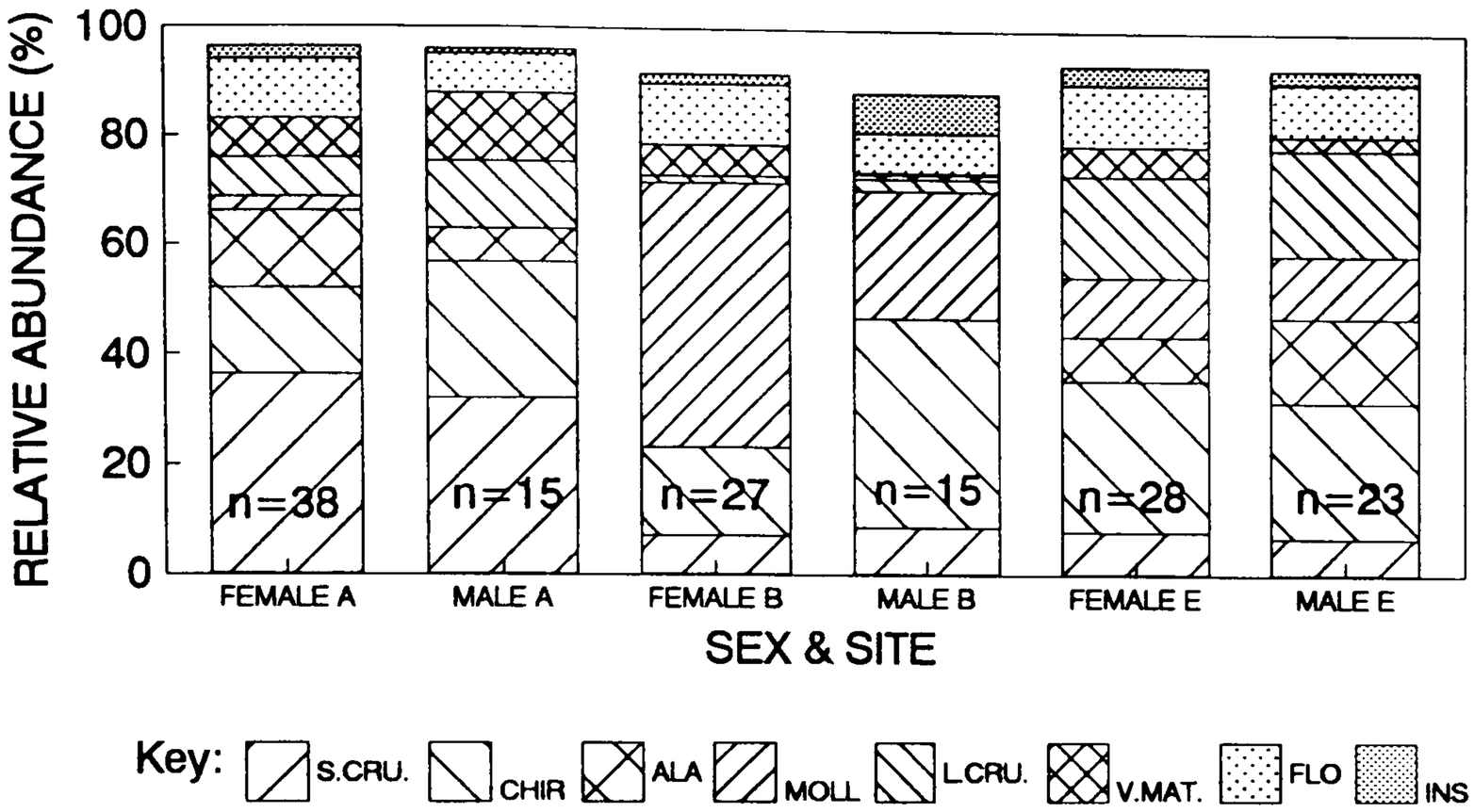



**Figure 5.4** Comparison of the percentage relative abundances of major food items in the stomachs of female and male sticklebacks at the three sites (A, B and E) in the River Wandle.

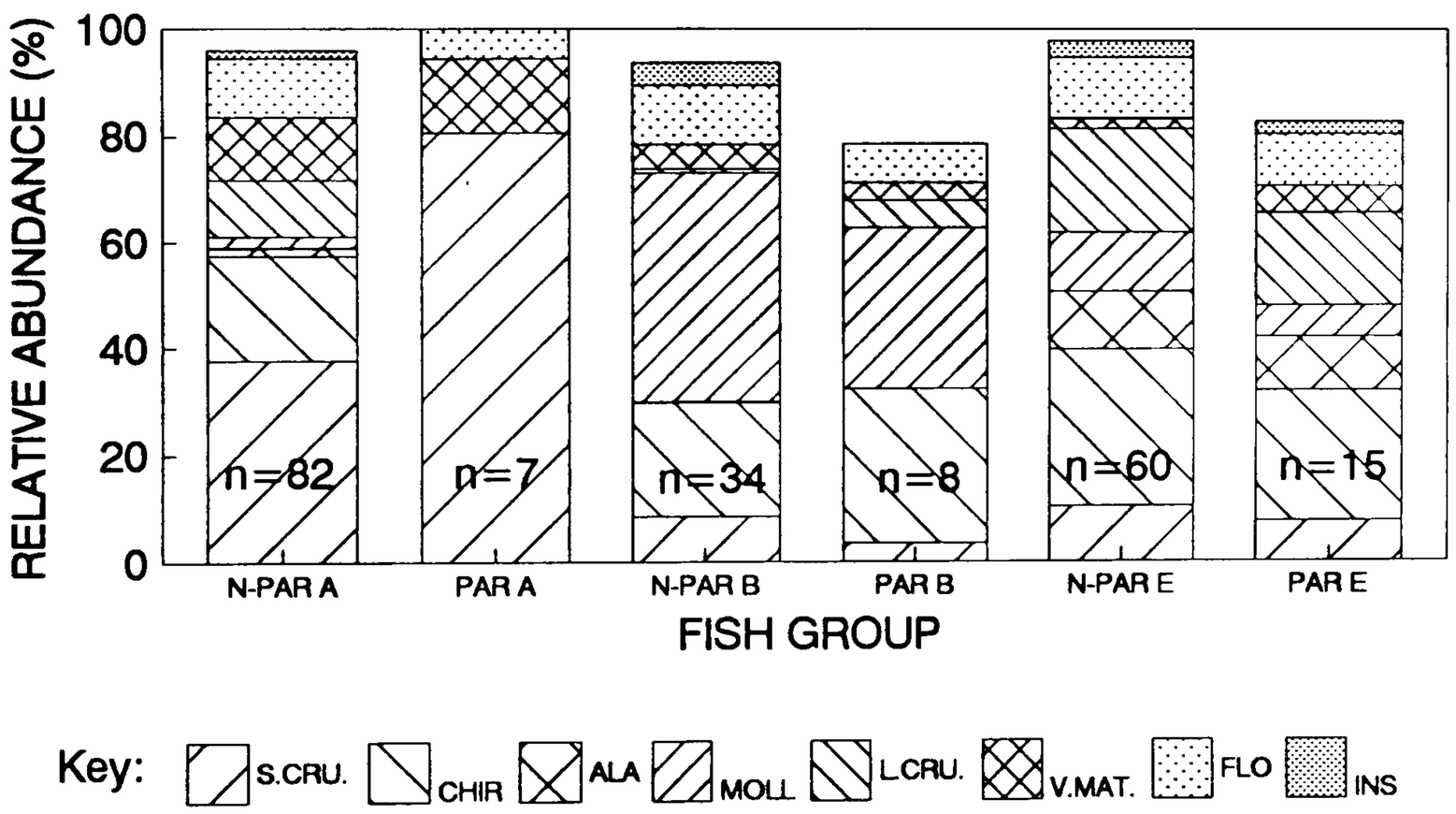



**Figure 5.5** Comparison of the percentage relative abundances of major food items in the stomachs of non-parasitised (N-PAR) and parasitised (PAR) sticklebacks at the three sites (A, B and E) in the River Wandle.

S.CRU.= Small crustaceans (Cladocera, Copepoda & Ostracoda)

L.CRU.= Larger crustaceans (Asellus, Gammarus and Crangonyx)

CHIR= Chironomid larvae & pupae, ALA= Filamentous algae

MOLL= Molluscs, V.MAT.= Vegetable material, INS= Insects

However, differences in the diet of the populations inhabiting the different environments of the three sampling sites are apparent. At site A, an unpolluted pond connected to the origin of the Croydon Arm of the Wandle, planktonic crustaceans, copepods and cladocerans, were the principal food items with chironomid larvae and algae present to a lesser extent. This compares with diets of the stickleback populations from Lake Windermere (Hynes 1950), from Llyn Frongoch reservoir (Allen and Wootton 1984) in Britain and from the Great Central Lake (Manzer 1976) in Canada (Table 5.8).

At sites B and E, two urban river sites, chironomid larvae and pupae were the most important food items. Similarly, in other British streams, e.g. Bere stream, Dorset (Mann and Orr 1969), River Endrick (Maitland 1965), River Kelvin, River Luggie and Aur Burn (Ukegbu and Hungtingford 1988), River Birket (Hynes 1950) and River Cam (Hartley 1940 in Hynes 1950), sticklebacks consumed significantly more chironomid larvae and pupae, and less planktonic crustaceans than populations in lakes and reservoirs (Table 5.8).

At site E, apart from chironomid larvae, the rest of food components were fairly equal in the diet, but the consumption of *Asellus* and *Gammarus* compares with other urban stream populations (Ukegbu and Hungtingford 1988). At site B, a surprisingly high percentage (27.9 P%) of molluscs, mainly snails, were found in the diet. Elsewhere, molluscs in the diet of sticklebacks has only been reported in winter when other food were in short supply (Mann and Orr 1969).

In the Wandle molluscs always occurred amongst in the diet, even amongst in the diet of small fish. This undoubtedly reflects the limited diversity of invertebrates available here but amongst which snails were relatively abundant. In general small sticklebacks ate small invertebrates but these included juveniles of invertebrates such as snails, amphipods and *Asellus* which would also be available to the larger adult fish sizes. As the fish grow larger the potential food items available increases.

Finally it is notable that filamentous algae were common in the diet at site A and E during spring, summer and autumn, and this has been only rarely recorded in the literature. It is generally accepted that stickleback are essentially carnivorous organism but it would appear

**Table 5.8** Summary the percentage of diet composition in stomachs of 3-spined sticklebacks in various literature.

| POPULATION / FOOD ITEMS | River Wandle A Pond | River Wandle B Running W. | River Wandle E | Llyn Frongoch Reservoir | Windermere Lake | Birket Stream | River Endrick | Bere Stream | Two Cam–bridgesh–ire Rivers | Cam River | Three Urban Streams | Great Centre Lake | Easdale Quarry Argyll | Danish Brachish Water |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish Form | | Leiurus | | ? | Leiurus* | Leiurus | ? | ? | Leiurus | | ? | Trachurus | Trachurus | Trachurus |
| Sample size | 102 | 65 | 88 | 240 | 120 | 1581 | 80 | 181 | 129 | 227 | >700 | 1088 | 55 | 736 |
| %EMP | 5.76 | 0.00 | 0.83 | ? | 6.7 | 5.3 | ? | ? | ? | ? | 9 | 7.4 | 10.9 | ? |
| %PAR | 7.09 | 16.44 | 14.28 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Mean fullness | 3.51 | 3.63 | 3.89 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Range of size | 15–54 | 14–64 | 17–57 | | 21–53 | 8–73 | ? | ? | ? | ? | ? | 15–86 | 38–98 | 2–110 |
| Method | | Point | | Point | Point | | Bulk | Numerical | Occurence | | Occurence | Bulk | Piont | ? |
| Crustacea | | | | | | | 8 | | | 24 | | | | |
| Aselius sp. | 1.3 | 0.4 | 9.8 | – | 3 | 11 | + | T | – | – | 30b | T | 73 | 41b |
| Amphipoda | 4.3 | 0.4 | 9.6 | – | – | – | + | T | – | – | + | T | – | + |
| Cladocera | 13.3 | 0.4 | 1.9 | 7.9 | 32 | 11 | + | 10 | 5d | + | | 45 | – | 10 |
| Copepoda | 19.8 | 15.3 | 7.5 | 36.3 | 40 | 19 | + | – | 23 | + | 22c | 31 | 5 | 27 |
| Ostracoda | 4.3 | – | – | 7.1 | 7 | 4 | + | 10 | + | + | + | T | – | 1 |
| Mollusca | 1.6 | 27.9 | 8.5 | 0.7 | – | 6 | 2 | T | T | 2 | 4 | T | – | 1 |
| Insects | | | | | | | | | | 51 | | | | |
| Chironomid larvae | 9.6 | 29.4 | 21.8 | 13.2 | 8 | 17 | 42a | 40a | 48a | + | 22a | 2 | – | – |
| Chironomid pupae | 7.2 | 5.8 | 7.2 | 4.1 | – | 4 | + | + | + | + | + | 6 | 3 | – |
| Insect unidentified | 2.2 | 3.3 | 4.2 | 4.3 | 3 | 5 | 20 | 20 | 2 | + | – | 4 | 4 | 4 |
| Ephemeroptera nymphys | ? | ? | ? | 13.9 | 1 | T | 23 | 20 | 10 | + | 4 | – | – | – |
| Polyzoan Statoblasts | 2.0 | 0.2 | 0.1 | – | – | T | – | – | – | – | – | – | – | – |
| Hydracarina | – | 1.8 | 0.6 | 0.3 | – | T | 1 | – | – | 1 | – | T | – | – |
| Nematoda | 0.1 | 1.3 | 2.7 | – | – | T | – | – | – | – | – | – | – | – |
| Annelida | – | – | – | 0.4 | 6 | 10 | 3 | – | – | 1 | – | – | 1 | 1 |
| Invertebrate eggs | 3.7 | 1.9 | 2.1 | – | T | T | – | – | – | – | 2 | 2e | – | – |
| Fish & stickleback's eggs | – | – | – | 0.8 | – | 1 | – | – | – | – | T | + | 2 | 8 |
| Filamentous algae | 11.5 | – | 10.7 | 3.5 | – | T | T | – | – | 14f | – | – | 4 | 1 |
| Other algae | – | – | – | – | – | 4 | – | – | T | 8 | – | – | – | – |
| Vegetable matter | 10.3 | 2.6 | 3.2 | 1.5 | T | 2 | – | – | T | + | 5 | – | 2 | T |
| Flock (fine bottom detritus) | 8.0 | 7.9 | 9.6 | 5.4 | – | T | – | – | – | – | – | – | 1 | – |
| Others (unidentified terrestrial) | 1.0 | 1.4 | 0.6 | 0.8 | T | 5 | T | T | – | – | – | 5 | 6 | 5 |
| REFERENCE Auther & Year | This study | | | Allen & Wootton, 1984 | Hynes, 1950 | Malt–land, 1965 | Mann & Orr, 1969 | Hartley, 1940 in | Ukegbu & Hungting | Manzer, 1976 | Hynes, 1950 | Blegvad, 1917 in |
| Region | London, U.K. | | | Mid–Wales | Northwest England | Scotland | Southern England | Hynes, 1950 England | ford,1988 Scotland | Vancouver Canada | Scotland | Hynes, 1950 Denmark |

* indicates contained semiarmrous form stickleback.
Blank indicates no record.
– indicates no found.
+ indicates recorded but merged in higher taxa.

a indicates chironomid larvae and pupae.
b indicates higher crustaceans, including Aselius and Gammarus.
c indicates small crastaceans, including copepods and ostracods.
d indicates cladocerans and ostracods
e indicates invertebrate and fish eggs.
f indicates filamentous alage and plant tissues.

that in habitats where food supply are restricted and variable that they will consumed a variety of food items, including vegetable matter. The diet of stickleback in the Wandle very much reflect the nature of the environment and the availability of food.

There are differences in the diet composition of sticklebacks at the 3 sites. Moreover, at the same site there are seasonal variations and diet shifts with age. Assuming that one route for the entry of significant quantities of metals into the sticklebacks could be via food, it is important to take into account the composition of the diet and the metal content of the different components of the diet. These factors will obviously have an important influence on the metal burden of the fish.

# CHAPTER SIX

# DISTRIBUTION OF COPPER AND CADMIUM IN THE ABIOTIC AND BIOTIC COMPONENTS IN THE RIVER WANDLE

## 6.1 INTRODUCTION

Investigations of the distribution of copper and cadmium in the various facets of the habitat of the 3-spined stickleback is essential for gaining an understanding of the possible route of entry of the metals to the fish.

There are two main routes by which the metal can enter fish. One is via the gills from the water, the other is via the gut from the ingestion of food. The study of both routes of entry has been examined in laboratory experiments with much of the work concentrating on direct uptake from the water (e.g. McKim & Benoit 1971, 1974, Kumada et al. 1973, Eaton 1974, Benoit et al. 1976, Smith et al. 1976, McCarty et al. 1978, Sangalang & Freeman 1979, Edgren & Notter 1980, Woodworth & Pascoe 1983, Thomas et al. 1983, Buckley et al. 1985, Haeslop & Schirmer 1985, Karlsson-Norrgren & Runn 1985, Pascoe et al. 1986, Radhakrishnaiah 1988, Buckley & Yoshida 1989, Ghosh & Chakrabarti 1990, Harrison & Klaverkamp 1990, Sankaraperumal et al. 1990, Vijayram et al. 1990, Dave & Xiu 1991). However, the relative importance of the routes of transfer of metals has not been demonstrated in the natural environment, where the situation is more complex and dissolved metal concentrations are usually close to or below the detection limits of routine method of analysis.

The assessment of a chemical's potential hazard to aquatic life and the establishment of water quality criteria rely primarily on standard acute and chronic toxicity tests (Alabaster & Llyod 1982). All of these tests are essentially based on exposure directly to contaminated water and they take little or no account of exposure via food intake. In natural systems many substances including many metals and lipophilic compounds, because of their physicochemical properties, partition mainly to the solid abiotic and biotic phases. Consequently food can be the main route of exposure.

The preliminary investigation reported in Chapter 3 showed that relatively high concentrations of Cu and Cd were to be found in the sediments and floc particularly at the contaminated sites. This chapter examines the distribution of Cu and Cd in the major biotic and abiotic components, particularly with respect to species difference, changes between separate surveys and intake of metals by the major food items of sticklebacks identified in the previous chapter. There are relatively few examples in the literature concerned with metal distribution in the natural abiotic and biotic components of freshwater ecosystems (Mathis & Cummings 1973, Mathis & Kevern 1975, Enk & Mathis 1977, Lewis 1980, Roch et al. 1985, Cabrera et al. 1987, Ajmal et al. 1988).

One of the earliest researches of this type was done by Mathis & Cummings (1973). They investigated the distribution of 8 elements, Cd, Cu, Co, Cr, Pb, Li, Ni and Zn, in an Illinois River receiving domestic and industrial wastes. It was shown that in most cases metal concentrations increased along the sequence; water, carnivorous fishes, omnivorous fishes, clams, worms, and then bottom sediment. There were some exceptions, for example Li was higher in water than in fishes; Cu in worms was similar to that in sediment; Zn was higher in clams than in worms; and Pb, Co and Cd levels in carnivorous and omnivorous fish were similar. In the eutrophic Lake Wintergreen, south-west Michigan (U.S.A.), Cd and Pb were found to be distributed, in ascending order, as follows: water, fish muscle, aquatic macrophytes, zooplankton, goose faeces and bottom sediment. In the case of Hg the highest concentrations were found in fish muscle tissue and its concentration in aquatic invertebrates was higher than in sediments. Some studies that have included invertebrates have found these organisms to have the highest concentrations of metals, e.g. Cd and Pb in Jubilee Creek, Illinois (Enk & Mathis 1977) and Cu, Zn, and Mn in Gila River, Arisona (Lewis 1980). Aquatic plants may also accumulate more metal than sediment, e.g. Cu distribution in Gila River and Kali Nadi, India (Lewis 1980, Ajmal et al. 1988), and Fe distribution in Guadiamar River, S.W. Spain (Cabrera et al 1987).

None of these researches set out to provide direct information about linkages between fish and the surrounding biota however. Nor do

these examples consider the relative importance of the routes of exposure to metals by fish and very few studies focus on this aspect. Hardisty et al (1974) demonstrated that fish accumulated significant amounts of Cd which was related to the percentage of crustaceans in their diets. Rainbow trout fed on metal enriched isopods (i.e. *Asellus aquaticus*) and snails (i.e. *Lymnaea truncatula*) in the River Augraben and Leiferer Graben, Italy, and it was suggested that this was the principal source of metals accumulated in the fish, whereas the absorption of metal from water through the gills played a secondary role (Dallinger & Kautzky 1985).

## 6.2 MATERIALS AND METHODS

Samples of aquatic animals, plants, water, sediments and floc were collected during the same seasonal surveys described previously for sticklebacks. All materials used were brought back from the field according to the sampling scheme given in Chapter 3.

Water samples were stored at $4^{o}C$ prior to analysis. Samples were filtered using membrane filter papers (Whatman, 0.45 um) and the filtered deposits were first dried at $70^{o}C$ for 2 days, weighed, and then digested with 5 ml of concentrated $HNO_3$ ("AristaR" grade, BDH). The determinations of copper and cadmium concentrations in the filtrated and unfiltered water samples were performed by directly injecting the samples into a graphite furnace of the AAS.

Samples of floc were centrifuged in 50 ml metal-free polythylene centrifuge tubes until all materials had settled. The pellets were transferred to petri dishes and dried at $105^{o}C$ for 2 days. The samples were then ground and passed through a stainless steel sieve with a mesh size of 1 mm and later prepared for as sediment samples for determination of copper and cadmium.

Sediment samples were dried, ground, and sieved in the same manner as the floc samples. The fraction that was smaller than 63 um was used for analysis. Approximately 1 g of each sample was weighed out into a narrow-necked boiling tube and digested according to the procedure given in Chapter 2.

Algae and aquatic plant samples were washed thoroughly several times with double distilled water (D.D.W.), immediately after they were brought back from field, and stored at 4$^{o}$C before further treatment in the next day. They were then blotted dry with filter paper and cut into small pieces, and separated into approximately 6 portions. Each portion was put into a 100 ml conical flask and dried at 105$^{o}$C to constant weight. Nitric acid was added to each sample and the samples were digested also according to the procedures described in Chapter 2.

Aquatic invertebrates were also rinsed with D.D.W. several times before they were frozen at -20$^{o}$C prior to analysis. Snails were removed from their shells and only the soft tissues were used for analysis. For other invertebrates the entire organism was used for the analysis. When possible small organisms were pooled together and divided into six replicates. Method for handling the small quantities of samples was the same as in Chapter 4. Also, the digestion procedures and metal analysis were followed as in Chapter 2.

## 6.3 RESULTS

### 6.3.1 Copper and Cadmium Concentrations in Water, Organic Detritus (Floc) and Sediment.

Comparison of the concentrations of Cu and Cd in the samples of water (total and dissolved), floc and sediments emphasises the marked partitioning of the metals to the solid phases in the system (Tables 6.1 and 6.2). Total concentrations (i.e. including dissolved and suspended particulate forms) of Cu and Cd in the water ranged between 1.7 to 20.8 ug/l and 0.04 to 0.09 ug/l, respectively, which from the point view of toxicity and water quality standards are considered to be quite low. However concentration are much higher in the sediments and floc samples with levels at sites B and E showing marked elevation.

In general the same overall trend of the abiotic factors are shown between the three sites (Figure 6.1). Elevated concentrations of Cd and Cu occurred at the two contaminated sites of B and E relative to the reference site A. Although Cu and Cd concentrations in floc samples at site A are very variable, with a six to eight fold difference

**Table 6.1** Concentrations of Cu and Cd in samples of water taken during each season (1989-1990) at the three sampling sites along the River Wandle. (The original data are listed in Appendix D.1).

| | | | COPPER | | CADMIUM | |
|---|---|---|---|---|---|---|
| | | | Concentration (ng/ml water) | | | |
| Site | Date | No | Mean | S.D. | Mean | S.D. |
| **Total Metal** | | | | | | |
| A | 21/07/89 | 6 | 6.3 | 1.7 | | |
| A | 12/10/89 | 6 | 4.1 | 0.2 | | |
| A | 10/01/90 | 6 | 1.7 | 0.1 | | |
| A | 25/04/90 | 6 | 2.5 | 0.9 | 0.04 | 0.02 |
| A | Annual | 24 | 3.6 | 0.9 | | |
| B | 21/07/89 | 6 | 17.5 | 1.4 | | |
| B | 12/10/89 | 6 | 10.0 | 0.2 | | |
| B | 10/01/90 | 6 | 8.7 | 3.0 | | |
| B | 25/04/90 | 6 | 20.8 | 6.5 | 0.09 | 0.02 |
| B | Annual | 24 | 14.2 | 3.4 | | |
| E | 21/07/89 | 6 | 7.6 | 0.8 | | |
| E | 12/10/89 | 6 | 7.4 | 0.5 | | |
| E | 10/01/90 | 6 | 6.0 | 0.4 | | |
| E | 25/04/90 | 6 | 6.4 | 1.1 | 0.06 | 0.03 |
| E | Annual | 24 | 6.9 | 0.7 | | |
| **Dissolved Metal** | | | | | | |
| A | 21/07/89 | 6 | 2.9 | 0.9 | | |
| A | 12/10/89 | 6 | 4.1 | 0.3 | | |
| A | 10/01/90 | 6 | 1.7 | 0.1 | | |
| A | 25/04/90 | 6 | 2.1 | 0.7 | 0.04 | 0.01 |
| A | Annual | 24 | 2.7 | 0.5 | | |
| B | 21/07/89 | 6 | 14.4 | 0.3 | | |
| B | 12/10/89 | 6 | 7.8 | 0.2 | | |
| B | 10/01/90 | 6 | 5.7 | 0.3 | | |
| B | 25/04/90 | 6 | 13.5 | 6.2 | 0.09 | 0.02 |
| B | Annual | 24 | 10.4 | 2.9 | | |
| E | 21/07/89 | 6 | 5.9 | 0.4 | | |
| E | 12/10/89 | 6 | 5.7 | 0.3 | | |
| E | 10/01/90 | 6 | 3.8 | 0.3 | | |
| E | 25/04/90 | 6 | 5.4 | 0.3 | 0.04 | 0.00 |
| E | Annual | 24 | 5.2 | 0.3 | | |

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

**Table 6.2** Concentrations of Cu and Cd in samples of organic detritus (floc) and sediment taken during each season (1989-1990) at the three sampling sites along the River Wandle. (The original data are listed in Appendix D.2 and D.3).

| | | | COPPER | | CADMIUM | |
|---|---|---|---|---|---|---|
| | | | Concentration (ug/g dry wt.) | | | |
| Site | Date | No | Mean | S.D. | Mean | S.D. |
| Floc | | | | | | |
| A | 21/07/89 | 6 | 431.92 | 7.25 | 33.097 | 0.387 |
| A | 12/10/89 | 6 | 325.42 | 6.07 | 21.017 | 0.537 |
| A | 10/01/90 | 6 | 71.49 | 1.88 | 2.687 | 0.180 |
| A | 25/04/90 | 6 | 38.88 | 0.96 | 1.251 | 0.268 |
| A | Annual | 24 | 216.93 | 4.52 | 14.513 | 0.343 |
| B | 21/07/89 | 6 | 684.61 | 18.96 | 15.818 | 0.476 |
| B | 12/10/89 | 6 | 758.03 | 9.14 | 16.703 | 0.633 |
| B | 10/01/90 | 6 | 414.87 | 4.56 | 13.989 | 0.318 |
| B | 25/04/90 | 6 | 307.44 | 2.73 | 9.182 | 0.513 |
| B | Annual | 24 | 541.24 | 10.12 | 13.923 | 0.464 |
| E | 21/07/89 | 6 | 366.88 | 5.31 | 13.613 | 0.436 |
| E | 12/10/89 | 6 | 516.98 | 2.97 | 14.920 | 0.300 |
| E | 10/01/90 | 6 | 392.28 | 4.63 | 15.668 | 0.370 |
| E | 25/04/90 | 6 | 371.03 | 5.53 | 14.070 | 0.537 |
| E | Annual | 24 | 411.79 | 4.40 | 14.568 | 0.391 |
| Sediment | | | | | | |
| A | 21/07/89 | 6 | 50.31 | 1.36 | 2.458 | 0.360 |
| A | 12/10/89 | 6 | 53.08 | 0.82 | 0.823 | 0.039 |
| A | 10/01/90 | 6 | 57.81 | 1.12 | 2.536 | 0.149 |
| A | 25/04/90 | 6 | 58.52 | 0.97 | 1.635 | 0.013 |
| A | Annual | 24 | 54.93 | 1.01 | 1.863 | 0.183 |
| B | 21/07/89 | 6 | 276.68 | 6.75 | 6.133 | 0.416 |
| B | 12/10/89 | 6 | 302.17 | 8.35 | 8.553 | 0.384 |
| B | 10/01/90 | 6 | 281.71 | 1.68 | 9.905 | 0.534 |
| B | 25/04/90 | 6 | 440.73 | 6.96 | 14.436 | 0.362 |
| B | Annual | 24 | 325.32 | 6.02 | 9.757 | 0.400 |
| E | 21/07/89 | 15 | 404.59 | 3.47 | 13.182 | 0.383 |
| E | 12/10/89 | 6 | 348.83 | 4.95 | 10.283 | 0.199 |
| E | 10/01/90 | 6 | 362.09 | 3.90 | 12.514 | 0.410 |
| E | 25/04/90 | 6 | 421.03 | 1.88 | 15.272 | 0.137 |
| E | Annual | 33 | 389.71 | 3.47 | 12.913 | 0.316 |

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

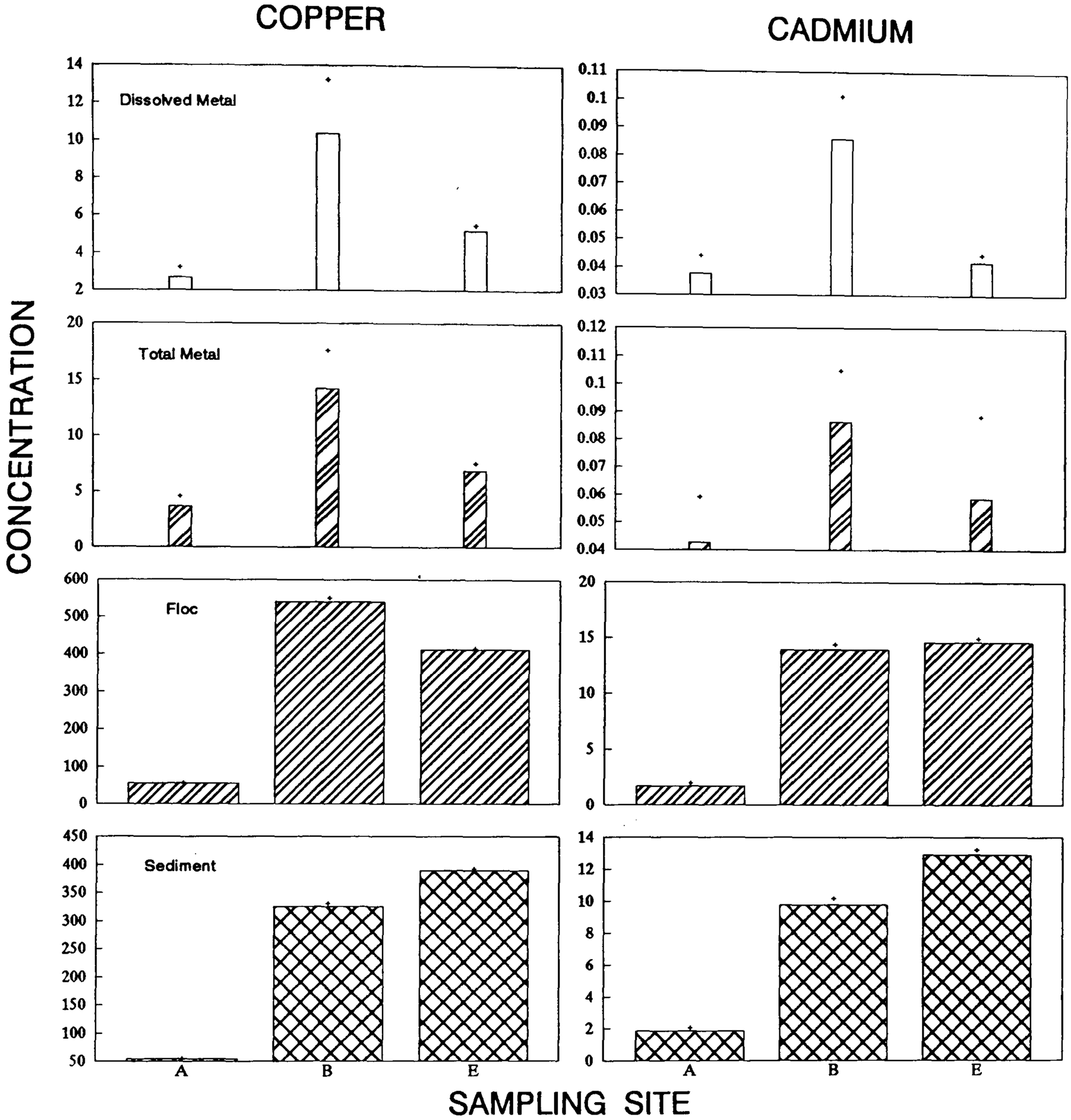



**Figure 6.1** Concentrations of Cu and Cd in samples of water, organic detritus (floc) and sediment at the three sites in the River Wandle. Histograms present the means and + indicates the standard deviation. The units for water samples are in ug/l and for floc and sediment samples are in ug/g dw.
(The original data are listed in Appendix D.1, D.2 and D.3).

between sampling periods for Cu and nearly 15 to 20 fold difference for Cd (Table 6.2). These results were rather surprising as from the fact that the first two sampling periods at site A gave the highest Cd concentrations of all other floc samples taken irrespective of site. Contamination from PVC buckets used in early sampling surveys for transporting the floc samples back to the laboratory may have been responsible. PVC is a well known source of cadmium. Therefore, the first two Cd results for floc were excluded in further presentation. Consequently, there was a tendency for metal levels in the abiotic components to be higher at site B than at site E, except for the sediment samples and Cd in the floc samples (Figure 6.1).

### 6.3.2 Copper and Cadmium Concentrations in the Aquatic Organisms.

Only three species were common to all three sites, and these were *Spirogyra* sp., *Lymnaea peregra*, and *Asellus aquaticus*. A number of other species were collected which were restricted to one or two of the sites (Tables 6.3 and 6.4).

Taking the three organisms common to all sites, the same overall trend is evident with elevated metal levels at sites B and E and significantly lower values at site A (Figure 6.2). There is some seasonal variation but an overall indication that the levels at site B are higher than that at site E. Most other organisms which are only present at sites B and E show the same trend (Tables 6.3 and 6.4). This may reflect a greater degree of bioavailability of the metals at B than at E because the total metal loading as shown by the sediments is very similar at these sites. The concentrations of Cu in *Lymnaea* and *Asellus* are particularly interesting. These organisms which have haemocyanin blood pigments have relatively high concentrations of Cu at the reference site but considerably higher concentrations (*Lymnaea* >150 ug/g and *Asellus* >300 ug/g) at the contaminated sites.

Site E is the only site with sufficient diversity of aquatic plants to allow any sort of comparison of Cu and Cd concentrations between different species. Table 6.5 shows the mean values of the data presented in Table 6.3 for the different species at site E. For both metals quite notable differences are found. There is a sevenfold

**Table 6.3** Concentrations of Cu and Cd in samples of algae and aquatic plants taken during each season (1989-1990) at the three sampling sites along the River Wandle. (The original data are listed in Appendix D.4).

| | | | COPPER | | CADMIUM | |
|---|---|---|---|---|---|---|
| | | | Concentration (ug/g dw) | | | |
| Site | Date | No | Mean | S.D. | Mean | S.D. |
| *Spirogyra sp.* | | | | | | |
| A | 21/07/89 | 6 | 4.46 | 0.70 | 0.357 | 0.026 |
| B | 12/10/89 | 1 | 29.09 | | | |
| E | 21/07/89 | 6 | 17.65 | 0.41 | 1.219 | 0.098 |
| E | 12/10/89 | 6 | 9.83 | 0.45 | 0.583 | 0.092 |
| E | Annual | 12 | 13.74 | 0.41 | 0.901 | 0.091 |
| *Cladophora sp.* | | | | | | |
| B | 21/07/89 | 10 | 26.81 | 1.93 | 3.071 | 0.545 |
| B | 12/10/89 | 6 | 47.47 | 2.32 | 4.147 | 0.493 |
| B | 10/01/90 | 6 | 146.40 | 2.60 | 4.114 | 0.188 |
| B | 25/04/90 | 6 | 26.67 | 0.73 | 1.674 | 0.030 |
| B | Annual | 28 | 56.83 | 1.89 | 3.226 | 0.388 |
| E | 21/07/89 | 6 | 46.51 | 1.06 | 2.279 | 0.335 |
| E | 12/10/89 | 6 | 48.12 | 1.78 | 9.576 | 0.256 |
| E | 25/04/90 | 6 | 47.30 | 2.50 | 1.555 | 0.050 |
| E | Annual | 18 | 47.31 | 1.76 | 4.470 | 0.230 |
| *Rhynchostegium riparioides* | | | | | | |
| B | 12/10/89 | 4 | 211.88 | 6.71 | 12.878 | 0.740 |
| E | 21/07/89 | 6 | 126.91 | 7.38 | 2.725 | 0.190 |
| E | 12/10/89 | 6 | 94.21 | 2.98 | 2.774 | 0.105 |
| E | 10/01/90 | 6 | 109.69 | 4.61 | 4.288 | 0.108 |
| E | 25/04/90 | 6 | 57.78 | 2.39 | 0.972 | 0.103 |
| E | Annual | 24 | 97.15 | 4.43 | 2.690 | 0.123 |
| *Callitriche sp.* | | | | | | |
| B | 21/07/89 | 6 | 57.02 | 2.41 | 4.560 | 0.196 |
| B | 12/10/89 | 4 | 79.71 | 2.56 | 7.487 | 0.225 |
| B | 10/01/90 | 6 | 98.92 | 3.25 | 10.921 | 0.282 |
| B | 25/04/90 | 6 | 60.40 | 1.56 | 6.632 | 0.295 |
| B | Annual | 22 | 73.49 | 2.33 | 7.392 | 0.237 |
| E | 12/10/89 | 6 | 96.82 | 3.60 | 5.810 | 0.195 |
| E | 10/01/90 | 6 | 114.14 | 3.25 | 9.014 | 0.182 |
| E | 25/04/90 | 6 | 37.63 | 1.48 | 1.932 | 0.150 |
| E | Annual | 18 | 82.87 | 2.75 | 5.585 | 0.166 |

(continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| *Elodea canadensis* | | | | | | |
| E | 21/07/89 | 6 | 61.58 | 2.43 | 1.593 | 0.172 |
| E | 12/10/89 | 5 | 100.09 | 2.27 | 5.651 | 0.199 |
| E | 10/01/90 | 6 | 104.71 | 1.63 | 7.359 | 0.228 |
| E | 25/04/90 | 6 | 56.98 | 2.69 | 7.418 | 0.190 |
| E | Annual | 23 | 80.00 | 2.13 | 5.499 | 0.184 |
| *Ranunculus fluitans* | | | | | | |
| E | 21/07/89 | 6 | 40.72 | 1.47 | 2.930 | 0.306 |
| E | 12/10/89 | 6 | 52.61 | 1.64 | 13.005 | 0.528 |
| E | 10/01/90 | 6 | 62.41 | 1.58 | 3.520 | 0.135 |
| E | 25/04/90 | 6 | 40.89 | 1.48 | 9.922 | 0.284 |
| E | Annual | 24 | 49.16 | 1.44 | 7.344 | 0.320 |
| *Potamogeton crispus* | | | | | | |
| B | 10/01/90 | 1 | 153.77 | | 4.633 | |
| E | 12/10/89 | 4 | 44.43 | 1.11 | 2.506 | 0.206 |
| E | 10/01/90 | 3 | 82.99 | 4.08 | 2.613 | 0.326 |
| E | 25/04/90 | 6 | 66.79 | 2.75 | 2.695 | 0.307 |
| E | Annual | 13 | 63.65 | 2.49 | 2.618 | 0.260 |
| *Apium nodiflorum (Water celery, leaves)* | | | | | | |
| E | 12/10/89 | 5 | 32.19 | 3.07 | 2.974 | 0.235 |
| E | 10/01/90 | 6 | 67.90 | 2.86 | 5.520 | 0.200 |
| E | 25/04/90 | 6 | 29.41 | 1.23 | 2.327 | 0.104 |
| E | Annual | 17 | 43.81 | 2.32 | 3.644 | 0.172 |
| *Apium nodiflorum (Water celery, roots)* | | | | | | |
| E | 12/10/89 | 5 | 56.71 | 1.33 | 7.281 | 0.748 |
| E | 10/01/90 | 6 | 74.19 | 2.54 | 10.851 | 0.860 |
| E | 25/04/90 | 6 | 94.44 | 3.15 | 16.166 | 0.637 |
| E | Annual | 17 | 76.20 | 2.36 | 11.677 | 0.706 |

(end)

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

**Table 6.4** Concentrations of Cu and Cd in samples of aquatic invertebrates taken during each season (1989-1990) at the three sampling sites along the River Wandle. (The original data are listed in Appendix D.5).

| Site | Date | No | COPPER (ug/g dw) Mean | S.D. | CADMIUM (ug/g dw) Mean | S.D. |
|---|---|---|---|---|---|---|
| *Haemopsis sanguisuga* | | | | | | |
| B | 21/07/89 | 5 | 108.61 | 16.77 | 1.288 | 0.540 |
| B | 12/10/89 | 3 | 64.81 | 18.84 | 1.283 | 0.365 |
| B | 10/01/90 | 1 | 59.43 | | 1.305 | |
| B | 25/04/90 | 8 | 66.41 | 18.22 | 1.340 | 0.315 |
| B | Annual | 17 | 78.13 | 16.12 | 1.313 | 0.365 |
| E | 25/04/90 | 1 | 42.30 | 14.33 | 1.653 | 1.104 |
| *Theromyzon tessulatum* | | | | | | |
| A | 25/04/90 | 1 | 4.40 | | 0.026 | |
| E | 21/07/89 | 3 | 47.13 | 3.26 | 3.902 | 0.823 |
| E | 12/10/89 | 5 | 37.64 | 8.22 | 3.241 | 0.447 |
| E | 10/01/90 | 2 | 36.43 | 6.88 | 4.775 | 0.504 |
| E | 25/04/90 | 1 | 41.63 | | 6.547 | |
| E | Annual | 11 | 40.37 | 5.82 | 4.000 | 0.491 |
| *Erpobdella octoculata* | | | | | | |
| E | 21/07/89 | 5 | 44.79 | 7.43 | 0.764 | 0.201 |
| E | 12/10/89 | 6 | 64.06 | 6.11 | 2.075 | 0.257 |
| E | 10/01/90 | 4 | 59.59 | 8.61 | 1.336 | 0.113 |
| E | 25/04/90 | 6 | 67.76 | 7.19 | 1.215 | 0.169 |
| E | Annual | 21 | 59.68 | 6.67 | 1.376 | 0.183 |
| *Lymnaea peregra* | | | | | | |
| A | 21/07/89 | 3 | 84.62 | 4.34 | 0.648 | 0.144 |
| A | 12/10/89 | 3 | 49.99 | 2.63 | 0.582 | 0.045 |
| A | 10/01/90 | 5 | 51.42 | 4.88 | 1.351 | 0.342 |
| A | 25/04/90 | 6 | 30.72 | 3.93 | 0.272 | 0.062 |
| A | Annual | 17 | 49.72 | 3.74 | 0.710 | 0.182 |
| B* | 21/07/89 | 5 | 344.71 | 17.18 | 2.068 | 0.414 |
| B* | 12/10/89 | 3 | 228.76 | 13.11 | 3.585 | 0.357 |
| B* | 10/01/90 | 10 | 221.27 | 17.35 | 3.113 | 0.268 |
| B* | 25/04/90 | 10 | 383.31 | 59.05 | 2.484 | 0.385 |
| B* | Annual | 28 | 301.99 | 36.32 | 2.752 | 0.329 |
| E | 21/07/89 | 9 | 150.54 | 26.57 | 1.352 | 0.335 |
| E | 12/10/89 | 10 | 624.51 | 28.84 | 1.847 | 0.227 |
| E | 10/01/90 | 10 | 188.72 | 22.40 | 1.925 | 0.233 |
| E | 25/04/90 | 10 | 221.66 | 23.13 | 1.384 | 0.229 |
| E | Annual | 39 | 300.10 | 24.31 | 1.634 | 0.247 |

(continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| *Lymnaea stagnalis* | | | | | | |
| E | 10/01/90 | 4 | 113.15 | 6.93 | 2.060 | 0.212 |
| E | 25/04/90 | 4 | 145.43 | 23.95 | 1.211 | 0.181 |
| E | Annual | 8 | 129.29 | 16.32 | 1.635 | 0.182 |
| *Physa fontinalis* | | | | | | |
| B | 21/07/89 | 1 | 2048.35 | | 6.182 | |
| B | 12/10/89 | 3 | 1515.94 | 55.73 | 3.025 | 0.402 |
| B | 10/01/90 | 1 | 1760.38 | | 3.238 | |
| B | 25/04/90 | 4 | 1106.13 | 50.97 | 5.528 | 0.573 |
| B | Annual | 9 | 1420.12 | 41.84 | 4.512 | 0.405 |
| E | 21/07/89 | 1 | 700.03 | | 3.269 | |
| E | 12/10/89 | 1 | 1369.11 | | 1.243 | |
| E | 10/01/90 | 2 | 422.78 | 23.31 | 4.025 | 0.862 |
| E | Annual | 4 | 728.68 | 13.46 | 3.141 | 0.498 |
| *Pisidium spp.* | | | | | | |
| A | 21/07/89 | 3 | 17.12 | 2.50 | 0.200 | 0.051 |
| A | 12/10/89 | 5 | 17.45 | 1.61 | 0.106 | 0.012 |
| A | 25/04/90 | 3 | 32.10 | 1.50 | 0.205 | 0.055 |
| A | Annual | 11 | 21.35 | 1.65 | 0.159 | 0.035 |
| B | 25/04/90 | 3 | 179.18 | 14.10 | 4.058 | 0.754 |
| *Gammarus pulex* | | | | | | |
| B | 25/04/90 | 3 | 88.97 | 6.02 | 1.680 | 0.188 |
| E | 21/07/89 | 6 | 87.01 | 3.18 | 1.496 | 0.285 |
| E | 12/10/89 | 6 | 80.84 | 2.43 | 0.670 | 0.026 |
| E | 10/01/90 | 11 | 81.67 | 3.22 | 1.208 | 0.211 |
| E | 25/04/90 | 10 | 66.61 | 5.36 | 0.991 | 0.088 |
| E | Annual | 33 | 77.93 | 3.72 | 1.097 | 0.170 |
| *Crangonyx pseudogracilis* | | | | | | |
| A | 21/07/89 | 1 | 94.29 | | 0.169 | |
| A | 25/04/90 | 4 | 22.92 | 5.09 | 0.301 | 0.040 |
| A | Annual | 5 | 37.20 | 4.40 | 0.274 | 0.035 |
| E | 21/07/89 | 3 | 98.51 | 5.96 | 1.775 | 0.133 |

(continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| *Asellus aquaticus* | | | | | | |
| A | 21/07/89 | 3 | 241.38 | 61.55 | 0.845 | 0.174 |
| A | 12/10/89 | 5 | 103.04 | 17.76 | 0.107 | 0.012 |
| A | 10/01/90 | 5 | 74.15 | 14.66 | 0.504 | 0.148 |
| A | 28/02/90 | 5 | 164.58 | 22.69 | 0.693 | 0.153 |
| A | 25/04/90 | 4 | 70.63 | 15.40 | 0.181 | 0.024 |
| A | Annual | 22 | 123.43 | 24.37 | 0.445 | 0.108 |
| B | 10/01/90 | 4 | 455.22 | 169.72 | 4.722 | 1.550 |
| B | 25/04/90 | 3 | 567.18 | 146.65 | 5.639 | 1.511 |
| B | Annual | 7 | 503.20 | 146.87 | 5.115 | 1.401 |
| E | 21/07/89 | 3 | 583.60 | 100.71 | 1.493 | 0.388 |
| E | 12/10/89 | 15 | 566.98 | 97.67 | 1.639 | 0.564 |
| E | 10/01/90 | 10 | 317.31 | 64.69 | 2.055 | 0.819 |
| E | 25/04/90 | 6 | 273.32 | 18.76 | 2.835 | 0.920 |
| E | Annual | 34 | 443.19 | 76.53 | 1.960 | 0.675 |
| *Chironomid larvae and pupae* | | | | | | |
| A | 21/07/89 | 6 | 30.44 | 2.15 | 0.465 | 0.093 |
| A | 12/10/89 | 6 | 18.92 | 2.30 | 0.662 | 0.116 |
| A | 10/01/90 | 6 | 16.11 | 1.30 | 0.441 | 0.114 |
| A | 25/04/90 | 6 | 13.59 | 0.99 | 0.152 | 0.029 |
| A | Annual | 24 | 19.77 | 1.65 | 0.430 | 0.088 |
| B | 21/07/89 | 4 | 55.31 | 1.75 | 1.775 | 0.446 |
| B | 10/01/90 | 3 | 45.46 | 1.48 | 5.989 | 0.884 |
| B | 25/04/90 | 3 | 47.64 | 4.33 | 5.112 | 0.972 |
| B | Annual | 10 | 50.05 | 2.38 | 4.040 | 0.671 |

(end)

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.
* : has been mixed with Potamopyrgus sp. and Bythinia sp.

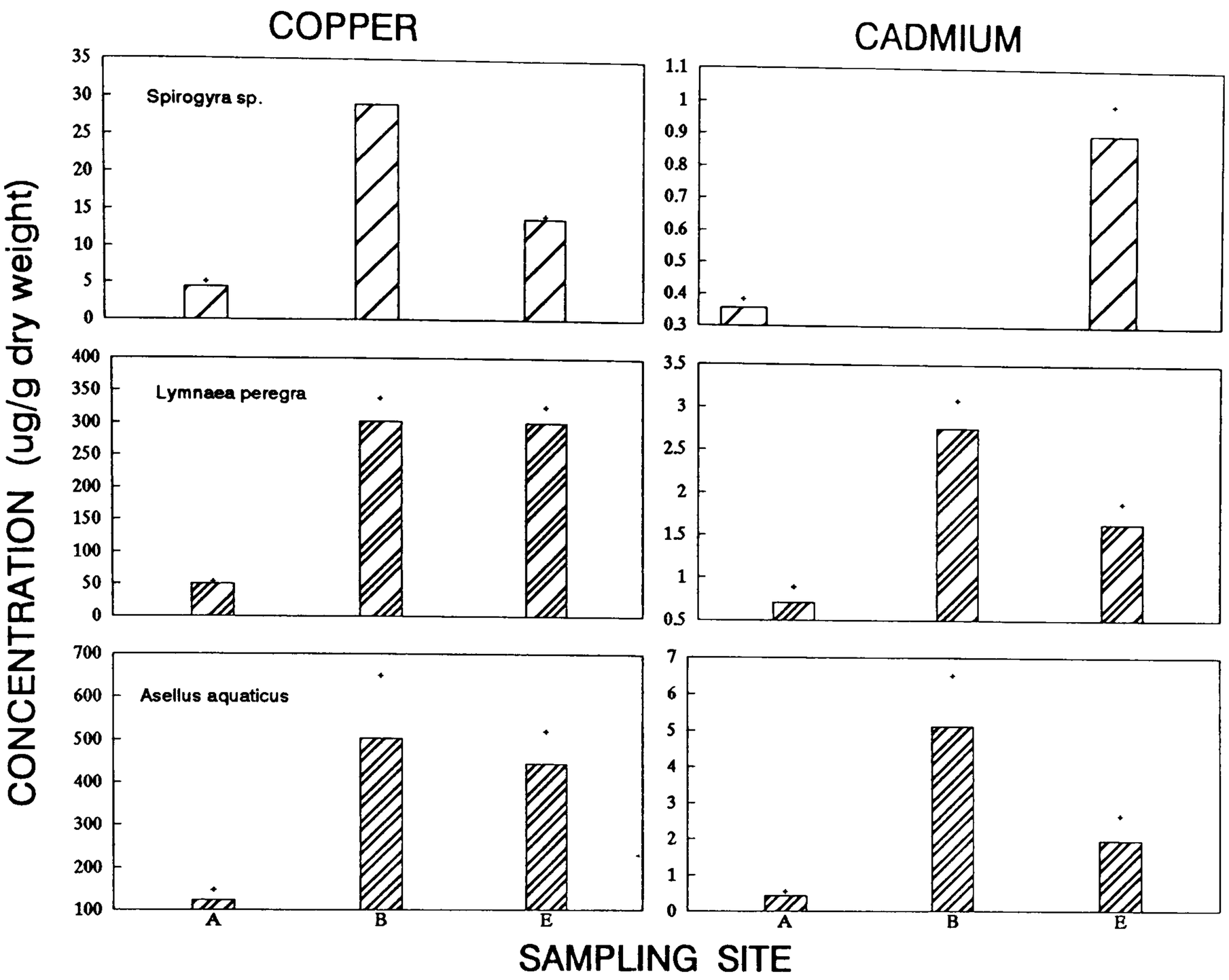



**Figure 6.2** Copper and cadmium concentrations in *Spirogyra* sp., *Lymnaea peregra* and *Asellus aquaticus* at the three sites in the River Wandle. Histograms present the means and + indicates the standard deviation. The units for all samples are in ug/g dw. (The original data are listed in Appendix D.4 and D.5).

**Table 6.5** Summary of mean concentrations of Cu and Cd in the various types of aquatic plants collected from contaminated site (Site E).

| Species | No | COPPER (ug/g dw) Mean | S.D. | CADMIUM (ug/g dw) Mean | S.D. |
|---|---|---|---|---|---|
| *Spirogyra sp.* | 12 | 13.7 | 0.4 | 0.90 | 0.09 |
| *Cladophora sp.* | 18 | 47.3 | 1.8 | 4.47 | 0.23 |
| *Rhynchostegium riparioides* | 24 | 97.1 | 4.4 | 2.69 | 0.12 |
| *Callitriche sp.* | 18 | 82.9 | 2.8 | 5.59 | 0.17 |
| *Elodea canadensis* | 23 | 80.0 | 2.1 | 5.50 | 0.18 |
| *Ranunculus fluitans* | 24 | 49.2 | 1.4 | 7.34 | 0.32 |
| *Potamogeton crispus* | 13 | 63.6 | 2.5 | 2.62 | 0.26 |
| *Apium nodiflorum* | | | | | |
| Leaves | 17 | 43.8 | 2.3 | 3.64 | 0.17 |
| Roots | 17 | 76.2 | 2.4 | 11.68 | 0.71 |

difference between the concentrations of Cu in *Spirogyra* to that in *Rhynchostegium*, for Cd there is an twelvefold difference between *Spirogyra* and roots of *Apium*. It is difficult to comment on these differences as various factors may be involved. For example differences in the surface roughness (i.e. the filamentous algae of *Spirogyra* has slimy surface but *Cladophora* has rough one) and can lead to marked difference in binding capacity. The plants were washed before analysis but these factors can influence the retentive characteristics of the surfaces. Actual uptake into plant cells will also vary between species and different parts of plants (i.e. the roots show higher concentrations than the leaves of *Apium*).

Site E also provides the greatest variety of invertebrates and it is the only site where metal concentrations in different species may be compared. The most outstanding feature is the range of concentrations of Cu, with those organisms with the blood pigment haemocyanin having the largest concentrations, although even within this group there is a large variation from *Physa* with nearly 730 ug/g Cu to *Gammarus* with less than 80 ug/g Cu. In the case of Cd the variation is smaller, with most values ranging between 1 to 2 ug/g, except for *Physa* and *Theromyzon* with double these concentrations (Table 6.6).

**Table 6.6** Summary of mean concentrations of Cu and Cd in the various types of aquatic invertebrates collected from contaminated site (Site E).

| Species | No | COPPER (ug/g dw) Mean | S.D. | CADMIUM (ug/g dw) Mean | S.D. |
|---|---|---|---|---|---|
| *Haemopsis sanguisuga* | 1 | 42.3 | 14.3 | 1.65 | 1.10 |
| *Theromyzon tessulatum* | 11 | 40.4 | 5.8 | 4.00 | 0.49 |
| *Erpobdella octoculata* | 21 | 59.7 | 6.7 | 1.38 | 0.18 |
| *Lymnaea peregra* | 39 | 300.1 | 24.3 | 1.63 | 0.25 |
| *Lymnaea stagnalis* | 8 | 129.3 | 16.3 | 1.64 | 0.18 |
| *Physa fontinalis* | 4 | 728.7 | 13.5 | 3.14 | 0.50 |
| *Gammarus pulex* | 33 | 77.9 | 3.7 | 1.10 | 0.17 |
| *Crangonyx pseudogracilis* | 3 | 98.5 | 6.0 | 1.77 | 0.13 |
| *Asellus aquaticus* | 34 | 443.2 | 76.5 | 1.96 | 0.67 |

## 6.4 DISCUSSION

### 6.4.1 Relative Distribution of Copper and Cadmium in Abiotic and Biotic Components of the Habitat of Stickleback Populations

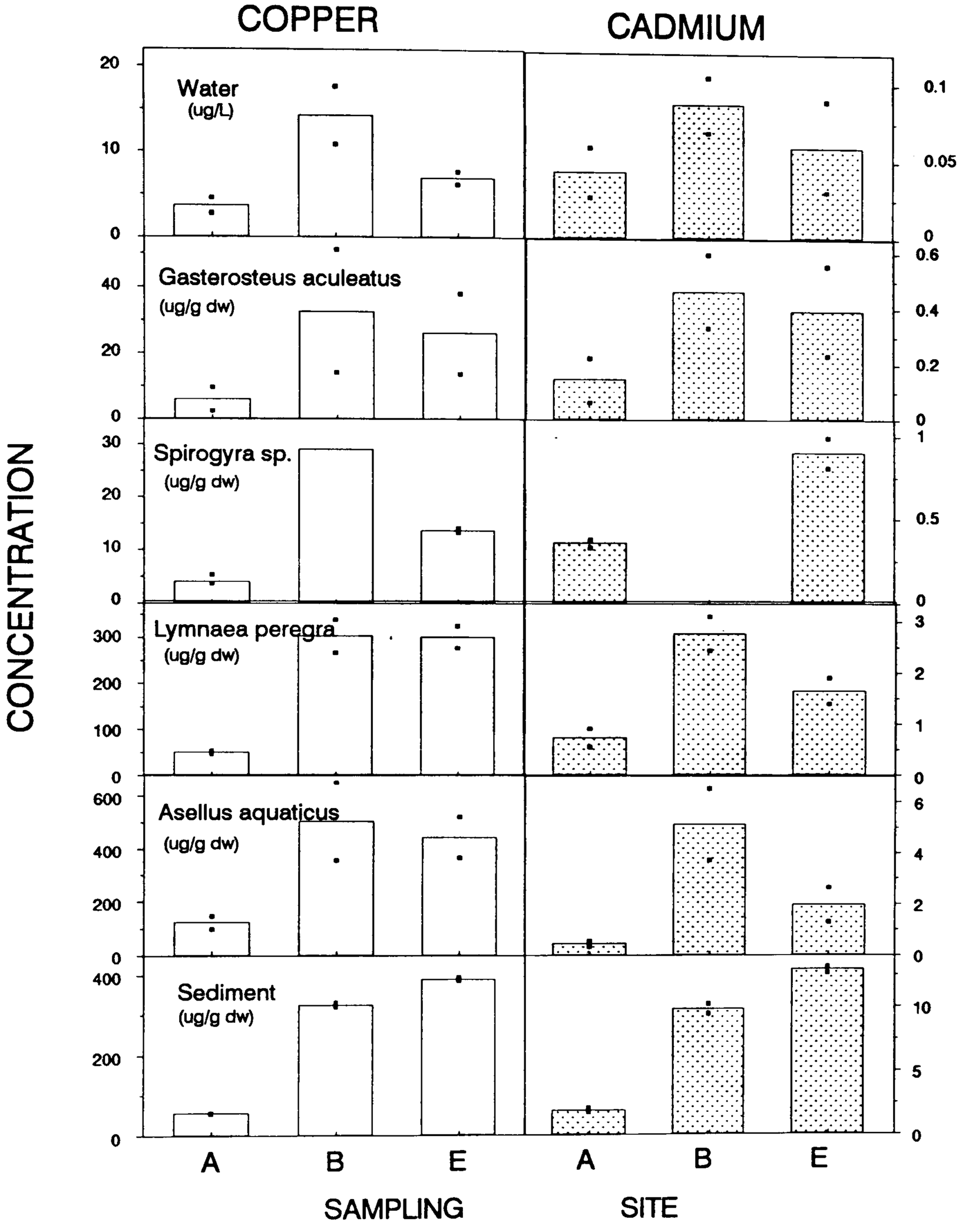



**Figure 6.3** Copper and cadmium concentrations at the different trophic levels in the River Wandle. Histograms present the means and "squares" indicate the standard deviations.

Figures 6.3 and 6.4 provide a summary of the mean concentrations of Cu and Cd in the different compartments and the organisms commonly found at all three sites, including the values for sticklebacks. Figure 6.3 summarises the site differences and shows the common trend between the reference site and the contaminated

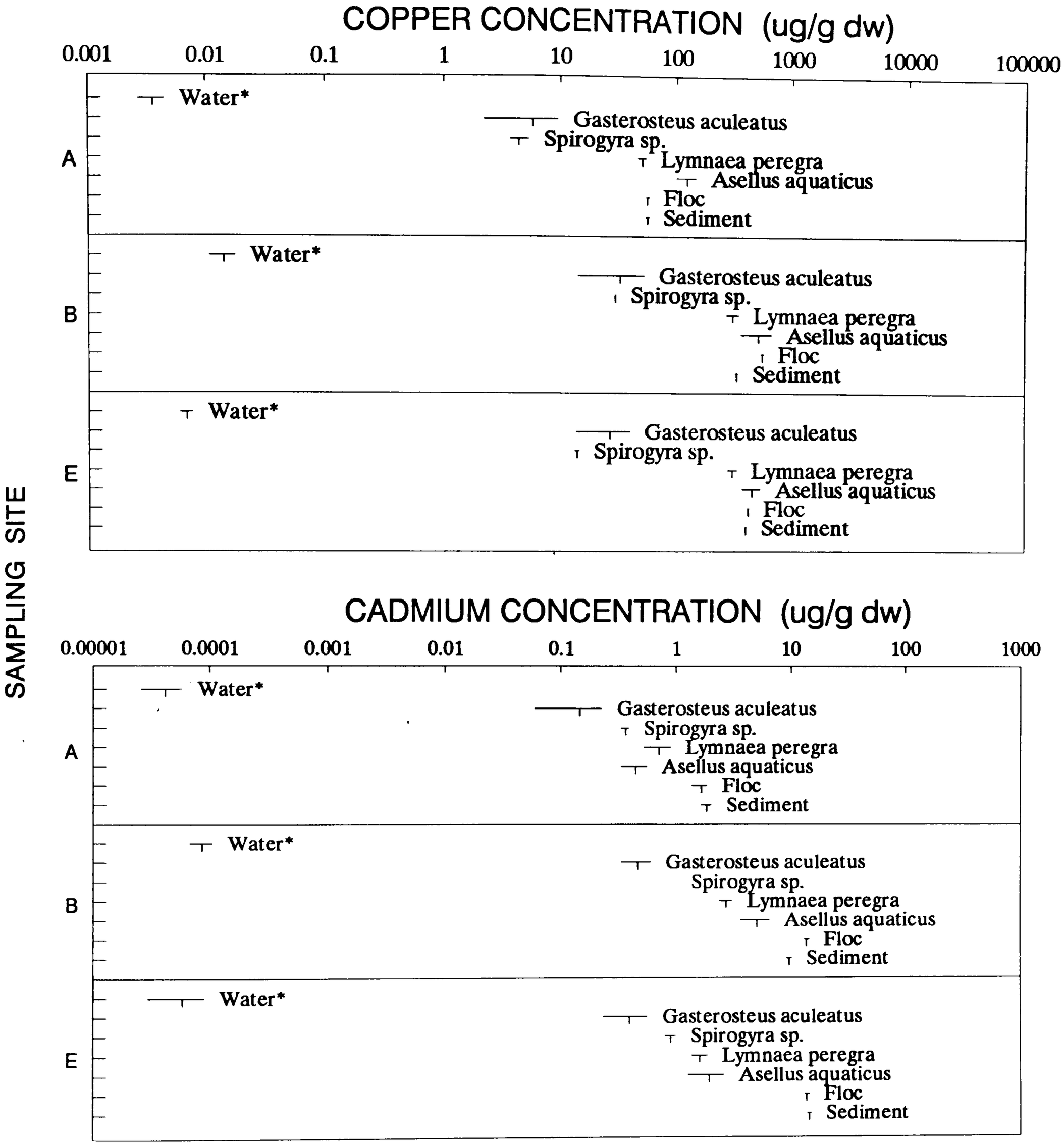



**Figure 6.4** The distributions of copper and cadmium in biota, water and sediment phases at the three sites (A, B and E) in the River Wandle. The vertical dashes present the means and the horizontal lines indicate the ranges of standard deviation. * shows the concentration in ug/ml.

sites for all the different types of samples. It also shows a divergence in trend at sites B and E; sediment concentrations for both elements are higher at E than B, whereas for the all other samples, concentrations are higher at B than E. It is thought that this reflects

differences in the bioavailable form of the metals between these two sites.

Figure 6.4 emphasises the rank order of Cu and Cd concentrations and shows that their concentrations in water, and floc and sediment were at the two extremes. Concentrations in water are generally over three orders of magnitude lower than the values of the other components in the system. For Cd sticklebacks have the next highest concentrations, whilst for Cu *Spirogyra* has concentrations very close to that in sticklebacks. The concentrations of Cu and Cd in the invertebrates, *Lymnaea* and *Asellus*, are intermediate between the high levels in the sediment and floc and those in stickleback and *Spirogyra*. The two figures (6.3 and 6.4) emphasise the fact that the sediment and the floc contain the greatest concentrations of metals.

In general terms, the relative distribution of Cu and Cd between the various organisms was found to be as follow;

(1) Copper:

*Spirogyra* < Stickleback < Chironomid < Various aquatic plants, Leeches < *Gammarus*, *Crangonyx* < *Lymnaea* < *Asellus* < *Physa*

(2) Cadmium:

Stickleback < *Spirogyra* < Chironomid, *Gammarus*, *Crangonyx*, *Lymnaea* < *Asellus*, *Physa* < Aquatic plants < Roots of aquatic plants

For Cu the aquatic invertebrates, *Physa*, *Asellus*, *Lymnaea*, tended to have higher values, more associated with the Cu levels in the sediment. By contrast, Cd in aquatic plants, particularly the roots of the plants, contained more Cd than any other members of the system. The distribution of Cu and Cd in the various organisms, water and particulate matter described above agree quite well with other published studies from America and Spain e.g. Mathis & Cummings 1973, Mathis & Kevern 1975, Cabrera et al. 1987.

### 6.4.2 Cu and Cd in the Waters

Copper and cadmium concentrations in water samples from 3 sites in the River Wandle are considered to be very low. Despite the fact that there was a significant difference between the reference site and the contaminated sites for Cd, the concentrations were close to a freshwater background level of 0.07 ug/l (Boyle et al. 1976, in Fornster and Wittmann 1983); within the background level, ranged 0.015-0.117 ug/l, for British rivers and streams in north-western England (Laxen 1984); and lower than the value of 0.183 $\pm$ 0.021 ug/l which has been used to categorise clean waters in River Brett and River Chelmer, Eastern England (Barak and Mason 1989).

Copper values are lower than 28 ug/l which is the water quality criterion for this metal for the protection of aquatic life in hard water of 300 mg $CaCO_3$/l (Mance et al. 1984). It will be recalled that water hardness in the River Wandle is in the range of 140-310 mg $CaCO_3$/l. However, the copper concentrations are higher than the background level of 1.8 ug/l given by Gibbs 1977 & Boyle 1978, in Fornster and Wittmann 1983.

Steele et al. (1990) using a behavioural assay involving zebrafish (*Brachydanio rerio*) and alanine as a chemical attractant demonstrated an avoidance response for Cu exposures of above 10 ug/l but not at 1 ug/l Cu. Dave and Xiu (1991) examined the hatching time of embryos and survival time of larvae of zebrafish exposed to Cu in water with an hardness of 100 mg/l $CaCO_3$. They found the "no-effect concentrations" were 0.05 ug/l and 0.21 ug/l, respectively. Brook trout (*Salvelinus fontinalis*) exposed to 32.5 ug/l Cu in Lake Superior water, produced eggs with lower hatching rates, and the survival and growth of alevins and juveniles was reduced in 17.4 ug/l Cu (McKim & Benoit 1971).

It is important to bear in mind that the River Wandle is a chalk stream, the relatively high $CaCO_3$ concentration has a big influence on the concentration of $Cu^{+2}$ and $Cd^{+2}$ ions in the water. The greatest proportion of the two elements are likely to be present as carbonates, and in other inorganic and organic complexes. The situation is further complicated by the fact that a high proportion of the riverflow at the contaminated sites is sewage effluent rich in organic matter, and Cu

and Cd are likely to be bound with organic substance (Stiff 1971a,b, Gardiner 1974a,b).

Notwithstanding these points, it is possible that the Cu concentrations found in the River Wandle particularly at the contaminated sites are sufficient to produce sub-lethal responses. This likelihood is increased if a significant intake of Cu also comes from ingestion of food. It is also possible however, that these "wild" populations have adapted to the elevated exposures of Cu and perhaps Cd.

### 6.4.3 Cu and Cd in the Sediments and Organic Detritus (Floc)

At the control site the Cu concentration in the sediment is similar to the published background level of 45 ug/g dw Cu. Cadmium is almost 5 times higher than the background level of 0.3 ug Cd/g dw given by Turekian and Wedepohl 1961, in Forstner and Wittmann 1983 and 3 times higher than 0.53 ug/g dw, reported for the rivers Brett and Chelmer, East Anglia, U.K. by Barak & Mason 1989. The concentrations of Cu and Cd at the two contaminated sites, are respectively 6 to 7 and 5 to 7 times greater than the values at the reference site. The most likely source of the metal is sewage effluent. A similar trend was found for the samples of floc, although the first two sampling dates at site A gave the highest Cd concentrations, and the Cu values were also elevated. It is not clear why these samples should be atypical. Cu concentrations in water samples for the same periods at site A were also elevated (Cd was not measured in these samples). There is also some indication from the invertebrates, particularly *Asellus*, that these early sampling data gave more elevated levels. Concentrations in the sediment, on the other hand, gave more uniform near background concentrations. As stated earlier the elevated concentrations could be a consequence of contamination caused by the sample carrying vessels e.g. PVC buckets or site A could receive intermittent contamination in some way.

Sediments act as a sink for metals in aquatic ecosystems (Mathis & Cummings 1973, Mathis & Kevern 1975), and concentrations in sediments are a good indication of the relative metal loading of the system (Duzzin et al. 1988). A number of studies have reported

relationships between metal levels in sediments and various organisms in the aquatic system; e.g. fish (Korzeniewski & Neugebauer 1991), annelida (Eyres & Pugh-Thomas 1978, Lang & Lang-Dobler 1979, Duzzin et al. 1988), mollusca (Tessier et al. 1984, Duzzin et al. 1988), crustaceans (Eyres & Pugh-Thomas 1978, Duzzin et al. 1988, Madigosky et al. 1991), insecta (Duzzin et al. 1988, Barak & Mason 1989, Krantzberg 1989, Young & Harvey 1991), as well as in macrophytes (Harding & Whitton 1978, Say et al. 1981, Schierup & Larsen 1981, Sprenger & McIntosh 1989). The data summarised in figures 6.3 and 6.4 confirm the relationship in that the trends found between sites for sediments is closely matched by the trends in the various organisms.

### 6.4.4 Cu and Cd in the Aquatic Plants and Algae

*Spirogyra* spp. did not show the high values of cadmium concentration as reported by Holsinger (1977) (in Fornster and Wittmann 1983). However, the site differences were marked, which indicated the difference of metal levels between them.

Leland and Carter (1984) found that concentrations of Cu in an oligotrophic stream with 5 ug/l and 10 ug/l resulted in changes of the species composition of periphyton and a reduction in the population density of *Spirogyra* spp. and *Cladophora* spp. In this study whilst copper concentrations in the water at the two contaminated sites were similar to the levels mentioned above, *Cladophora* spp. were abundant throughout the year of sampling in site B and E.

Cladophoraceae are generally absent from completely quiet waters (Whitton 1970), and so it is not surprising that *Cladophora* sp. was absent at site A, Waddon pond. *Cladophora* sp. in the Wandle accumulated some of the highest copper levels found in the literature (56.8 ug/g dw and 47.3 ug/g dw in site B and E respectively). Cadmium levels are similar to values reported by Keeney et al. (1976) for Lake Ontario, but they are an order of magnitude higher than most other published values (Ready 1972 & Bayerische 1977 in Fornster and Wittmann 1983, Abo-Rady 1980).

The only bryophyte examined was *Rhynchostegium riparioides*. The Cu concentrations of 212 ug/g dw and 127 ug/g dw at sites B and E are some of the highest values recorded in the literature, but the Cd concentrations are similar to those reported by Say et al. (1981) for the same species from River Etherw, Manchester, U.K.

The higher plants investigated were either submerged-rooted, emergent, or floating plant types, all had the elevated copper and cadmium levels which were nearly one order of magnitude greater than those of the same species of similar macrophytes reported in the literatures (Heydt 1977 & Bayerische 1977 in Fornster and Wittmann 1983, Abo-Rady 1980, Sprenger & McIntosh 1989).

The results of Cu and Cd in plants substantiate several facts regarding macrophytes in polluted aquatic environments as reported by Haslam (1990); (1) metal levels in higher plants are higher than in algae, (2) metal accumulation in bryophytes tends to be more than in angiosperms, (3) within a plant, such as *Apium nodiflorum*, more Cu and Cd tends to accumulate in lower parts (roots) than that in upper parts (leaves), (4) different species of plants tend to accumulate metals to different degrees.

However it is important to stress that the study did not distinguish between externally bound metals, including surface bound particulates and the internal concentration of metals. The total amount of metals in plants and the relative distribution between external and internal concentrations varies considerably from one plant type to another. It is generally acknowledged that washing with distilled water has only a limited affect in distinguishing between these two fractions. The degree of surface roughness of plant surfaces is important in the retention of metal particulates. Metal ions and simple polar complexes may be tightly bound to the cell wall and the "Donnan free space" (Kwan & Smith 1991). Efficient removal of these fractions may only be achieved by applying displacement solution containing a ligand and/or competitive ions.

### 6.4.5 Cu and Cd in the Aquatic Invertebrates

Except for *Lymnaea peregra*, the Cd concentrations of the various aquatic invertebrates at the reference site were similar to the background values reported for the rivers Brett and Chelmer, Eastern England (Barak and Mason 1989). The Cd level of *Lymnaea peregra* in site A was three times higher than these values. *Gammarus pulex* at the contaminated sites contained Cd concentrations similar to those of Barak and Mason. The Cd concentrations of mollusca at the contaminated sites were 5 times higher than those in Illinois River, U.S.A. (Mathis & Cummings 1973). *Asellus aquaticus* concentrations were similar to samples from the Elsenz River, F.R.G. (Prosi 1977 quoted by Forstner & Wittmann 1983).

The Cu concentrations of mollusca and *Asellus* at the reference site were higher than the results of Mathis and Cummings (1977) for the Illinois river and of Prosi for Elsenz river (1977 quoted by Forstner & Wittmann 1983). At the contaminated sites the metal levels of leeches, mollusca, *Asellus*, and chironomid larvae and pupae were similar to those found for these organisms in River Irwell, Lancashire, U.K. (Eyres & Pugh-Thomas 1978), although they were lower than measurements reported for the same river while it was considered as one at the most polluted river in Europe.

One of the most interesting features of the concentrations of Cu in the invertebrates is the particularly high values found in those forms which have the blood pigment heamocyanin. These organisms apparently accumulate very high levels at the contaminated sites. As far as can be ascertained limited work has been done on the uptake kinetics of Cu in these organisms (Rainbow & White 1989, Rainbow et al. 1990).

### 6.4.6 Assessment of Metal Exposure in the Diet of Sticklebacks

Taking into account the low values of Cu and Cd in the water and the relatively high concentrations found in the various organisms and organic detritus, it is evident that sticklebacks are exposed to considerable amount of Cu and Cd through the diet. Whether or not significant amounts are absorbed and constitute a major part of the

body burden of sticklebacks is another matter which will be considered in the next chapter.

At this juncture it is important to try and combine the results of this chapter with those of the previous one, which considered the composition of the diet of sticklebacks. In Chapter 5 it was found that the composition of the diet varied between sites, between sampling periods and in different age groups of sticklebacks.

Table 6.7 provides a summary of mean metal concentrations at each site and the percentage abundance of the organisms in the diet. It is unfortunate that there are a few notable omissions in the table. The most important is the absence of metal data from the small crustaceans at all three sites and Chironomids at site E. They were exceedingly difficult to separate from the organic detritus and generally presenting such small quantities so that it was virtually impossible to obtain sufficient 'tissue' for the determination of metals. Also living higher plant material was not available at site A.

Table 6.7 emphasises the fact that for any one food type, the concentration of Cu and Cd is greater at the contaminated sites of B and E than at the reference site A. This can be most clearly seen for intakes of organic detritus or floc, where it constitutes about the same fraction of the diet at each site and the metals are significantly higher at sites B and E. However over and above this well established trend is that the change in the composition of diet form one site to the next can have a major influence on the metal input into sticklebacks. This is most clearly shown at site B, where Mollusca make up nearly 30% of the diet, and since the species making up this group have some of the highest metal levels, it will further enhance the metal intake into the fish. Over 30% of the diet at this site consists of chironomids which are a further significant source of metals. At site E nearly 30% of the diet consists of the three groups, *Asellus*, Amphipoda and Mollusca and these together make a major contribution to the metal input to the fish. On the other hand the composition of the diet at site A is such that the major items tend to have a lower metal concentration e.g. vegetable matter and algae. Molluscs with high metal levels are a small part of the diet. However small crustaceans make up nearly 40% of the diet but as explained their possible contributions to metal intake was not analysed. The literature has

**Table 6.7** Concentrations of Cu and Cd (ug/g dw) in the major food items of the stickleback populations and in the fish from the River Wandle, P% = percentage relative abundance of food item in stomach of stickleback (see chapter 5).

| | Site A | | | Site B | | | Site E* | | |
|---|---|---|---|---|---|---|---|---|---|
| | P% | Cu | Cd | P% | Cu | Cd | P% | Cu | Cd |
| *Asellus aquaticus* | 1.3 | 123±24 | 0.24±0.11 | 0.4 | 503±147 | 5.1±1.4 | 9.8 | 443±77 | 2.0±0.7 |
| Amphipoda | 4.3 | – [2] 37±4 | – 0.27±0.04 | [1] 0.4 | 89±6 | 1.7±0.2 | 9.6 | 78±4<br>98±6 | 1.1±0.2<br>1.8±0.1 |
| Small Crustaceans | **37.4** | **?** | **?** | 15.7 | ? | ? | 9.4 | ? | ? |
| Mollusca | 1.6 | [3] 50±4<br>– [5] 21±2 | 0.71±0.18<br>– 0.16±0.04 | **27.9** | **302±36**<br>[4] **1420±42**<br>**179±14** | **2.8±0.3**<br>**4.5±0.4**<br>**4.1±0.8** | 8.5 | 300±24<br>729±13 | 1.6±0.2<br>3.1±0.5 |
| Chironomid larvae & pupae | 16.8 | 20±2 | 0.43±0.09 | **35.2** | **50±2** | **4.0±0.7** | **29.0** | ? | ? |
| Vegetable matter & algae | **21.8** | **?**<br>[6] **4.5±0.7** | **?**<br>**0.36±0.03** | 2.6 | [7] 57–154<br>29 | 3.2–7.4<br>– | 13.9 | 44–83<br>13.7±0.4 | 2.6–11.7<br>0.9±0.09 |
| Floc | 8.0 | 217±5 | 14.5±0.3 | 7.9 | 541±10 | 13.9±0.5 | 9.6 | 412±4 | 14.6±0.4 |
| No. of examined stickleback | (102) | | | (65) | | | (88) | | |
| Non–parasitised stickleback | (75) | 5.0±1.7 | 0.12±1.81 | (63) | 27.3±1.8 | 0.43±1.44 | (79) | 23.1±1.7 | 0.33±1.83 |
| Parasitised stickleback | (21) | 5.1±2.1 | 0.08±2.19 | (35) | 37.1±2.5 | 0.34±2.06 | (25) | 16.3±2.1 | 0.14±3.05 |

*1 : Gammarus pulex*
*2 : Crangonyx pseudogracilis*
*3 : Lymnaea peregra*
*4 : Physa fontinalis*
*5 : Pisidium spp.*
*6 : Spirogyra sp.*
*7 : mean range of all vegetations*

very few examples of zooplanktonic metal levels. Cd concentration of zooplankton was measured in Wintergreen, an eutrophic lake, in Michigan (U.S.A.). The value was one order of magnitude higher than that of 5 species of fishes (Mathis & Kevern 1975). Roch et al. (1985) examined the Cu and Cd concentrations in zooplankton and stickleback from Buttle Lake and Campbell River Drainage, Vancouver (Canada). They found the Cu and Cd concentrations in zooplankton were over 4 times and 5 times, respectively, higher than in sticklebacks. Assuming that Cu and Cd in zooplankton relative to sticklebacks is the same in the Wandle as in above study, it would mean that this group has similar Cu and Cd concentrations to the Amphipoda.

It will also be recalled from Chapter 5 that the diet of sticklebacks was found to change from one sampling period to the next. For example, vegetable matter at site A increased to 44 % of the diet in April 1990

and small crustaceans were proportionally lower. At site B in October 1989 nearly 60 % of the food consisted of molluscs, whereas in July 1989 they were a smaller component (19 %), and 40 % of the diet was then chironomids. Sticklebacks at site E have a more diverse diet and the food items are more equally represented in the diet. Nevertheless in July 1989 chironomids were 17 % and floc was 30% of the diet. In subsequent surveys the fish consistently had 30 - 36 % of chironomids in the diet but floc had decreased to 12 % and less. It was also noted in Chapter 5 that the composition of the diet changed with size of the fish, with a tendency for a higher proportion of larger organisms to be in the diet of older fish. For example, small crustaceans make up a smaller proportion of the diet in larger and older fish at all sites. At site B this is offset with a greater intake of molluscs.

The overall conclusion is that the diet of sticklebacks constitutes a major route of exposure for Cu and Cd. However it is also clear that the magnitude of the exposure can be enhanced or reduced depending on the composition of the diet. This can result from differences in the availability of food at different locations, changes in the availability of food from season to season and changes in the type of food consumed by different age groups of fish.

The next question is to ascertain if the metals consumed in food are a significant source of the body burden of Cu and Cd in the stickleback. One aspect that should be considered is the availability of the metals in the food and the fraction that is absorbed across the gastro-intestinal tract. The only direct way of examining this is to set up controlled laboratory experiments which examine the amount of metal consumed in food and the amount excreted. Additionally the speciation of the metals consumed in the foods and the faeces can be measured and this can be related to the forms of metals that are absorbed across the alimentary canal. This approach was not possible in the present study. An alternative approach is to examine the tissue distribution of the metals in the stickleback at each of the sites in order to determine which tissues accumulate the metals. For example accumulation in the gills would indicate that uptake of metals directly from water is the main route of entry into the fish (Karlsson-Norrgren & Runn 1985, Harrison & Klaverkamp 1990). Alternatively accumulation in the intestine and liver would stress the importance of metal uptake through the gut. This method of approach is the subject of the next chapter.

# CHAPTER SEVEN

# COPPER AND CADMIUM DISTRIBUTION IN THE ORGANS OF THE 3-SPINED STICKLEBACK

## 7.1 INTRODUCTION

### 7.1.1 The Distribution of Metals Within the Fish Body

Metal accumulation is not equally distributed in the fish body. The patterns of distribution vary according to many factors including the type and concentration of heavy metals, fish species, and route of entry and exposure duration (Table 7.1). Gills, kidney, and liver are major target organs for accumulation of Cu and Cd taken in via the water. Dietary sources of Cu and Cd are mainly deposited in the intestine, kidney, and liver.

It has been shown that metals taken in directly from water accumulate in specific tissues such as gills, in proportion to the magnitude of exposure, but it has also been found that by increasing the length of the exposure the metals can become redistributed and accumulated in other tissues, e.g. kidney. Norey et al., (1990a) exposed pike to a series of Cd concentrations (60, 125, 225 ug Cd/l) for different periods of time. Initially the major proportion of the body burden was in the gills but after 11 weeks exposure approximately equivalent loads were present in liver, gill and kidney tissues. At this point in time the liver had accumulated up to 50% of total burden. In Rainbow trout and stone loach exposed to 3 ug Cd/l for more than 133 days and 119 days, respectively, the metal was mainly deposited in gills initially. At the end of the experiment, rainbow trout which is a Cd sensitive species had most Cd in the livers and kidney, and the stone loach, a Cd-tolerant species, the metal was mainly in the liver (Norey et al.. 1990b, 1990c). Also, acute water-borne copper exposure to *Clarias lazera* and *Labeo rohita* resulted in Cu accumulation in the gills (El-Domiaty 1987, Radhakrichnaish 1988). Brook trout, *Salvelinus fontinalis*, exposed to Cu concentration (2.7 - 9.4 ug/l) below the "maximum acceptable total concentration' in soft water covering two generations for up to two years showed no copper

**Table 7.1** Summary of published literature showing the influence of the route of exposure of Cu and Cd on the distribution of the metals in the tissues of fish.

| Fish Species | Source of Metal | Exposure Dose & Duration | Metal Accumulation Organ* G | K | L | I | M | Other | Accumulation Sequence | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Water-Borne Metal Exposure** | | | | | | | | | | |
| Rainbow Trout *Salmo gairdneri* | 109 Cd | 56 day | + | + | | | | | | Harrison & Klaverkamp 1990 |
| Rainbow Trout | Cd | 9 ug/l for 0-36 wk or 9,18,36,54 ug/l for 2 mo | + | + | + | | | | | Thomas et al. 1983 |
| Rainbow Trout | Cd | 3 ug/l | + | + | + | | | | G → K & L | Norey et al 1990b,c |
| Lake Whitefish *Corego clupeaformis* | 109 Cd | 56 day | + | + | | | | | | Harrison & Klaverkamp 1990 |
| Stone Loach *Noemacheilus barbatulus* | Cd | 3 ug/l | + | + | + | | | | G K L → L | Norey et al 1990b,c |
| Pike *Esox lucius* | Cd | 60, 125, 225 ug/l for 11 wk | + | + | + | | | | G → GKL → L | Norey et al 1990a |
| Zebrafish Brachydanio rerio | 109 Cd | Inital 21 day | + | | | + | | Heart | | Karlsson-Norrgren & Runn 1985 |
| | | 21 to 42 day | | + | + | | + | Gonad | | |
| Coho Salmom *Oncorhynchus kisutch* | STPE Cd** | 2 ug/l for 30 day | + | + | + | | | | | Buckley & Yoshida 1989 |
| *Clarias lazera* | Cu | 3.2 mg/l for 24, 48, 72, 96 hr | | + | + | | | | | El-Domiaty 1987 |
| *Labeo rohita* | Cu | lethal for 1, 2 day | + | | + | | + | Brain | G>B>M>L | Radhakrichnaish 1988 |
| *Labeo rohita* | Cu | sublethal for 1, 7, 15, 30 day | + | | + | | + | Brain | G>L>M>B | Radhakrichnaish 1988 |
| Brook Trout *Salvelinus fontinalis* | Cu | 2.7, 4.5, 6.1, 9.4 ug/l for 2 yr | 0 | 0 | 0 | | 0 | | | McKim & Benoit 1974 |
| **Dietary Metal Exposure** | | | | | | | | | | |
| Rainbow Trout | 109 Cd | 56 day | | + | + | + | | | | Harrison & Klaverkamp 1990 |
| Rainbow Trout | Cd | 5 mg/kg fish per day for 15 d | | + | + | | | Spleen | | Crespo et al. 1986 |
| Rainbow Trout | Cd | ? for 12 wk | | + | | + | | | I → K | Kumada et al. 1980 |
| Lake Whitefish | 109 Cd | 56 day | | + | + | + | | | | Harrison & Klaverkamp 1990 |
| Zebrafish | 109 Cd | one 109 Cd labelled guppy per fish per day for 5 day | | | | + | | | | Karlsson-Norrgren & Runn 1985 |
| Eel *Anguilla anguilla* | Cd | Gammarids exposed to 20 ug Cd/l for 5 day contained 22 ug Cd/g dw and was fed the fish for 5 day | | + | + | + | | | | Haesloop & Schirmer 1985 |
| Rainbow Trout | Cu | 15 & 150 mg/kg for 20 wk | | | | + | | | | Knox et al. 1982 |
| Rainbow Trout | Cu | 2.5 & 500 mg/kg for 20 wk | | | | + | | | | Knox et al. 1984 |
| Channel Catfish *Ictalurus punctatus* | Cu | 32 mg/kg for 16 wk | | | | + | | | | Murai et al. 1981 |
| Channel Catfish | Cu | 40 mg/kg for 16 wk | | | | + | | | | Gatlin & Wilson 1986 |

* G = Gills, K = Kidney, L = Liver, I = Intestine, M = Muscle, Other = other organs.
** STPE Cd means Cadmium in Sewage Treatment Plant Effluent.

accumulation in gills, liver, kidney and muscle in either first- or second generation fish (McKim & Benoit 1974).

The dietary intake of copper and cadmium reveal different patterns. Evidence that the food source of copper causes elevated liver concentrations in rainbow trout was shown by Murai et al.. (1981) and Gatlin & Wilson (1986), and in channel catfish by Knox et al.. (1982, 1984). On the other hand, dietary intake of cadmium results in accumulations in the intestine, liver and kidney. It seems that there is not major single target organ for orally administered Cd in fish. Although, an elevated intestine cadmium concentration is the first sign of dietary cadmium exposure. Kumada et al. (1980) found that Cd accumulation after Cd dietary treatment for 12 weeks took place first in the gastrointestinal tract and then in the kidney in rainbow trout.

According to the above laboratory experiments, in fish exposed to copper or cadmium in water, the gills generally show the highest concentrations whereas fish exposed to the metals in food show elevated levels in intestine, and obviously in gut contents and faecal pellets.

In the natural environment fish are exposed to metals both from water via the gills and via the gastrointestinal tract, and in food which is only taken in via digestive system.

One study has suggested that elevated levels found in the gills and intestinal tract are the result of direct exposure in water via the gills and the intestinal tract. Woodworth & Pascoe (1983) demonstrated that in naturally exposed sticklebacks, high concentrations of cadmium were found in the gill and in the gut.

However other studies have emphasised the importance of food as a source of elevated metals in the intestinal tract. For example in a field study of rainbow trout in the rivers Augraben and Leiferer Graben in Italy by Dallinger & Kautzky (1985) found that Pb, Cr, Ni, Mn and Zn accumulated in the gut and gills, Cu in gut and liver, and Cd in gut and kidney. The authors provided evidence that the main source of the metals in the fish was from food and that from the water was minimal. In a marine environment, intestinal uptake is also

considered to be the main route of metal in take in fish (Voyer et al.. 1975, Stromberg et al.. 1983). Hardisty et al.. (1974b) working with fish from the metal contaminated Severn Estuary and Bristol Channel, England, revealed that Zn accumulation in larger flounder was related to dietary Zn and a positive correlation was found between the Cd concentration in six species of fishes and the proportion of crustaceans consumed.

Studies examined the relative importance of metal taken up either directly from water or from food are limited. Harrison and Klaverkamp (1989) found that rainbow trout and lake whitefish (*Coregonus clupeaformis*) retained more cadmium in the liver and kidney in 'food-exposed' fish than 'water-exposed' fish. In another study in which a subtoxic pulse of $^{109}Cd$ was administered either via the immersion medium or via the diet to female zebrafish (*Brachydanio rerio*) produced a different pattern of accumulation. Direct exposure to Cd in the water resulted initially in accumulation in the gills, alimentary canal and heart, but after 21 days concentrations in these tissues decreased markedly but in the subsequent period of 21-42 days the metal became concentrated in the liver, kidney, ovaries and muscle before eventually being eliminated from the fish. However, after exposure to dietary $^{109}Cd$ the accumulation was mainly in the alimentary canal and only less than 5% was retained in the fish body (Karlsson-Norrgren & Runn 1985).

In the previous chapter it was established that Cu and Cd in the food constitutes a major route of entry of the metals into the fish. Attention was also drawn to the fact that the composition of the diet may have a further influence in the amounts of Cu and Cd taken via the food route. It was therefore decided to ascertain if the metals taken in via food contribute to the metal body burden in the fish by examining the distribution of the metals in the tissues of stickleback from the non-contaminated and contaminated sites.

### 7.1.2 Metal Binding-Proteins in Fish

Metallothioneins, are a group of low-molecular-weight cytopasmic metalloproteins whose apoprotein, thionein, is induced by exposure to a number of metal including cadmium, copper, mercury, and zinc.

They play an important role in the transport and storage of heavy metals. It is generally thought that they provide a protective role against the toxic effects of these metals by sequestering and thus reducing the amount of the free metal ions which would otherwise cause adverse effects in organisms.

Metallothioneins (MTTs) or metal-binding proteins (MBPs) are found mainly in liver tissues of metal exposed organisms. The induction of MTTs (or MBPs) have been used to indicate elevated metals levels in a variety of organisms (Olafson et al.. 1979, Shaikh & Hirayama 1979, Hamilton & Mehrle 1986, Henning 1986, Overnell & Abdullah 1988, Hogstrand et al.. 1989). Roch et al.. (1982) investigated metallothionein in the livers of rainbow trout (*Salmo gairdneri*) caught in metal-contaminated lakes of the Campbell River watershed, Ontario, Canada. They found that the amount of metallothionein in liver cytosol fraction closely corresponded with the metal levels in the lakes. In another study significantly increased MT levels were found in livers of blue-striped grunt, *Haemulon sciurus*, and tomtate, *H. aurolineatum*, caught in heavy-metal-polluted areas of Hamilton Harbour and Castle Harbour. The increased MTT levels correlated with the hepatic zinc and copper concentrations in these fish (Hogstrand & Haux 1990). The existence of metallothionein in the fish from natural environments has also been reported, e.g., carp, *Cyprinus carpio*, captured in Nagara river and breeding ponds of Japan (Kito et al.. 1982b). Hogstrand et al.. (1989) examined MTTs in perch (*Perca fluviatilis*), caught along a Cu/Zn gradient in a river flowing past a brassworks. Significant correlations were found between hepatic Cu and Zn concentrations and MTT levels. As a result they proposed that MTT could be used as an indicator of heavy metals in water.

As the liver is one of the main tissues in which metals such as Cu and Cd accumulate, a preliminary study was made to investigate the formation of Cu and Cd proteins in the livers of sticklebacks. This has been studied in the hepatic cytosol of the sticklebacks from the contaminated sites by gel filtration.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Investigation of Cu and Cd Distribution in the Stickleback

Seventeen of the larger sticklebacks (Total length 54-67 mm) that were collected in the surveys of 12th Oct. 1989, 10th Jan. 1990 and 25th Apr. 1990 from three sampling sites were dissected individually in a sterilised petri dish. A new scalpel blade and acid-washed forceps were used for each fish. The length of each one was measured, sex was determined and it was examined for the signs of *Schistocephalus* infection. The following organs were separated; gill, liver, gonad, digestive tract, body carcase, kidney (including spleen), and fat (including other connected tissues). The gut contents were squeezed from the digestive tract.

Methods for obtaining accurate weights of tiny organs and tissues fractions were described in Chapter 4. Samples were prepared for metal analysis according to the procedure described in Chapter 2, section 2.2.

In order to compare, the 17 fish were divided into 3 groups: (i) non-parasitised fish with low metal content (i.e. fish from site A), (ii) non-parasitised fish with high metal content, (i.e. fish from sites B and E.), (iii) parasitised fish with high metal content.

### 7.2.2 Investigation of Metal Binding Proteins in the Liver of Sticklebacks

The livers of 30 to 50 sticklebacks were freshly dissected, pooled and quickly frozen in liquid nitrogen, and then freeze-dried for 4 days at -40$^{o}$C, and stored at -20$^{o}$C for further analysis.

Approximately 0.3 g of freeze-dried fish liver was weighed into a glass blender with 3 ml of extraction buffer (40 mM Tri-HCl, 50 mM $NH_4Cl$, and 5 mM 2-Mercaptoethanol, pH=7.8) to which was added 4 mM of PMSF (phenylmethanesulphonyl fluoride) and 1.4 mM of E64 (L-trans-epoxysuccinyl leucylamino (4-guanidino)-butane). Then an electronic homogenizer was used with a glass pestle. In the extracting procedure the sample was always kept cold in an ice box. Afterwards the pinkish

milky solution was transferred to an ultracentrifuge tube and centrifuged at 100,000 G for 1.5 hours at $4^{o}C$.

The supernatant was transferred into a tube and petroleum ether added to extract the fat. The samples were filtered with a series of membrane filters of pore sizes, 5 um, 2 um, 1.3 um and 0.45 um. The extractant was degassed for about one hour, and then about 3 ml was loaded onto a double-linked Sephadex G-75 (6.5 cm x 50 cm) and eluted with the buffer. Elutant volumes of every 4.5 ml were collected giving 95 fractions in total.

Protein was determined by the Bradford method (Bradford 1976, Darbre 1986). Cu and Cd concentrations were measured by flameless AAS. Haemoglobin, Ovalbumin, Myoglobin and Cytochrome C with molecular weights of 64000, 45000, 16000, and 12000, respectively, were used to calibrate the column. This method followed that of Marshell & Inglis (1986).

## 7.3 RESULTS

### 7.3.1 The Cu and Cd Distribution in the Organs of the Stickleback

(A) Comparisons based on Cu and Cd concentrations

The copper and cadmium concentrations in different fish tissues are shown in Figures 7.1 and 7.2. The food in the gut had the highest values, followed by the liver. For each group of fish the metal concentrations in the different tissues were compared by one-way ANOVA. With the exception of copper in the low-metal group, all other groups showed some significant difference ($P<0.5$) in the metal concentrations in the different tissues. The multiple range test highlighted the fact that for Cu, the concentrations in the liver and food were markedly different to the other tissues but that for cadmium, only food was notably different (Table 7.2).

Table 7.3 compares the metal concentrations in the same tissues between the three groups of fish. It can be seen that the liver and kidney show weakly significant differences ($P<0.5$) for both metals. Neither of the metals were significantly different in the gills. The

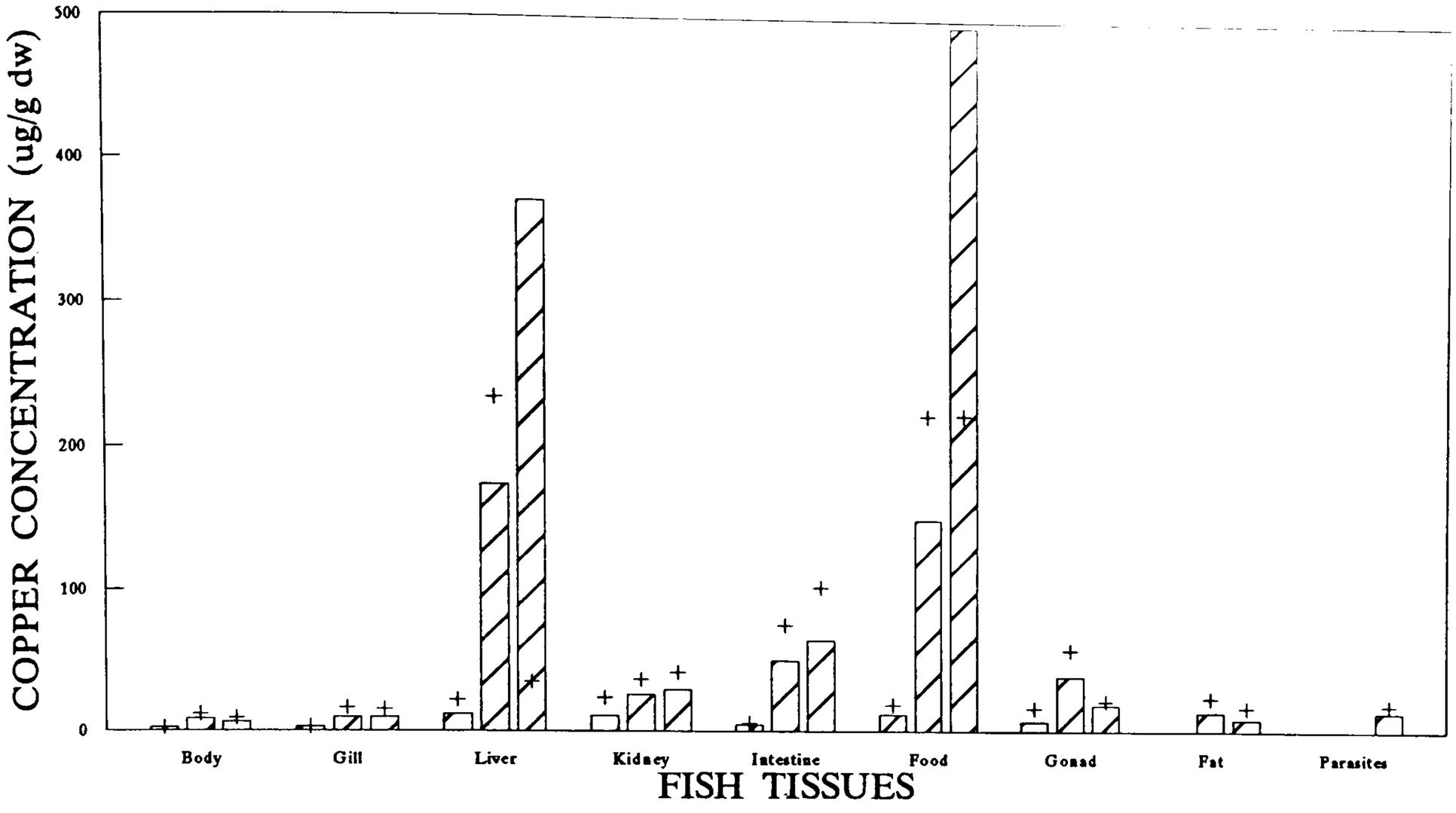



**Figure 7.1** Distribution of copper concentrations in the tissues of three groups of sticklebacks; (1) left bar: low-metal non-parasitised fish, (2) middle bar: high-metal non-parasitised fish, (3) right bar: high-metal parasitised fish. + indicates one standard deviation. (The original data are listed in Appendix E.2).

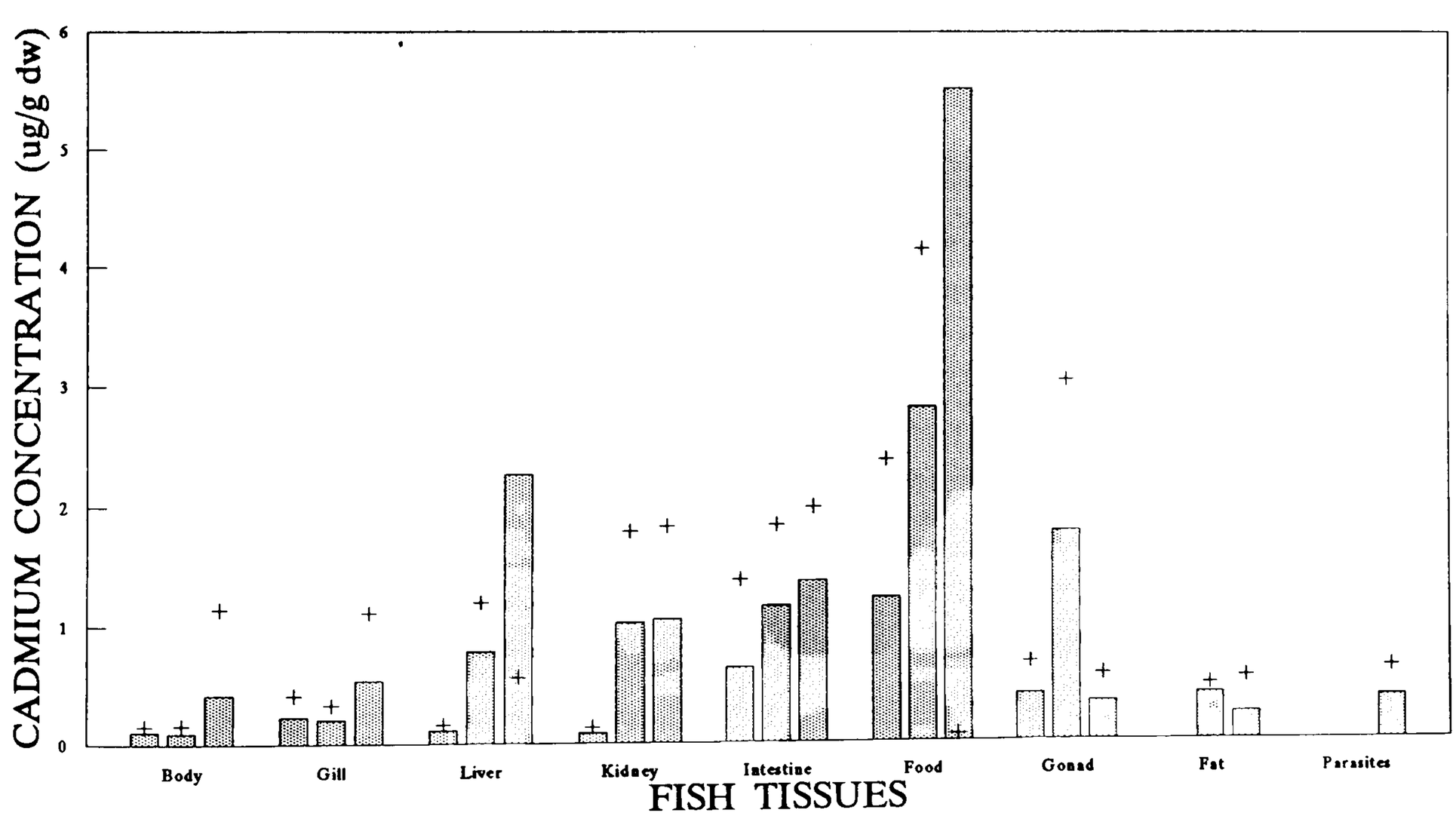



**Figure 7.2** Distribution of cadmium concentrations in the tissues of three groups of sticklebacks; (1) left bar: low-metal non-parasitised fish, (2) middle bar: high-metal non-parasitised fish, (3) right bar: high-metal parasitised fish. + indicates one standard deviation. (The original data are listed in Appendix E.2).

**Table 7.2** Comparison of Cu and Cd distribution in tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. The arithmetical means, S.D. and 95% lower /upper confidence intervals (C.Int.), together with one-way ANOVA and multiple range test. * = P<0.05, ** = P<0.01, *** = P<0.001, **** =P<0.0001, and a, b, c, & d, indicate the ascending groups of means. (The ANOVA tables are listed in Appendix E.4).

| Fish Group | Tissue | Sample Size | Mean | S.D. | Lower Con. Int. | Upper Con. Int. | Sig. Level | Multiple Range Test |
|---|---|---|---|---|---|---|---|---|
| Copper Concentration | | | | | | | | |
| LNP | Body | 5 | 2.15 | 0.37 | −2.67 | 6.98 | n.s. | |
| | Gill | 5 | 2.77 | 0.42 | −2.06 | 7.59 | | |
| | Gonad | 5 | 7.02 | 9.12 | 2.20 | 11.85 | | |
| | Kidney | 5 | 10.67 | 12.86 | 5.84 | 15.50 | | |
| | Liver | 5 | 12.05 | 9.88 | 7.22 | 16.88 | | |
| | Intestine | 5 | 4.59 | 0.88 | −0.23 | 9.42 | | |
| | Food | 5 | 12.04 | 6.45 | 7.21 | 16.87 | | |
| HNP | Body | 6 | 8.58 | 3.41 | −13.63 | 30.79 | **** | a |
| | Gill | 6 | 9.75 | 6.63 | −12.46 | 31.95 | | a |
| | Gonad | 6 | 38.72 | 18.79 | 16.51 | 60.93 | | a |
| | Kidney | 6 | 26.06 | 10.44 | 3.85 | 48.27 | | a |
| | Liver | 6 | 174.45 | 60.33 | 152.24 | 196.66 | | b |
| | Intestine | 6 | 49.66 | 25.34 | 27.45 | 71.87 | | a |
| | Food | 6 | 149.85 | 72.45 | 127.64 | 172.06 | | b |
| HP | Body | 6 | 6.53 | 2.76 | −83.22 | 96.28 | **** | a |
| | Gill | 6 | 9.77 | 5.51 | −79.98 | 99.53 | | a |
| | Gonad | 4 | 18.40 | 3.32 | −91.52 | 128.33 | | a |
| | Kidney | 6 | 29.46 | 11.98 | −60.30 | 119.21 | | a |
| | Liver | 6 | 370.95 | 336.20 | 281.19 | 460.70 | | b |
| | Intestine | 6 | 64.34 | 37.92 | −25.41 | 154.09 | | a |
| | Food | 5 | 495.63 | 272.67 | 397.32 | 593.95 | | b |
| | Cestode | 6 | 13.70 | 5.09 | −76.05 | 103.45 | | a |
| Cadmium Concentration | | | | | | | | |
| LNP | Body | 5 | 0.1072 | 0.0466 | −0.2377 | 0.4520 | * | a |
| | Gill | 5 | 0.2274 | 0.1780 | −0.1174 | 0.5722 | | a |
| | Gonad | 5 | 0.3967 | 0.2673 | 0.0519 | 0.7415 | | a |
| | Kidney | 5 | 0.0870 | 0.0446 | −0.2578 | 0.4319 | | a |
| | Liver | 5 | 0.1108 | 0.0517 | −0.2341 | 0.4556 | | a |
| | Intestine | 5 | 0.6389 | 0.7434 | 0.2941 | 0.9838 | | a b |
| | Food | 5 | 1.2274 | 1.1490 | 0.8826 | 1.5723 | | b |
| HNP | Body | 6 | 0.0915 | 0.0696 | −0.3822 | 0.5652 | **** | a |
| | Gill | 6 | 0.2041 | 0.1202 | −0.2696 | 0.6778 | | a |
| | Gonad | 6 | 1.7783 | 1.2546 | 1.3046 | 2.2520 | | c |
| | Kidney | 6 | 1.0265 | 0.7645 | 0.5528 | 1.5002 | | a b c |
| | Liver | 6 | 0.7859 | 0.4137 | 0.3122 | 1.2596 | | a b |
| | Intestine | 6 | 1.1608 | 0.6811 | 0.6871 | 1.6344 | | b c |
| | Food | 6 | 2.8154 | 1.3260 | 2.3407 | 3.2881 | | d |
| HP | Body | 6 | 0.4445 | 0.7163 | −0.7100 | 1.5991 | ** | a |
| | Gill | 6 | 0.5317 | 0.5839 | −0.6228 | 1.6862 | | a |
| | Gonad | 4 | 0.3257 | 0.2392 | −1.0883 | 1.7397 | | a |
| | Kidney | 6 | 1.0507 | 0.7811 | −0.1039 | 2.2052 | | a |
| | Liver | 6 | 2.2652 | 1.7130 | 1.1107 | 3.4197 | | a |
| | Intestine | 6 | 1.3658 | 0.6235 | 0.2113 | 2.5203 | | a |
| | Food | 5 | 5.5194 | 5.4696 | 4.2547 | 6.7841 | | b |
| | Cestode | 6 | 0.3664 | 0.2512 | −0.7882 | 1.5209 | | a |

**Table 7.3** Comparison of Cu and Cd concentrations in the same tissue of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. The arithmetical means, S.D. and 95% lower/upper confidence intervals (C.Int.), together with one-way ANOVA and multiple range test. * = P<0.05, ** = P<0.01, *** = P<0.001, **** =P<0.0001, and a, b, c, & d, indicate the ascending groups of means. (The ANOVA tables are listed in Appendix E.5).

| Tissue | Fish Group | Sample Size | Mean | S.D. | Lower Con. Int. | Upper Con. Int. | Sig. Level | Multiple Range Test |
|---|---|---|---|---|---|---|---|---|
| Copper Concentration | | | | | | | | |
| Body | LNP | 5 | 2.15 | 0.37 | 0.37 | 4.67 | ** | a |
| | HNP | 6 | 8.58 | 3.41 | 6.28 | 10.88 | | b |
| | HP | 6 | 6.53 | 2.76 | 4.23 | 8.83 | | b |
| Gill | LNP | 5 | 2.77 | 0.42 | −2.18 | 7.71 | n.s. | |
| | HNP | 6 | 9.75 | 5.51 | 5.23 | 14.26 | | |
| | HP | 6 | 9.77 | 6.63 | 5.26 | 14.29 | | |
| Liver | LNP | 5 | 12.05 | 9.88 | −183.86 | 207.96 | * | a |
| | HNP | 6 | 174.45 | 60.33 | −4.39 | 353.28 | | a b |
| | HP | 6 | 370.95 | 336.20 | 192.11 | 549.78 | | b |
| Kidney | LNP | 5 | 10.69 | 12.86 | −0.58 | 21.92 | * | a |
| | HNP | 6 | 26.06 | 10.44 | 15.79 | 36.33 | | b |
| | HP | 6 | 29.46 | 11.98 | 19.19 | 37.73 | | b |
| Intestine | LNP | 5 | 4.59 | 0.88 | −21.56 | 30.75 | ** | a |
| | HNP | 6 | 49.66 | 25.34 | 25.79 | 73.53 | | b |
| | HP | 6 | 64.34 | 37.92 | 40.47 | 88.21 | | b |
| Cadmium Concentration | | | | | | | | |
| Body | LNP | 5 | 0.1072 | 0.0466 | −0.3062 | 0.5205 | n.s. | |
| | HNP | 6 | 0.0915 | 0.0696 | −0.2858 | 0.4688 | | |
| | HP | 6 | 0.4446 | 0.7163 | 0.0672 | 0.8219 | | |
| Gill | LNP | 5 | 0.2274 | 0.1780 | −0.1264 | 0.5812 | n.s. | |
| | HNP | 6 | 0.2041 | 0.1202 | −0.1188 | 0.5270 | | |
| | HP | 6 | 0.5317 | 0.5839 | 0.2088 | 0.8546 | | |
| Liver | LNP | 5 | 0.1108 | 0.0517 | −0.9000 | 1.1215 | * | a |
| | HNP | 6 | 0.7859 | 0.4137 | −0.1368 | 1.7086 | | a |
| | HP | 6 | 2.2652 | 1.7130 | 1.3425 | 3.1879 | | b |
| Kidney | LNP | 5 | 0.0870 | 0.0446 | −0.5400 | 0.7141 | * | a |
| | HNP | 6 | 1.0265 | 0.7645 | 0.4541 | 1.5990 | | b |
| | HP | 6 | 1.0507 | 0.7811 | 0.4782 | 1.6231 | | b |
| Intestine | LNP | 5 | 0.6389 | 0.7434 | −0.0134 | 1.2913 | n.s. | |
| | HNP | 6 | 1.1608 | 0.6811 | 0.5652 | 1.7563 | | |
| | HP | 6 | 1.3658 | 0.6235 | 0.7703 | 1.9613 | | |

copper concentrations of the body carcase and intestine in the high-metal content groups were markedly higher than those of the reference group, but not in the case of cadmium concentrations (Table 7.3).

(B) <u>Comparisons based on percentages of the total amounts of metal in tissues</u>

The concentration in each tissue was multipled by the tissue dry weight to give the total amount of Cu and Cd. The percentage distribution of copper and cadmium in the tissues of fish revealed that most of the increased body burden of metals in the fish at the contaminated sites was in the liver and intestine (Table 7.4; Figures 7.3 and 7.4).

One way ANOVA analysis of the percentages of metals in the tissues between the different groups of fish (Table 7.4) revealed that: (1) In the high-metal groups the liver and intestine contained a significantly greater percentage of copper. (2) In the low-metal group the percentage of Cu in the body, gill and kidney were significantly greater. (3) In the high-metal groups the liver and intestine had significantly greater percentages of cadmium.

### 7.3.2 Metal Binding Protein in the Liver of Stickleback from High Metal Content Sites in the River Wandle

Figure 7.5 shows the result of the gel filtration with Sephadex G-75 of the stickleback liver cytosols. The void volume (Vo) was about 125 ml and bed volume (Vt) was at 440 ml. The main features of the chromatogram are the two protein peaks. One of these is located at ca 140 ml of elution volume (Ve) which is at the high-molecular protein end and is off the resolution of the column. The other is located at ca 267 ml of Ve which is towards the low-molecular end of the column. There are also distinct Cu and Cd peaks corresponding with the first protein peak and then a second Cu peak immediately following the second protein peak. Unfortunately it is not known whether the latter are separate entities or the result of an error in the collection procedure.

**Table 7.4** Comparison of percentages of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. The arithmetical means, S.D. and 95% lower/upper confidence intervals (C.Int.), together with one-way ANOVA and multiple range test. * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001, and a, b, c, & d, indicate the ascending groups of means. (The ANOVA tables are listed in Appendix E.6).

| Tissue | Fish Group | Sample Size | Mean | S.D. | Lower Con. Int. | Upper Con. Int. | Sig. Level | Multiple Range Test |
|---|---|---|---|---|---|---|---|---|
| Percentage of the Total Amount of Copper | | | | | | | | |
| Body | LNP | 5 | 46.50 | 16.01 | 36.99 | 56.01 | ** | b |
| | HNP | 6 | 35.93 | 7.00 | 27.25 | 44.61 | | b |
| | HP | 6 | 17.95 | 4.61 | 9.27 | 26.64 | | a |
| Gill | LNP | 5 | 3.03 | 1.10 | 2.24 | 3.82 | * | b |
| | HNP | 6 | 2.17 | 0.85 | 1.44 | 2.89 | | a b |
| | HP | 6 | 1.38 | 0.48 | 0.66 | 2.11 | | a |
| Liver | LNP | 5 | 25.92 | 14.19 | 15.31 | 36.53 | ** | a |
| | HNP | 6 | 47.67 | 6.69 | 37.98 | 57.36 | | b |
| | HP | 6 | 54.56 | 11.69 | 44.87 | 64.24 | | b |
| Kidney | LNP | 5 | 3.51 | 2.09 | 2.40 | 4.62 | ** | b |
| | HNP | 6 | 1.17 | 0.42 | 0.16 | 2.18 | | a |
| | HP | 6 | 1.19 | 0.26 | 0.18 | 2.21 | | a |
| Intestine | LNP | 5 | 4.82 | 1.36 | 1.80 | 7.84 | * | a |
| | HNP | 6 | 10.91 | 3.34 | 8.15 | 13.66 | | b |
| | HP | 6 | 10.73 | 3.88 | 7.98 | 13.49 | | b |
| Percentage of the Total Amount of Cadmium | | | | | | | | |
| Body | LNP | 5 | 44.49 | 19.92 | 28.51 | 60.47 | n.s. | |
| | HNP | 6 | 33.02 | 10.88 | 18.43 | 47.61 | | |
| | HP | 6 | 37.07 | 18.47 | 22.48 | 51.65 | | |
| Gill | LNP | 5 | 4.66 | 3.50 | 1.60 | 7.71 | n.s. | |
| | HNP | 6 | 4.65 | 1.81 | 1.86 | 7.44 | | |
| | HP | 6 | 4.22 | 3.92 | 1.44 | 7.01 | | |
| Liver | LNP | 5 | 6.65 | 5.29 | 0.26 | 13.03 | * | a |
| | HNP | 6 | 21.42 | 3.40 | 15.59 | 27.25 | | b |
| | HP | 6 | 21.08 | 9.49 | 15.26 | 26.91 | | b |
| Kidney | LNP | 5 | 1.94 | 3.44 | −0.39 | 4.28 | n.s. | |
| | HNP | 6 | 4.53 | 2.29 | 2.39 | 6.66 | | |
| | HP | 6 | 2.35 | 1.38 | 0.22 | 4.48 | | |
| Intestine | LNP | 5 | 11.54 | 9.25 | 4.65 | 18.43 | * | a |
| | HNP | 6 | 24.86 | 4.64 | 18.58 | 31.15 | | b |
| | HP | 6 | 15.71 | 7.37 | 9.42 | 21.99 | | a |

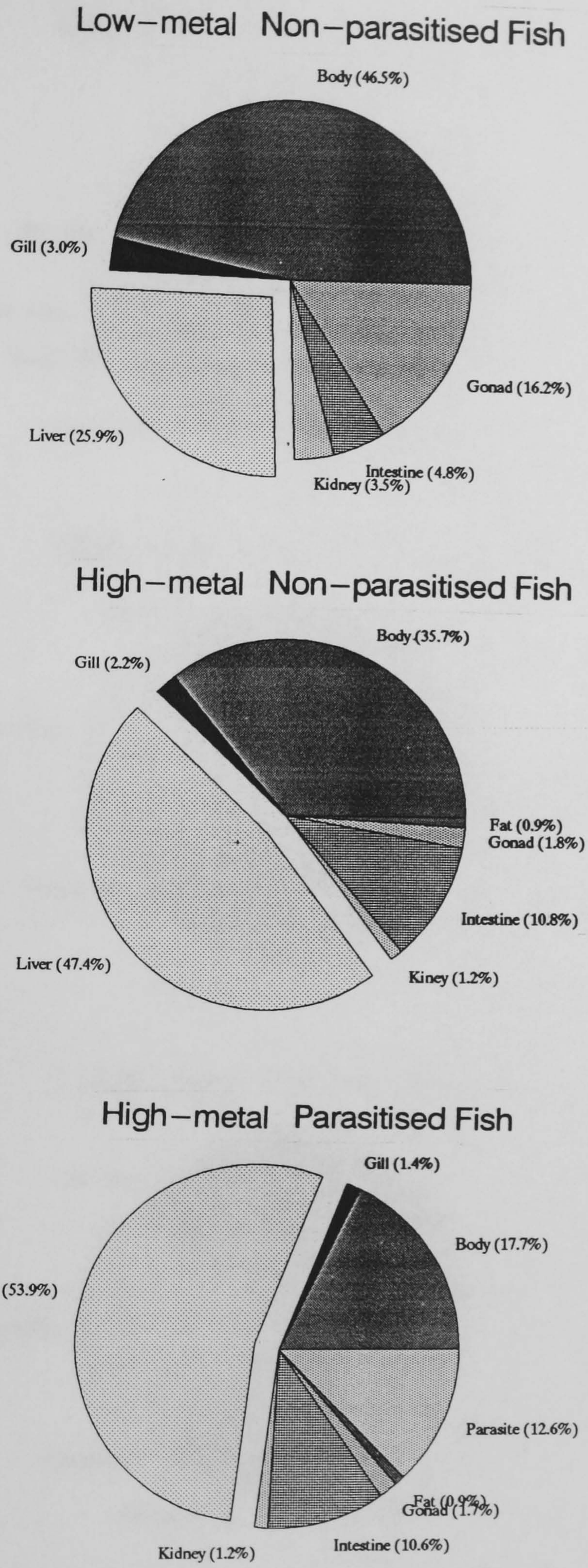
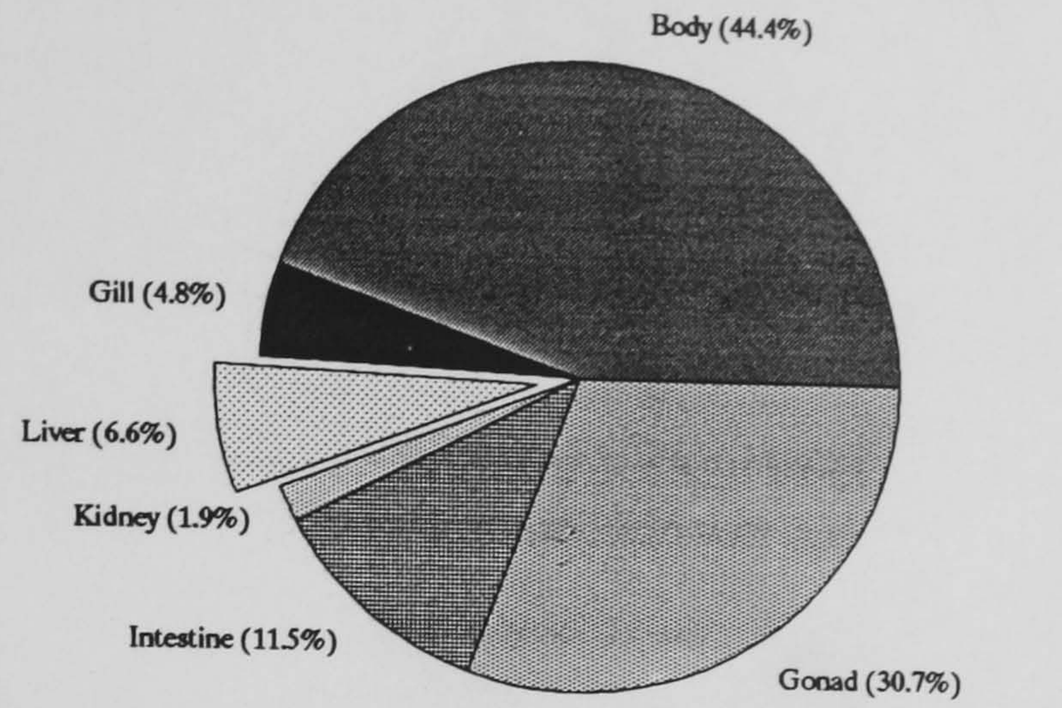
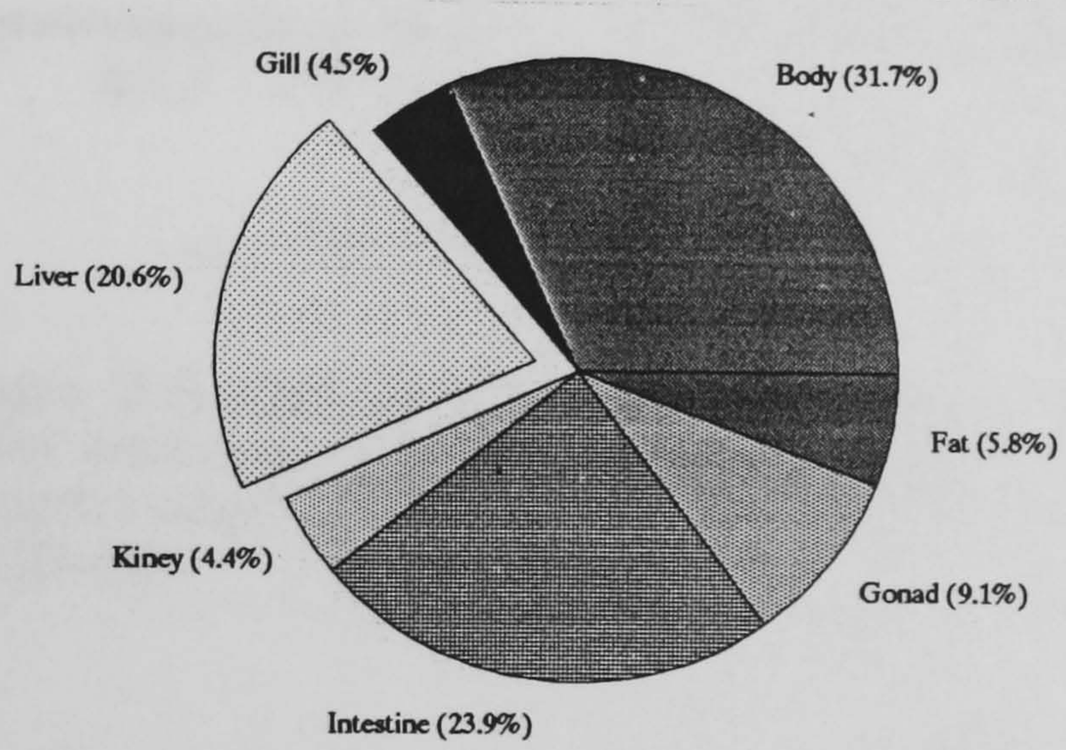



**Figure 7.3** Percentage of the total amount of copper in the tissues of three groups of sticklebacks; low-metal non-parasitised fish, high-metal non-parasitised fish, and high-metal parasitised fish. (The original data are listed in Appendix E.3).



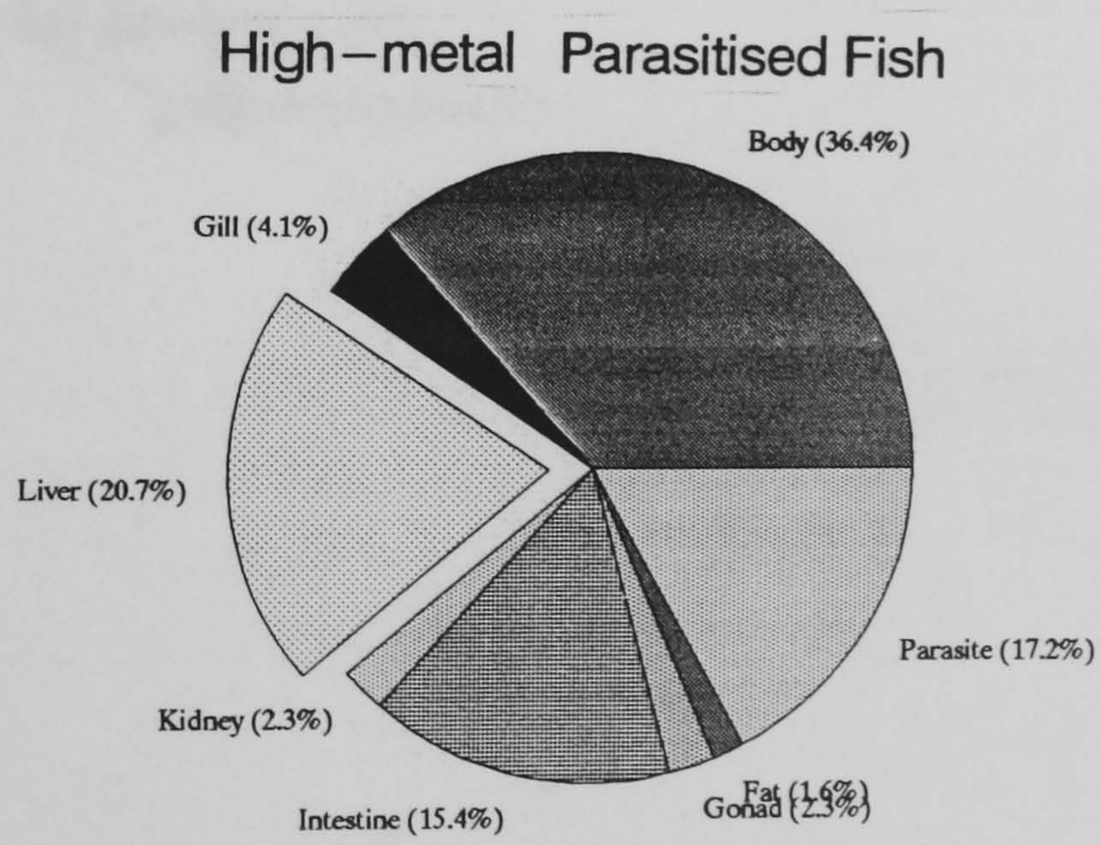




**Figure 7.4** Percentage of the total amount of cadmium in the tissues of three groups of sticklebacks; low-metal non-parasitised fish, high-metal non-parasitised fish, and high-metal parasitised fish. (The original data are listed in Appendix E.3).

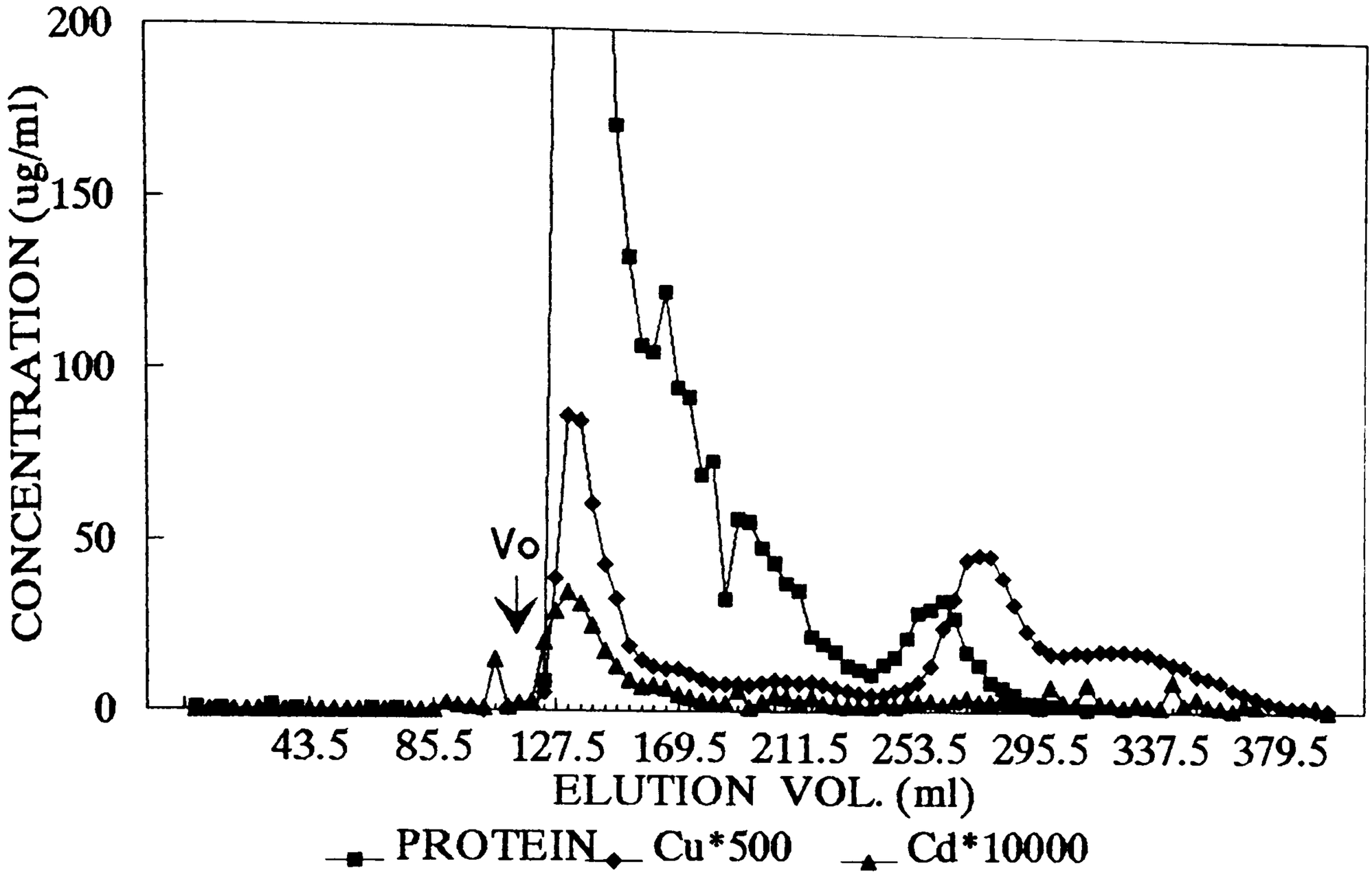



**Figure 7.5** Gel filtration elution profiles (Sephadex G-75) of buffer extracts of the liver of sticklebacks from the metal contaminated sites. (Vo: void volume is 125 ml, Vt: bed volume is 440 ml).

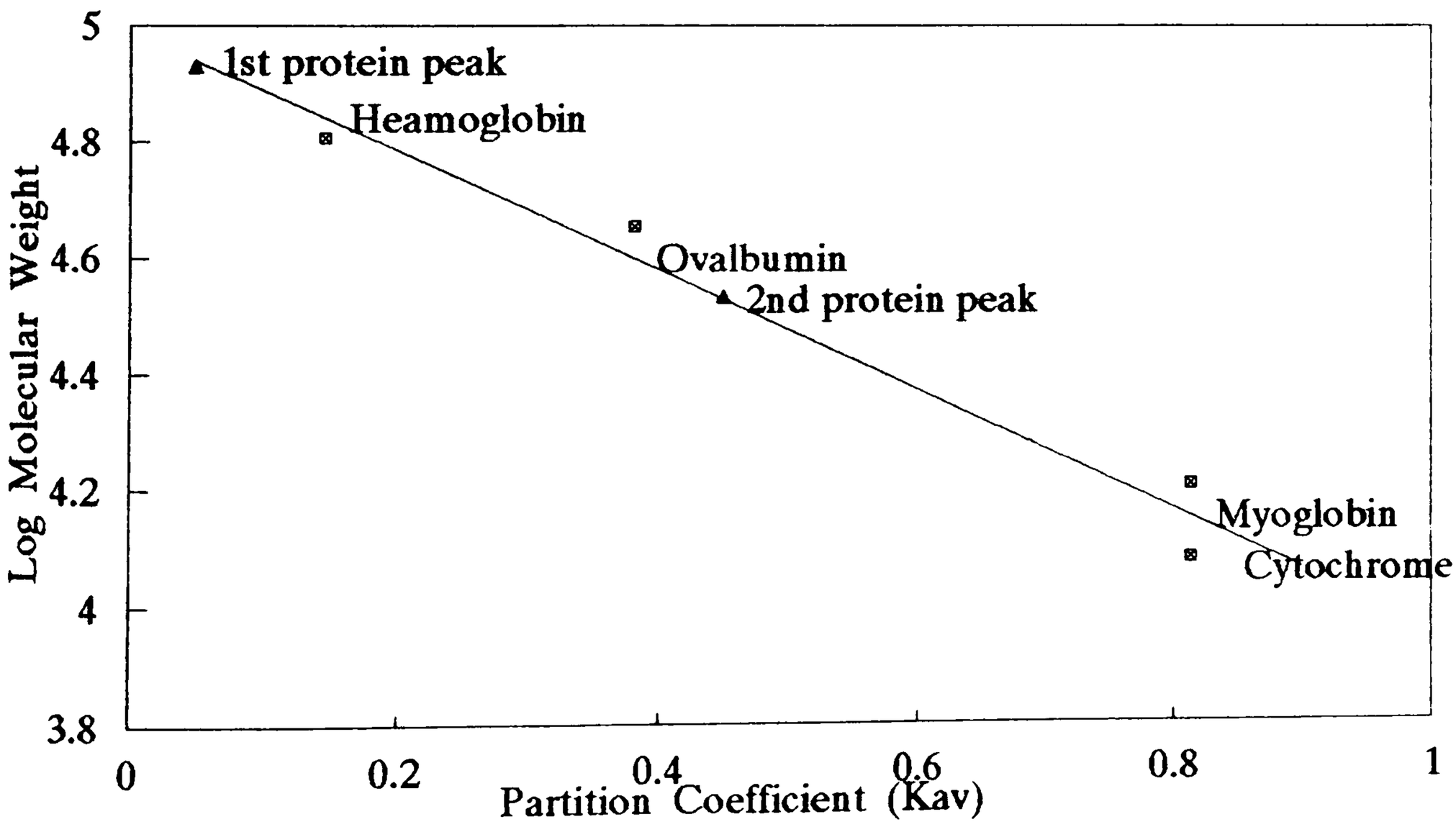



**Figure 7.6** Calibration curve of gel filtration column (Sephadex G-75), Kav = (Ve-Vo)/(Vt-Vo).

The size of the two protein peaks were estimated from the calibration graph of protein standards shown in Figure 7.6. The molecular weight of the second protein peak is about 39,800 Daltons. The first huge protein peak indicates a group of proteins with a molecular weight larger than 80,000 Daltons.

## 7.4 DISCUSSION

Table 7.1 in the introduction highlighted the fact that the pattern of accumulation of Cu and Cd in tissues of fish depended on the route of exposure. Various studies have shown that direct uptake from water generally results in elevated concentrations firstly in the gill tissue and then also in the liver and kidney. For example, Norey et al. (1990a,b,c) demonstrated that rainbow trout, stone loach and pike, exposed to long-term water-borne Cd revealed a phenomenon of shifting metal-deposit-organs, initially the gills then the liver and kidney. However, in experiments where Cu and Cd have been administered in the diet the main tissues showing metal accumulations were the intestine, liver and kidney. For example, rainbow trout fed Cd-labelled food showed intestine, liver and kidney accumulation (Kumada et al. 1980, Crespo et al. 1986, and Harrison & Klaverkamp 1990), and the same fish with orally administered Cu revealed an elevated Cu concentration in the liver (Knox et al. 1982, 1984).

The present study has clearly shown that the tissues where Cu and Cd accumulate are the intestine and notably the liver. From estimates of the proportions of the total metal in each tissue it was shown that most of the increase in the body burden of Cu and Cd in sticklebacks was accounted for by the increase of these metals in the liver.

It is also important to note that the gills of the stickleback did not show any accumulation of Cu and Cd. Furthermore it was established that Cu and Cd in the gills were not significantly different between (1) tissues in the same group of fish, (2) gill concentration in different groups of fish, and (3) the relative amounts in different groups of fish.

This is in contrast to the situation of Cu and Cd in the liver and intestine, where it was shown that concentrations in these tissues in

fish from contaminated sites were significantly greater than (1) other tissues in fish from the same sites, and (2) in the same tissue with respect to the reference site.

McKim and Benoit (1974) exposed two generations of brook trout to very low background of metals (2.7, 4.5, 6.1 and 9.4 ug/l) for two years and found no significant difference in metal accumulation in the various tissues. It was concluded that long-term water-borne metal exposure at background levels does not result in metal accumulation. It will be recalled from Chapter 6 that the highest dissolved Cu measured in the River Wandle water was 14 ug/l (total Cu = 21 ug/l). It seems unlikely therefore that the direct uptake of Cu (and possibly Cd) from water will account for the elevated concentration of the metal found in Wandle fish.

The distribution of Cu and Cd in tissues of parasitised fish was very similar to that of the non-parasitised fish from contaminated sites. Unfortunately no parasitised fish were available from site A for comparison. It would appear that the route of entry of the metals was the same in both groups of fish.

Various laboratory-based experiments and some field studies have shown that elevated metal concentration in the liver of fish are accompanied by an increase in MTT (e.g. Noel-Lambot et al. 1978, Yamamoto et al. 1978, Beattie & Pascoe 1979, Dixon & Sprague 1981, Kito et al. 1982a,b, Takeda & Shimizu 1982, Thomas et al. 1983, Roch & McCarter 1984, Benson & Birdge 1985, Hidalgo & Flos 1986). As stated earlier MTT appears to have a self-regulatory role for dealing with excess metal intakes. For example the common Dab, *Limanda limanda*, fed a casein based diet containing copper at a concentration of 1.4 mg Cu/kg (dry weight) for 20 weeks, resulted in increased synthesis of MTT. It was found that exposed fish had three times as much as MTT-bound copper in the livers than the control fish (Overnell and McIntosh 1988).

The elevated Cu and Cd concentrations found in the livers of fish from the contaminated sites provided an ideal opportunity to determine whether MTT could be found in a natural population. From the limited data obtained no evidence of Cd binding MTT was found. Also it is not clear if the second peak of Cu coincides with the second protein peak

in Figure 7.5. Furthermore the estimated molecular weight of 39,800 is much larger than those quoted for typical Cu-MTTs. Clearly this aspect of the work needs considerable development.

The overall conclusion from the results presented in this Chapter is that the diet is an important source of Cu and Cd in the stickleback of the River Wandle. The previous Chapter clearly showed that the fish were exposed to considerable amounts of the metals in the diet. The tissue distribution of Cu and Cd in this Chapter strongly suggest that food is the main source of metals burden in the fish.

However in the field situation it is very difficult to establish beyond doubt the relative importance of metals uptake via the gills and the alimentary canal. For example this study has only looked at the total concentration of metals, and nothing is known of the different forms of each metals in either the water or in the food. Furthermore very little is known about the relative efficiency of the absorption of different metals across the gill surfaces and lining of the gastrointestinal tract. The problem is further confounded by a fish such as the stickleback which is an opportunistic feeder and so obtains its food from a variety of sources.

It is acknowledged that an unequivocal demonstration of the importance of the food route for metal uptake is still lacking. It is suggested that future work should determine the species of Cu and Cd in the water and in the food and hence the forms in which they are available for uptake by the stickleback.

# CHAPTER EIGHT

## A SUMMARY DISCUSSION AND CONCLUSION

The main part of the project was formulated from the results of the pilot study reported in Chapter Three. It will be recalled that the concentrations of a number of metals were found to be elevated in the sediments at several sites. In conjunction with these results, preliminary analysis of metals in sticklebacks showed a similar trend to the metal concentrations in sediments.

The results of the pilot study raised a number of questions concerning the route and rate of supply of metals to sticklebacks and the accumulations of metals in relation to the growth of the fish. The main study of Cu and Cd in stickleback and in the habitat of the fish at a few sites was designed to tackle these questions. Early on in the study it was also realised that because the Wandle is a hard water river, the dissolved forms of Cu and Cd would be very low and a greater part of the metal load would be in the solid phases of the system. Consequently it was realised that it could not be assumed that the metal burdens in the fish arose directly from the water.

### 8.1 Development of Methods for Determining Cu and Cd

In a study of this nature it is important that the analytical techniques should have a high order of accuracy and precision. This was a particularly challenging task in a programme that involved a very large number of determinations and a very wide range of different types of samples. At one end of the range/spectrum there was the problem of determining ultra-trace concentrations in water and at the other end there were sediments that contained relatively high concentrations of Cu and Cd. The diverse range of organisms with their different types of tissues presented a number of matrix problems for metal determination by AAS.

As reported in Chapter Two, each type of sample was subjected to a quality control procedure, that involved use of standard additions and internationally recognised reference materials. Not surprisingly the

determination of Cu and Cd by flame AAS did not present any problems. Also it was relatively easy to develop drying-ashing-atomisation programme for the determination of Cu by flameless or graphite AAS. This is because Cu can withstand high ashing temperatures (900$^{o}$C) and so the potential interference by organic matter at the atomisation stage can be easily removed during the ashing stage of the programme. The results of analysis of the reference material show that the precision of the Cu measurements in this study were very good.

By contrast, Cd by flameless AAS presents a number of problems because it is a relatively volatile element and ashing temperatures even as low as 300-400$^{o}$C can result in loss of the element during the ashing stage but not all organic matter would be totally decomposed at these temperatures. Consequently there is the problem of loss of analyte and interference of the absorption signal as carbonaceous fumes can be emitted during atomisation. Great care was taken to ensure that all Cd in a sample was converted to a more stable phosphate form by the addition of $H_3PO_4$, which permitted a higher ashing temperature to be used. The addition of the surfactant, Triton-100 served to increase the precision of the analysis. Nevertheless the Cd results were not totally satisfactory and it was rather surprising to find that whilst standard additions produced parallel plots for a number of different types of samples, Lake Sediment stood out as the one type of sample in which there was still some interference in the absorption signal. This was never fully resolved since it was felt that in the study there would not be sediment samples needed to be exampled Cd concentration by flameless AAS.

## 8.2 General Consideration of the Sampling Sites and the Stickleback Populations

In the four surveys undertaken during 1989-1990 over 600 fish were collected from the sampling sites. This large sample size provided an opportunity to examine the complex relationships between growth and metal accumulation in fish from each of the three sites. Also the intensity of parasite infection by *Schistocephalus solidus* of about 20% permitted a unique opportunity to treat the infected fish as separate group in which to examine growth, metal accumulation and the

relationship between the metal content of the most fish and the parasite.

Because of the importance of site comparison in the project it is worthwhile discussing some general aspects of the sites. It was emphasised in the General Introduction that much of the flow of the River Wandle is due to sewage effluent and it is believed that this may be the major source of the metals, although there are a number of miscellaneous small inputs along the river. It remains chronically polluted and throughout most of its length according to the National Water Council Classification Scheme, (1990) it is class 3: poor. Early observations supported by the surveys of the main part of the project showed that Site A near Croydon is a relative impoverished site. Hynes (1960) described the typical changes in water quality below an organic input and the shifting occurence and abundances of selected organisms. *Cladophora* (the blanket weed) is widely associated with mild organic pollution or with the early stages of recovery from more severe pollution. It was common in this study of site B. As nutrients are used up or diluted, so it becomes less common as was the case at site E. Moreover, benthic invertebrates also show a clear pattern of response to the polluted condition. As conditions improve, blood worms, larvae of the midge *Chironomus*, became established, such as at site B where they were easily collected but not at site E. As further amelioration occurs, large populations of the isopod *Asellus* build up and as the stream gradually re-oxygenates, the clean water fauna increases in diversity and abundance as is found at site E which revealed a higher diversity of benthos and aquatic plants. These findings confirm the NWC classification of the Croydon arm which is rated at 2:fair improving to 1B:good at the junction with the Carlshalton arm before falling to the and staying at 3:poor below the Beddington sewage effluent discharge (NRA 1991).

These differences are borne out by the food found in the gut of sticklebacks. It is well known that sticklebacks are opportunistic feeders and quite a diverse range of food items in the fish revealed a number of differences, not least of which was the difference in the composition of diet found between the sites. At site A the fish depended more on small crustaceans and chironomids, at site B the diet of younger fish mainly consisted of small crustaceans and

chironomids but in older fish molluscs made up a significant proportion of the diet. Their diet at site E was much more varied and included chironomids, small crustaceans, molluscs, and amphipods.

Notwithstanding these difference, the growth of fish was similar at each of the sites. The basis of this statement is in the regressions between the length and dry weight of the fish at each site. Analysis of covariance showed that there was no difference in the regressions between all age groups at sites A and E and the $0^+$ age group at site B. Although it was found that the older fish at site B, the $1^+$ age group in particular, did not conform to the same growth pattern.

Using the length frequency method, it was established in Chapter Four that sticklebacks generally lived for three to four years. The age structure at the three sites did not fit the same pattern. Sites A and E had a higher proportion of younger fish than site B. In the later/last survey the older fish had essentially disappeared for no apparent reason. Considering the progression of the age structure through the successive surveys the common feature is the increasing dominance of the $0^+$ age group through the year that runs from one recruiting season to the next. Thus the four successive seasonally-based surveys essentially sampled the increasing size of the $0^+$ age group at each site.

## 8.3 Cu and Cd in Stickleback

Some general but nevertheless important points to emerge from the determination of Cu and Cd in stickleback include the following:

**(1)** The body burdens and concentrations of Cu and Cd were significantly elevated at the metal contaminated site.

**(2)** With reference to the literature it was established that the concentrations of Cu and Cd at the reference site were closed to typical background concentrations. At the metal contaminated sites, Cu concentrations were consider to be in the range of grossly polluted/contaminated, although Cd was classed as being moderately elevated.

**(3)** The study is one of only a few that have demonstrated elevated levels of metals in fish from a hard-water body.

One of the most significant features of the results of Cu and Cd in sticklebacks was the contrasting relationships found for body burdens of metals and the concentration of metal in different sizes of fish. Excellent regression models were established between the body burdens of Cu or Cd and the length or dry weight of fish at each site. The relationship clearly showed that the body burden of metal increased with size and therefore the age of fish. However the concentrations of Cu or Cd versus fish length or fish dry weight were more widely scattered and where a significant relationship was found the concentration of metal decreased with age. Further analysis using analysis of variance and the multiple range test between age groups indicated that the concentrations of Cu and Cd reached a peak in 'middle-aged' fish and concentrations decreased in the older fish. It is suggested that a more intensive sampling programme, perhaps monthly, would be needed to examine these trends further. The possible explanations put forward in Chapter Four for these differences between body burden and concentrations of metals are based on the idea that growth of the fish exerts a dilution effect on the concentration of metals in tissues. Also it will be recalled from Chapter Seven that the liver was found to the main organ of metal accumulation and much of the increased body burden in the fish is in this organ. Therefore concentration measurements based on a whole fish would mask what is happening in the liver and the concentration of metal would appear to have less relationship to the size of fish and even exhibit a decrease with the size of fish.

The published literature on metals, and other pollutants, in fish are almost totally based on concentration measurements. Internationally agreed standards are also based on concentrations. Probably the most well known example is that of mercury. In fish for human consumption the WHO maximum recommended concentration is 0.5 ug/g (fresh weight) and the EC value is 0.3 ug/g Hg. Although when it comes to an assessment of consumption of Hg it is estimated as the total intake of metal.

This study has cast some doubt on the value of concentration measurements. Moriarty (1984) came to the same conclusion and

emphasised the importance of body burden-age relationships as a basis of comparison. However the Wandle study has taken this a step further by highlighting that regression equation of metal body burdens and size of fish are not only site specific but also in some cases differences can be encountered between different age groups and between parasitised and non-parasitised fish at the same location. It is argued that there is a strong case for the more general use of metal body burdens as monitoring and regulatory tools. However it is emphasised that for each population of fish careful study should be made of the relationships between metal body burden and size in the various sub-groups such as different age groups and between parasitised and non-parasitised fish.

It is acknowledged that a sudden switch from concentration measurements to body burdens of metals in fish (and other organisms) is unlikely to occur. However it is clear that comparison of concentrations measurements is a dubious exercise without consideration of the age of fish. Sticklebacks are relatively short-lived fish and in long-lived fish age standardised concentrations of metals would be even more important.

Another important point that emerges from this study is the possible use of body burdens to assess the toxicity of a metal. A fundamental relationship in toxicology is that between exposure, body burden and toxicity. This is particularly relevant in situations involving persistent toxicants such as metals, chronic exposures and exposures which have more than one route of supply. The body burden represents an integrated picture of exposure over a period of time. There are many examples in which the body burden or some indices of body burden of a chemical is related to the severity of poisoning. For mercury, lead and cadmium dose-response relationships have been established which related such parameters as blood, hair, urine and teeth concentrations of the metal to the signs and symptoms of poisoning.

Throughout aquatic toxicology, exposure or water concentration is related to the toxic response which is usually fatality e.g. $LC_{50}$ and $LD_{50}$. One of the most important issues is that or relating laboratory toxicity data to the fields situation. For many chemicals and in particular metals, the basic problem is that of assessing exposure in the field. This study has shown that Cu and Cd are mainly found in

the sediments and organic detritus. The concentrations of the metals in the water were quite low and the most bioavailable free metal ion concentration would be less than, and probably much less than, the total concentration. Also in this study it has been shown that sticklebacks are exposed to significant quantities of metal through the food route. In addition, it is probable that exposure will vary over short distances and with time. This is can be due to variation of the flow or volume of water, and changes in source strength. Consequently it is very difficult to quantify metal exposure in the field with any degree of certainty and hence equally to translate laboratory derived $LC_{50}$ values and chronic sub-lethal toxic responses to the field situation. Nevertheless water quality standards are based on determining the 'no observable effect concentration' by laboratory experimentation and the 'expected environmental exposure' which is essentially an estimate of the water concentration.

In view of the results obtained from the Wandle study of Cu and Cd in sticklebacks it is surprising that so little use has been made of body burden or target tissue burden to metals as an integrated measure of exposure. It is conceivable that this parameter could be extremely useful in translating laboratory-based dose-response relationships to the field situation. As a result routine monitoring could be based on body burdens and serve an important regulatory purpose.

## 8.4 Influence of Parasite Infection on the Cu and Cd Contents of Sticklebacks.

A unique aspect of the study has been the investigation of the influence of parasite infection on the growth and metal accumulation of sticklebacks. In the discussion of Chapter Four, it was recognised that the nature of the infection by the cestode parasite was not typical of the found in other studies. The incidence of infection of around 20% was lower than that reported for other populations where incidences as high as 90 and even 100% have been found. Secondly the intensity of infection of about one or two worms per fish was very much smaller than in other populations where up to 130 worms per fish has been reported. On the other hand the biomass of parasite per fish as indicated by the parasite index is similar to that found in other studies. The other significant feature of the Wandle study is that the

length-weight relationships of parasitised fish were significantly different to the non-parasitised fish. The parasitised fish were lighter than the non-parasitised counterparts while they were in same length.

Consequently it is important to bear in mind that the picture found for parasitised fish in the Wandle may not be typical of that to be found elsewhere. For instance, sticklebacks with a high intensity of infection may show very different patterns of metal accumulation than the parasitised fish in the Wandle. The Wandle study has highlighted some differences between non-parasitised and parasitised fish, although some of them are not easy to explain. Using the length standardised metal body burdens reported in Table 4.30, there is an indication that the Cu and Cd body burden of parasitised fish is less than that in non-parasitised fish. The one exception is the body burden of Cu at site B where the parasitised fish have a greater body burden. From consideration of the regression equations for body burdens of Cu or Cd versus dry weights in the two groups of fish, there is an indication that the pattern of metal accumulation is different. It will be recalled that for non-parasitised fish the equations were significantly different, but for parasitised fish only the intercepts (and not the slopes) were significantly different.

It is possible that the intake and absorption of metal in parasitised fish is different from the non-parasitised group. It has been reported that parasitised fish have a greater $O_2$ and food consumption but if anything this would have enhanced the site differences in metal burden and as a consequence the regressions would have been more likely to have been significantly different in parasitised fish. Alternatively it is possible that the intake of metal is similar in the two groups of fish but some of the metal is diverted to the parasite. This seems more clear cut for Cd than for Cu. The Cd body burden in the cestode parasite increased in proportion to age and exposure. As a result the regression slopes are significantly different between sites, and Cd concentration in the cestode parasite is always higher than that in the host fish. However for Cu the picture is different, and the content of Cu in the cestode parasite which is always lower than in the host fish, appears to be regulated.

Pascoe and Cram (1977) have shown that parasitised sticklebacks are more susceptible to Cd. However the toxicity determinations were

based on Cd concentrations in the water. It would be very interesting to ascertain the body burden of Cd and other metals that produced the same toxic response. From this it could be determined whether any difference in sensitivity was a function of exposure and absorption of metal or if it demonstrated a true synergistic effect of one stress compounding another.

The interaction of pollution and fish diseases is a key issue at the present time and very little is known about the interaction of different types of natural and artificial stresses. It is felt that the Wandle study has provided an important base on which to mount a subsequent study concerned specifically with accumulation and toxicity of toxins and parasite infection.

## 8.5 Evaluation of Cu and Cd Exposure to Stickleback Populations

The second part of the Wandle study which is presented in Chapters 5, 6, and 7 has been concerned with the assessment of exposure of Cu and Cd to the stickleback populations at the three sites. Essentially this has been a qualitative assessment, based on determining the composition of the diet of sticklebacks, and measuring the concentrations of Cu and Cd in the water, sediments and organic detritus and in a variety of organisms which were considered as potential food items.

There are relatively few examples in the literature of detailed studies of the distribution of metals in the various biotic and abiotic components of aquatic systems (Mathis & Cummings 1973, Mathis & Kevern 1975, Enk & Mathis 1977, Dallinger & Kautzky 1985). A general point to emerge during sampling was that relatively few organisms were common to the three sites and this made direct comparison between sites more difficult. The response for differences in the composition of the communities over such small distances are probably related to the nature of the habitat and the water quality.

Figure 6.3 in Chapter 6 showing the Cu and Cd concentrations in water, sediments, *Asellus aquaticus*, *Lymnaea peregra*, *Spirogyra* sp. and *Gasterosteus aculeatus* highlight the trend that was found for all types of samples. The samples from the contaminated sites B and E

have elevated concentrations relative to those at site A. It is important to note that this trend was also found for the water samples. It was also found that whilst Cu and Cd concentrations in sediment were higher at site E than at site B, in most of the other types of sample the values were higher at site B. It is thought that this indicates differences in the complexed and bound forms of the metal at the two sites. The other interesting feature was the high Cu concentrations that were found in invertebrates which have haemocyanin blood pigment. *Lymnaea peregra* is good example of this group with Cu concentrations of 50, 302, and 300 ug/g at sites A, B and E, respectively. Surprisingly very little is known about the dynamics of copper accumulation in this groups of organisms. It seems as though there is very little metabolic regulation of Cu which contrasts with the situation in stickleback where tissue concentrations of Cu appear to be under some degree of control.

The distributions of Cu and Cd between the various components at each site are more or less as predicted. Figures 8.1 and 8.2 have been constructed from the data presented in Chapter 6 and they show the distribution of the metals in different trophic levels of the system. The relative distributions of Cu and Cd between the sediments, organic detritus and the water emphasise the physical and chemical characteristics of the River Wandle. The surfaces of sediment particle and organic detritus provide numerous sites for metal absorption. The alkaline pH conditions which occur in hard-waters promoted the absorption of Cu and Cd to solid surfaces. Consequently metal concentrations are high in the sediments and organic detritus but quite low in the water. Despite the fact that site difference existed in water samples, it was established in Chapter Six that Cd water concentrations at all sites were slightly elevated were only just above background values at the contaminated sites. Figures 8.1 and 8.2 (as well as Figure 6.4) also emphasise the point that water concentrations are at least 3 order of magnitude less than the concentration in other parts of the system. Comparing the concentrations in biota, it can be seen that sticklebacks tend to have the lowest concentrations of Cu and Cd. This fits in with the established view that metals other than perhaps mercury in the form of methyl mercury, are not biomagnified through aquatic food chains. It would also appear that stickleback are

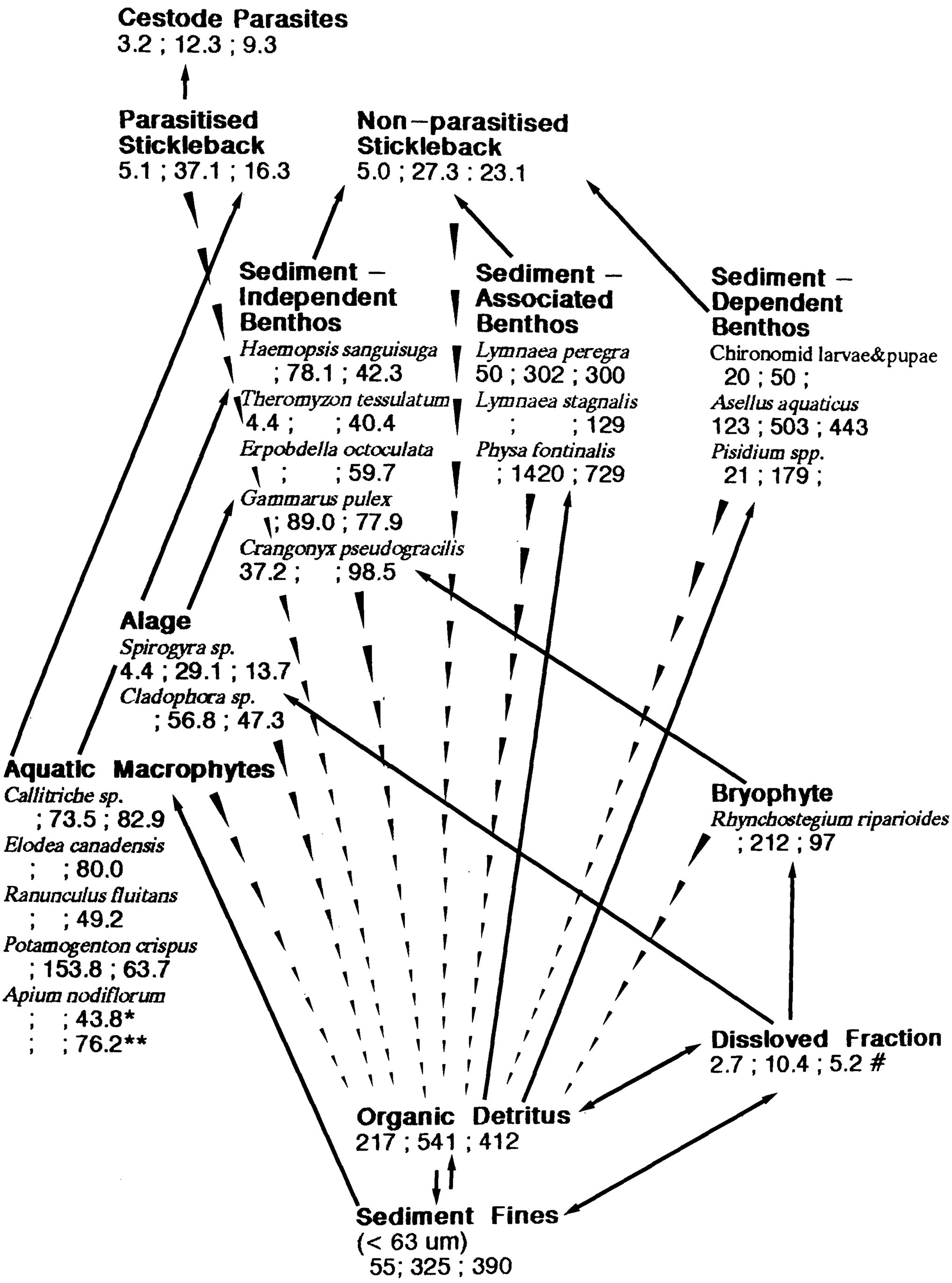



**Figure 8.1** Copper distribution in a urban river system. Values represent in sequence order as sites A, B, and E in the River Wandle. The unit is in ug/g dry weight, except # in ug/l.
* indicates the leave part, ** indicates the root part.

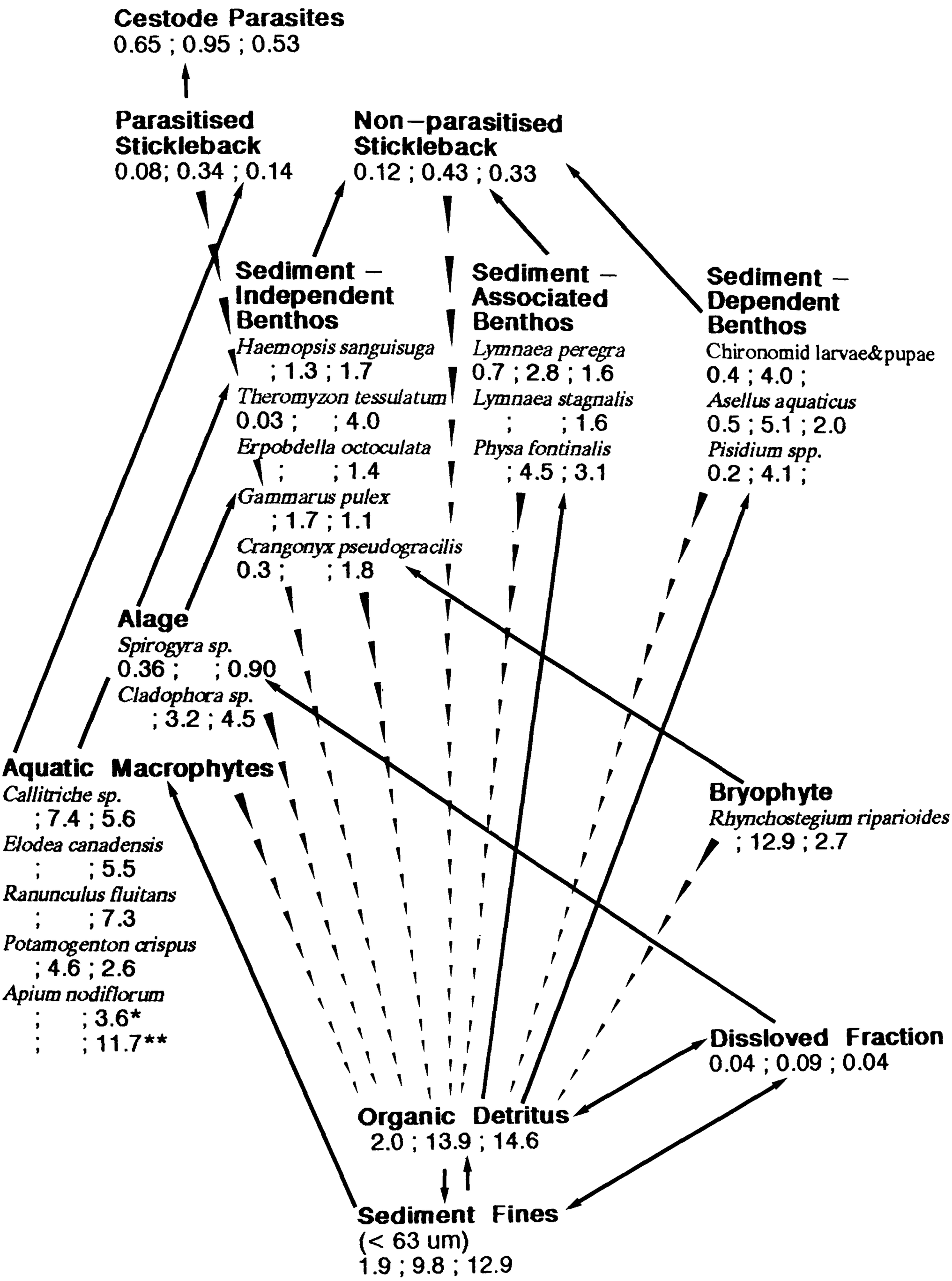



**Figure 8.2** Cadmium distribution in a urban river system. Values represent in sequence order as sites A, B, and E in the River Wandle. The unit is in ug/g dry weight, except # in ug/l. * indicates the leave part, ** indicates the root part.

able to regulate Cu and Cd uptake more strictly than the other organisms in the system.

The main objective of Chapters 6 and 7 was to determine if the Cu and Cd taken via food accounted for a significant part if not all of the metal body burden in the fish. Or to put it the other way round; could the Cu and Cd concentrations in the water account for the body burdens of metal in the fish ? After all there are site differences in the dissolved concentrations of the metals and the volume of water passed over the gills may be sufficient to produce the concentrations and body burdens found in the fish. Clearly this cannot be disproved, and the literature on this subject is not very helpful. It has already been stated that all of the Cd dissolved concentrations are in the background range. However some of the biota at the contaminated sites have markedly elevated levels of Cd and in Chapter Four it was established that the Cd concentration in stickleback at the contaminated site could be considered as being moderately contaminated. In the case of Cu, water concentrations are more elevated than Cd and one study quoted in Chapter Six recorded a behavioural response in zebrafish exposed to 10 ug Cu/l in water in a laboratory study (Steele et al. 1990). However McKim and Benoit (1974) examined the uptake Cu of brook trout exposed to water concentrations of 4.5, 6.1 and 9.4 ug Cu/l for two years and did not find elevated levels in the fish at these low concentrations. Also it is important to remember that the dissolved Cu concentration in the Wandle would have included a number of different forms as well as free metal ions.

The significant feature of Chapters Five and Six is that stickleback are exposed to considerable quantities of Cu and Cd in the diet. This comes from consideration of the composition of the diet and the measurement Cu and Cd in most of the food items. It is unfortunate that Cu and Cd were not determined in small crustaceans which constituted such an important part of the diet particularly in fish from site A. This was because these small organisms are very difficult to collect and separate from suspended particulate matter. The other aspect that is evident from this part of the study is that Cd and Cu exposure can increased as a result of a change in the diet. This was most clearly shown for Cu, since fish at site A consumed more plant

material and chironomids, whereas at site B, the older fish especially, consumed more molluscs which had much higher concentrations of Cu.

Although it has been established that considerable amounts of Cu and Cd are taken in with food, the results of Chapter 5 and 6 do not show if/whether the Cu and Cd content of stickleback is derived from the food route and/or the water route. One way of pursuing this aspect further would be to examine the absorption of metal from the food of sticklebacks. For example, it may be possible to develop micro-analytical method to actually determine the concentration of metal in successive parts of the gastrointestinal tract. This could be complemented with laboratory studies examining the metals in food, in the gut contents and in faeces to estimate the amount of metal absorbed.

The final part of the study was concerned with examining the tissue distribution of Cu and Cd in sticklebacks. It was felt this approach would shed further light on the route of supply of the metals to the fish. As pointed out in Chapter Seven, it is quite well established that if the gills are the main route of uptake then this tissue would have elevated metal concentrations. On the other hand, studies which have examined metal absorption from the diet have shown the intestine and the liver are the main tissues of accumulation. In fact the picture that emerged was even clearer than expected. The Cu and Cd concentration in the gills was not significantly greater between the reference site and the contaminated sites but the intestine and the liver were significantly different for both metals. It was shown that most of the increased body burdens of Cu and Cd were due to the accumulation of the metals in the liver. It was therefore concluded that, although the evidence was indirect, it nevertheless pointed strongly to the food route as being a significant source of the body burden of Cu and Cd in sticklebacks.

The overall conclusions are that the body burdens of Cu and Cd in sticklebacks at certain locations in the River Wandle are elevated markedly as a consequence of discharges of these metals in sewage effluents. The concentrations of Cu are as high as values quoted in other studies that were considered to be grossly contaminated. Cadmium values are typical of urban rivers and they are moderately

elevated. This raises the possibility that the fish and perhaps other organisms in the Wandle may be exposed to levels which are detrimental to the health of the organisms. Although it is conceded that measurements of the growth of the stickleback populations over a period of one year did not show a decrease at the metal contaminated sites. Alternatively it is possible that the sticklebacks at the contaminated sites are more tolerant to the elevated metal exposure regime. One well known response to prolonged metal exposure is an increased production of metal binding proteins e.g. metallothioneins in the liver. This has received only a preliminary consideration in the present study and it is though that it is an area which could be further developed. The possible interaction of toxic metal stress and parasite infection is largely unknown. This study has drawn attention to some difference but it is not known if these are typical of other situations and it is not known whether fish infected with parasites are more susceptible to metal stress. Further more, even assuming that sticklebacks are not under metal stress at the present time it is important to ascertain what further increases in metal input would produce a toxic response. It is clear that in situations similar to the River Wandle, in which laboratory studies are needed to clarify an existing problem or to assess a potential problem, then this should be undertaken be equating metal body burden with a toxic response. This is all the more essential where metals enter the fish both via the water across the gills and via food through the intestine. It should also be the basis for assessing the interaction of natural and pollutant stresses, and for examing the sensitivity to metal toxicity in different species of fish.

# REFERENCE


**Abel, P.** (1989). Water Pollution Biology. Ellis Horwood Ltd., Chichester, 231 pp..

**Abo-Rady, M.D.K.** (1979). The levels of heavy metals (Cd, Cu, Hg, Ni, Pb, Zn) in brook trout from the River Leine in the Area of Gottingen (West Germany). Z Lebensm Unters Forsch 168:259-263.

**Abo-Rady, M.D.K.** (1980). Aquatic macrophytes as indicator for heavy metal pollution in the River Leine (West Germany). Arch. Hydrobiol. 89(3):387-404.

**Ajmal, M., R. Uddin and A.U. Khan** (1988). Heavy metals in water, sediments, plants and fish of Kali Nadi U.P. (India). Environ. Internat. 14:515-523.

**Alabaster, J.S. and R. Lloyd** (1982). Water Quality Criteria for Freshwater Fish. 2nd Edition. FAO, Butterworths, 361 pp.

**Allen, J.R.M. and R.J. Wootton** (1982a). Effect of food on the growth of carcase, liver and ovary in female *Gasterosteus aculeatus* L. J. Fish Biol. 21:537-547.

**Allen, J.R.M. and R.J. Wootton** (1982b). Age, growth and rate of food consumption in an upland population of the three-spined stickleback, *Gasterosteus aculeatus* L. J. Fish Biol. 21:95-105.

**Allen, J.R.M. and R.J. Wootton** (1984). Temporal patterns in diet and rate of food consumption of the three-spined stickleback (*Gasterosteus aculeatus* L.) in Llyn Frongoch, an upland Welsh lake. Freshwat. Biol. 14:335-346.

**Andersson, P., and H. Borg** (1988). Effects of liming on the distribution of cadmium in water, sediment, and organisms in Swedish lake. Can. J. Fish. Aquat. Sci. 45:1154-1162.

**Arme, C. and R.W. Owen** (1967). Infection of the three-spined stickleback, *Gasterosteus aculeatus* L., with the plerocercoid larvae of *Schistocephalus solidus* (Muller, 1776), with special reference to pathological effects. Parasitology 57:301-314.

**Armstrong, F.A.J. and A. Lutz** (1977a). Lake Superior 1974: PCB, chlorinated insecticides, heavy metals and radioactivity in offshore fish. Fish. Mar. Serv. Tech. Rep. 693: vi+17 p.

**Armstrong, F.A.J. and A. Lutz** (1977b). Lake Huron 1974: PCB, chlorinated insecticides, heavy metals and radioactivity in offshore fish. Fish. Mar. Serv. Tech. Rep. 692: v+14 p.

**Badsha, K.S. and C.R. Goldspink** (1982). Preliminary observations on the heavy metal content of four species of freshwater fish in NW England. J. Fish Biol. 21:251-267.

**Badsha, K.S. and C.R. Goldspink** (1988). Heavy metal levels in three species of fish in Tjeukemeer, A Dutch Polder Lake. Chemosphere 17(2):459-463.

**Barak, N.A-E. and C.F. Mason** (1989). Heavy metals in water, sediment and invertebrates from rivers in Eastern England. Chemosphere 19(10/11):1709-1714.

**Barak, N.A-E. and C.F. Mason** (1990a). Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Sci. Total Environ. 92:249-256.

**Barak, N.A-E. and C.F. Mason** (1990b). Mercury, cadmium and lead concentrations in five species of freshwater fish from Eastern England. Sci. Total Environ. 92:257-263.

**Beattie, J.H. and D. Pascoe** (1979). A cadmium-binding protein in rainbow trout. Toxicol. Letters 4:241-246.

**Bebbington, G.N., N.J. Mackay, R. Chvojka, R.J. Williams, A. Dunn and E.H. Auty** (1977). Heavy metals, selenium and arsenic in Nine species of Australian commercial fish. Aust. J. Mar. Freshwater Res. 28:277-286.

**Bendell-Young, L.I., H.H. Harvey and J.F. Young** (1986). Accumulation of cadmium by white suckers (*Catostomus commersoni*) in relation to fish growth and lake acidification. Can. J. Fish. Aquat. Sci. 43:806-811.

**Benoit, D.A., E.N. Leonard, G.M. Christenson and J.T. Fiandt** (1976). Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 105:550-560.

**Benson, W.H. and W.J. Birge** (1985). Heavy metal tolerance and metallothionein induction in fathead minnows: Results from field and laboratory investigations. Environ. Toxicol. Chem. 4:209-217.

**Binnemann, P.H., U. Sandmeyer and E. Schmuck** (1983). Contents of heavy metals, organochlorine pesticides, PCB and volatile halogenated hydrocarbons in fish samples from the "Hoch- and Oberrhine" and Lake Constance. Z Lebensm Unters Forsch 176:253-261.

**Blake, C.J.** (1980). Sample preparation methods for the analysis of metals in foods by atomic absorption spectrometry - A literature review. The British Food Manufacturing Industries Research Association, Scientific & Technical Surveys No. 122, October 1980.

**Bradley, R.W. and J.R. Morris** (1986). Heavy metals in fish from a series of metal-contaminated lakes near Sudbury, Ontario. Water Air Soil Pollut. 27:341-354.

**Bradford, M.M.** (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 12:248-254.

**Brafield, A.E. and P. Matthiessen** (1976). Oxygen consumption by sticklebacks (Gasterosteus aculeatus L.) exposed to zinc. J. Fish Biol. 9:359-370.

**Brodie, K. and J, Rowland** (1981). A simple fault-finding guide for flame atomic absorption spectrometry. Varian Instruments at Work, No. AA-12, February 981.

**Brown, B.T. and B.M. Rattigan** (1979). Toxicity of soluble copper and other metal ions to *Elodea canadensis*. Environ. Pollut. ? :304-315.

**Buckley, J.A., G.A. Wells and R.T. Aquino** (1985). Toxicities of total and chelex-labile cadmium to salmon in solutions of natural water and diluted sewage with potentially different cadmium complexing capacities. Wat. Res. 19:1549-1554.

**Buckley, J.A. and G.A. Yoshida** (1989). Effects of treated sewage on the availability of cadmium to coho salmon. Chem. Spec. Biolavail. 1(1):25-30.

**Bull, K.** (1975). Routine heavy metal analysis of biological samples. Science chelsea, J. Res. Chel. 7:23-25.

**Cabrera, F., M. Soldevilla, R. Cordon and P. de Arambarri** (1987). Heavy metal pollution in the Guadiamar River and the Guadalquivir estuary (South West Spain). Chemospere 16(2/3):463-468.

**Cairns, V.W., P.V. Hodson, and J.O. Nriagu** (1984). CONTAMINANT EFFECTS ON FISHERIES. John Wiley & Sons. 333 pp.

**Calamari, D., R. Marchetti, and G. Vailati** (1979). Influence of water hardness on cadmium toxicity to *Salmo gairdneri* Rich. Wat. Res. 14:1421-1426.

**Carpene, E., O. Cattani, G.P. Serrasanetti, G. Fedrizze and P. Cortesi** (1990). Zinc and copper in fish from natural waters and rearing ponds in Northern Italy. J. Fish Biol. 37:293-299.

**Carroll, J.J., S.J. Ellis, and W.S. Oliver** (1979). Influences of hardness constituents on the acute toxicity of cadmium to brook trout (*Salvenlinus fontinalis*). Bull. Environ. Contam. Toxicol. 22:575-581.

**Cassie, R.M.** (1954). Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshwater Res. 5:513-522.

**Chappell, L.M.** (1969). The parasites of the three-spined stickleback, *Gasterosteus aculeatus* L., from a Yorkshire pond. I. Seasonal variation of parasite fauna. J. Fish Biol. 1:137-152.

**Chernoff, B. and J.K. Dooley** (1979). Heavy metals in relation to the biology of the mummichog, *Fundulus heteroclitus*. J. Fish Biol. 14:309-328.

**Clark, E.R. and J.A.L. Fraser** (1983). The survival and growth of six species of freshwater fish, in tapwater and diluted and undiluted effluent from sewage percolating filters. J. Fish Biol. 22:431-445.

**Clarkson, T.W., L.Friberg, G.F. Nordberg and P.R. Sager** (1988). Biological Monitoring of Toxic Metals. Plenum Press, New York and London. 663 pp.

**Clubb, R.W., A.R. Gaufin and J.L. Lords** (1975). Acute cadmium toxicity studies upon nine species of aquatic insects. Environ. Res. 9:332-341.

**Cock, M.J.W.** (1978). The assessment of preference. J. Anim. Ecol. 47:805-816.

**Coombs, T.L.** (1980). Heavy metal pollutants in the aquatic environment. In; (Gilles, R. ed.) Animals and environmental fitness. Pergaman Press, Oxford, New York, pp.283-302.

**Crespo, S., G. Nonnotte, D.A. Colin, C. Leray, L. Nonnotte and A. Aubree** (1986). Morphological and functional alterations induced in trout intestine by dietary cadmium and lead. J. Fish Biol. 28:69-80.

**Cross, F.A., L.H. Hardy, N.Y. Jones and R.T. Barber** (1973). Relation between total body weight and concentrations of manganese, iron, copper, zinc, and mercury in whiter muscle of bluefish (*Pomatomus saltatrix*) and a bathyl-demersal fish, *Antimora rostrata*. J. Fish Res. Board Can. 30:1287-1291.

**Culver, B.R., J.F. Lech and N.K. Prahan** (1975). Trace metal analysis of foods by non-flame atomic absorption spectroscopy. Fd Technol. 1975(Mar.):16-25.

**Dallinger, R., F. Prosi, H. Segner, and H. Back** (1987). Contaminated food and uptake of heavy metals by fish: A review and a proposal for further research. Oecologia 73:91-98.

**Dallinger, R and H. Kautzky** (1985). The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): A field study. Oecologia 67:82-89.

**Darbre, A** (1986). Analytical Methods. In: Practical Protein Chemistry - A Handbook. (A. Darbre ed.), John Wiley and Sons Ltd. pp. 227-335.

**Dave, G. and R. Xiu** (1991). Toxicity of mercury, copper, nickel, lead, and cobalt to embryos and larvae of zebrafish, *Brachydanio rerio*. Arch. Environ. Contam. Toxicol. 21:126-134.

**Denton, G.R.W. and C. Burdon-Jones** (1986). Trace metal in fish from the Great Barrier Reef. Marine Pollut. Bull. 17(5):201-209.

**Dixon, D.G. and J.B. Sprague** (1981). Copper bioaccumulation and hepatoprotein synthesis during acclimation to copper by juvenile rainbow trout. Aquat. Toxicol. 1:69-81.

**Douben, P.T.** (1989). Lead and cadmium in stone loach (*Noemacheilus barbatulus* L.) from three rivers in Derbyshire. Ecotoxicol. Environ. Saf. 18:35-58.

**Duzzin, B., B. Pavoni and R. Donazzolo** (1988). Macroinvertebrate communities and sediments as pollution indicators for heavy metals in the River Adige (Italy). Wat. Res. 22(11):1353-1363.

**Eaton, J.G.** (1974). Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans. Am. Fish. Soc. 103:729-735.

**Edgren, M. and M. Notter** (1980). Cadmium uptake by fingerlings of perch (*Perca fluviatilis*) studied by Cd-115m at two different temperatures. Bull. Environ. Contam. Toxicol. 24:647-651.

**El-Domiaty, N.A.** (1987). Stress response of juvenile *Calrias lazera* elicited by copper. Comp. Biochem. Physiol. 88C(2):259-262.

**Elder, J.F.** (1975). Complexation side reactions involving trace metals in natural water systems. Limnol. Oceanogr. 20:96-102.

**Enk, M.D. and B.J. Mathis** (1977). Distribution of cadmium and lead in a stream ecosystem. Hydrobiologia 52(2/3):153-158.

**Eustace, I.J.** (1974). Zinc, cadmium, copper, and manganese in species of finfish and shellfish caught in the Derwent Estuary, Tasmania. Aust. J. mar. Freshwat. Res. 25:209-220.

**Evans, M.S.** (1988). Toxic Contaminants and Ecosystem Health: A Great Lakes Focus. John Wiley & Sons. 600 pp.

**Eyres, J.P. and M. Pugh-Thomas** (1978). Heavy metal pollution of the River Irwell (Lancarshire, UK) demonstrated by analysis of substrata materials and macroinvertebrate tissue. Environ. Pollut. 16(2):129-136.

**Falandysz, J.** (1985). Trace metals in flatfish from the Southern Baltic, 1983. Z Lebensm Unters Forsch 181:117-120.

**Falandysz, J.** (1986). Trace metals in sprats from the southern Baltic, 1983. Z Lebensm Unters Forsch 182:40-43.

**Florence, T.M.** (1982). The speciation of trace elements in waters. Talanta 29:345-364.

**Forstner, U. and F. Prosi** (1979). Heavy metal pollution in freshwater ecosystem. In; (Rovera, O. ed.) Biological aspects of freshwater pollution. Comission of the European Communities. Pergaman Press, pp.129-161.

**Forstner U. and G.T.W. Wittmann** (1983). Metal Pollution in the Aquatic Environment. Spring-Verlag, Berlin, Heidelberg, New York, Tokyo. 486 pp.

**Gamble, D.S. and M. Schnitzer** (1974). The chemistry of fulvic acid and its reactions with metal ions. In: Trace Metals and Metal-Organic Interactions in Natural Waters (Edited by Singer P.C.) 380 pp. Ann Arbor Science, Michigan.

**Gardiner, J.** (1974a). The chemistry of cadmium in natural water - I. A study of cadmium complex formation using the cadmium specific-ion electrode. Wat. Res. 8:23-30.

**Gardiner, J.** (1974b). The chemistry of cadmium in natural water - II. The absorption of cadmium on river muds and naturally occurring solids. Wat. Res. 8:157-164.

**Gatlin, III. D.M. and R.P. Wilson** (1986). Dietary copper requirement of fingering channel catfish. Aquaculture 54:277-285.

**Ghosh, A.R. and P. Chakrabarti** (1990). Toxicity of arsenic and cadmium to a freshwater fish, *Notopterus notopterus*. Environ. Ecol. 8(2):576-579.

**Giesy, J.P.Jr., G.J. Leversee and D.R. Williams** (1977). Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphnidae) and *Gambusia affinis* (Poeciliidae). Wat. Res. 11:1013-1020.

**Giesy, J.P.Jr. and J.G. Wiener** (1977). Frequency distribution of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. 106:393-403.

**Gilber, T.R. and J.P. Kakareka** (1985). Analytical Chemistry. In; Fundamental Aquatic Toxicology (Rand, G.M. and S.R. Petrocelli eds.) Hemisphere Publishing Corporation, Washington.

**Giordano, R., P. Arata, L. Ciaralli, S. Rinaldi, M. Giani, A.M. Cicera and S. Costantini** (1991). Heavy metals in mussels and fish from Italian coastal waters. Mar. Pollut. Bull. 22(1):10-14.

**Godin, J.G.J. and C.D. Sproul** (1988). Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. Can. J. Zool. 66:2360-2367.

**Gokhale, K.S. and N.N. Patil** (1989). Relationship of metals in different organs of *Mystus gulio* (Ham.) with environmental parameters and biology of fish. J. Environ. Biol. 10(3):265-271.

**Haesloop, U. and M. Schirmer** (1985). Accumulation of orally administered cadmium by the eel (*Anguilla anguilla*). Chemosphere 14:1627-1634.

**Hamilton, S.J. and P.M. Mehrle** (1986). Metallothionein in fish: Review of its importance in assessing stress from metal contaminants. Trans. Am. Fish. Soc. 115:596-609.

**Harding, L. and D. Goyette** (1989). Metals in Northeast Pacific coastal sediments and fish, shrimp, and prawn tissues. Mar. Pollut. Bull. 20(4):187-189.

**Harding, J.P.C. and B.A. Whitton** (1978). Zinc, cadmium, and lead in water, sediments and submerged plants of the Derwent Reservoir, northern England. Wat. Res. 12:307-316.

**Harding J.P.** (1947). The use of probability paper for the graphical analysis of polymodal frequency distributions. J. mar. Biol. Ass. U.K. 28:141-153.

**Hardisty, M.W., R.J. Huggins, S. Kartar and M. Sainsbury** (1974a). Ecological implications of heavy metal in fish from the Severn Estuary. Mar. Pollut. Ecol. 5:12-15.

**Hardisty, M.W., S. Kartar and M. Sainsbury** (1974b). Dietary habits and heavy metal concentrations in fish from the Severn Estuary and Bristol Channel. Mar. Pollut. Ecol. 5:61-63.

**Harrison, S.E. and J.F. Klaverkamp** (1989). Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaforms* Mitchill). Environ. Toxicol. Chem. 8:87-97.

**Harrison, S.E. and J.E. Klaverkamp** (1990). Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and in sediments from lakes near the smelter at Flin Flon, Manitoba. Environ. Toxicol. Chem. 9(7):941-956.

**Hartung, R.** (1973). Biological effects of heavy metal pollutants in water. Adv. Exp. Med. Biol. 40:161-172.

**Haslam, S.M.** (1982). A proposed method for monitoring river pollution using macrophytes. Environ. Technol. Letters. 3:19-34.

**Haslam, S.M.** (1990). The effect of pollution on plants. In; River Pollution : An ecological perspective. Belhaven Press, London and New York, pp. 39-67.

**Harding, J.P.C. and B.A. Whitton** (1981). Accumulation of zinc, cadmium and lead by field populations of *Lemamea*. Wat. Res. 15:301-319.

**Hawkins, W.E., L.G. Tate and T.G. Sarphie** (1980). Acute effects of cadmium on the spot, *Leistomus xanthurus* : tissue distribution and rental ultrastructure. J. Tox. Environ. Hlth. 6:283-295.

**Hellawell, J.M.** (1986). Biological indicator of freshwater pollution and environmental management. London: Elsevier Applied Science Publishers.

**Hennig, H.F-K.O.** (1986). Metal-binding proteins as metal pollution indicators. Environ. Hlth Persp. 65:175-187.

**Hernandez, H., J. Medina, J. Ansuategui, M. Conesa** (1990). Heavy metal concentrations in some marine organisms from the Mediterranean Sea (Castellon, Spain): Metal accumulation in different tissues. Sci. Mar. 54(2):113-129.

**Hidalgo, J. and R. Flos** (1986). Dogfish metallothionein - I. Purification and characterization and comparison with rat metallothionein. Comp. Biochem. Physiol. 83C:93-103.

**Hogstrand, C., G. Lithner and C. Haux** (1989). Relationship between metallothionein, copper and zinc in perch (*Perca fluviatilis*) environmental exposed to heavy metals. Mar. Environ. Res. 28:179-182.

**Hogstrand, C. and C. Haux** (1990). Metallothionein as an indicator of heavy metal exposure in two subtropical fish species. J. Exp. Mar. Biol. Ecol. 138(1/2):69-84.

**Holm, G., L. Norrgren and O. Linden** (1991). Reproductive and histopathological effects of long-term experimental exposure to bis(tributyltin)oxide(TBTO) on the three-spined stickleback, *Gasterosteus aculeatus* Linnaeus. J. Fish Biol. 38:373-386.

**Hopkins, C.A. and J.D. Smyth** (1951). Notes on the morphology and life history of *Schistocephalus solidus* (Cestoda: Diphyllobothriidae). Parasitology 41:283-291.

**Howarth, R.S. and J.B. Sprague** (1978). Copper lethality to rainbow trout in waters of various hardness and pH. Wat. Res. 12:455-462.

**Huynh-Ngoc, L., N.E. Whitehead, and B. Oregioni** (1988). Cadmium in the Rhone River. Wat. Res. 22(3):571-576.

**Hynes, H.B.N.** (1950). The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. J. Anim. Ecol. 19:36-58.

**Hynes, H.B.N.** (1960). The Ecology of Polluted Waters. Liverpool University Press, 284 pp.

**Hyslop, E.J.** (1980). Stomach contents analysis - A review of methods and their application. J. Fish Biol. 17:411-429.

**Issaq, H.J.** (1979). Effect of matrix on the determination of volatile metals in biological samples by flameless atomic absorption spectrometry with the graphite tube atomizer. Anal. Chem. 51(6):657-659.

**Janben E. and H. Brune** (1984). Determination of mercury, lead and cadmium residues in fish from the Rhine and Main by flameless atomic absorption. Z Lebensm Unters Forsch 178:168-172.

**Joensen, J. and B. Korsgaard** (1986). Uptake, time-course distribution and elimination of cadmium in embryos and tissues of the pregnant *Zoarces viviparus* (L) after intraovarian loading. J. Fish Biol. 28:61-68.

**John, J., E. Gjessing, M. Grande and B. Salbu** (1987). Influence of aquatic humus and pH on the uptake and depuration of cadmium by the Atlantic salmon (*Salmo salar*). Sci. Total Environ. 62:253-265.

**Jones, J.R.E.** (1935). The toxic action of heavy metal salts on the three-spined stickleback (*Gasterosteus aculeatus*). J. Exp. Biol. 12:165-173.

**Jones, J.R.E.** (1938). The relative toxicity of salts of lead, zinc and copper to the stickleback (*Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. J. Exp. Biol. 15:394-407.

**Jones, J.R.E.** (1939). The relation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (*Gasterosteus aculeatus* L.). J. Exp. Biol. 16:425-437.

**Jones, J.R.E.** (1947a). The oxygen consumption of *Gasterosteus aculeatus* L. in toxic solutions. J. Exp. Biol. 23:298-311.

**Jones, J.R.E.** (1947b). The reactions of *Pygosteus pungitius* L. to toxic solutions. J. Exp. Biol. 24:110-122.

**Jones, J.R.E.** (1948). A further study of the reactions of fish to toxic solutions. J. Exp. Biol. 25:22-34.

**Jones, J.R.E.** (1952). The reactions of fish to water of low oxygen concentration. J. Exp. Biol. 29:403-415.

**Jones, J.W. and H.B.N. Hynes** (1950). The age and growth of *Gasterosteus aculeatus, Pygosteus pungitius* and *Spinachia vulgaris,* as shown by their otoliths. J. Anim. Ecol. 17:59-73.

**Karlsson-Norrgren, L. and P. Runn** (1985). Cadmium dynamics in fish: pulse studies with $^{109}Cd$ in female zebrafish, *Brachydanio rerio.* J. Fish Biol. 27:571-581.

**Keeney, W.L., W.G. Breck, G.W. Vanloon and J.A. Page** (1976). The determination of trace metals in *Cladophora glomerata - C. glomerata* as a potential biological monitor. Wat. Res. 10:981-984.

**Kelly, M.G. and B.A. Whitton** (1989). Interspecific differences in Zn, Cd and Pb accumulation by freshwater algae and bryophtes. Hydrobiologia 175(1):1-11.

**Kennedey, C.R.** (1974). A checklist of British and Irish freshwater parasites with notes on their distribution. J. Fish Biol. 6:613-644.

**Kelso, J.R.M. and R. Frank** (1974). Organochlorine residues, mercury, copper and cadmium in yellow perch, white bass and smallmouth bass, Long Point Bay, Lake Erie. Trans. Am. Fish. Soc. 3:577-581.

**Kinkade, M.L. and H.E. Erdman** (1975). The influence of hardness components ($Ca^{+2}$ and $Mg^{+2}$) in water on the uptake and concentration of cadmium in a simulated freshwater ecosystem. Environ. Res. 10:308-313.

**Kito, H., Y.Ose, V.Mizuhira, T.Sato, T.Ishikawa and T. Tazawa** (1982a). Separation and purification of (Cd, Cu, Zn) - metallothionein in carp hepato-pancreas. Comp. Biolchem. Physiol. 73C(1):121-127.

**Kito, H., T. Tazawa, Y. Ose, T. Sato and T.Ishikawa** (1982b). Formation of metallothionein in fish. Comp. Biochem. Physiol. 73C(1):129-134.

**Knox, D., C.B. Cowey, and J.W. Adron** (1982). Effects of dietary copper and copper:zinc ration on rainbow trout, *Salmo gairdneri.* Aquaculture 27:111-119.

**Knox, E., C.B. Cowey and J.W. Adron** (1984). Effects of dietary zinc intake upon copper metabolism in rainbow trout (*Salmo gairdneri*). Aquaculture 40:199-207.

**Korzeniewski, K. and E. Neugebauer** (1991). Heavy metals contamination in the Polish Zone of Southern Baltic. Mar. Pollut. Bull. 23:687-689.

**Krantzberg, G.** (1989). Accumulation of essential and nonessential metals by Chironomid larvae in relation to physical and chemical properties of the elements. Can. J. Fish. Aquat. Sci. 46:1755-1761.

**Kumada, H., S. Kimura, M. Yokote and Y. Matida** (1973). Acute and chronic toxicity, uptake and retention of cadmium in freshwater organisms. Bull. Freshwat. Fish Res. Lab. 22:157-165.

**Kumada, H., S. Kimura and M. Yokote** (1980). Accumulation and biological effects of cadmium in rainbow trout. Bull. Jpn. Soc. Sci. Fish. 46:97-103.

**Kwan, K.H.M.** (1988). Comparative study on the whole plant toxicity, uptake kinetics and accumulated forms of thallium and cadmium in *Lemna minor* L. Ph.D. thesis. University of London.

**Kwan, K.H.M. and S. Smith** (1991). Some aspects of the kinetics of cadmium and thallium uptake by fronds of *Lemna minor* L. New Phytol. 117:91-102.

**Lang, C. and B. Lang-Dobler** (1979). The chemical environment of Tubificid and Lumbriculid worms according to the pollution level of the sediment. Hydrobiologia 65(3):273-282.

**Larsson, A** (1975). Some biochemical effects of cadmium on fish. In: Sublethal Effects of Toxic Chemicals on Aquatic Animals (Koeman, J.H. and W.A. Strik eds.), PP.3-13. Elsevier Sci. Publ., Amsterdam.

**Law, A.T. and A. Singh** (1988). Heavy metals in fishes in the Kelang Estuary, Malaysia. Malayan Nat. J. 41(4):505-513.

**Law, A.T. and A. Singh** (1991). Relationships between heavy metal content and body weight of fish from the Kelang Estuary, Malaysia. Mar. Pollut. Bull. 22(2):86-89.

**Laxen, D.P.H.** (1984). Cadmium in freshwaters: concentration and chemistry. Freshwater Biol. 14:587-585.

**Legore, R.S. and D.M. Desvoigne** (1973). Absence of acute effects on threespine sticklebacks (*Gasterosteus aculeatus*) and coho salmon (*Oncorhynchus kisutch*) exposed to resuspended harbor sediment contaminants. J. Fish. Res. Bd Can. 30(8):1240-1242.

**Leland, H. and J.L. Carter** (1984). Effects of copper on species composition of periphyton in a Sierra Nevada, California, stream. Freshwat. Biol. 14:281-296.

**Leland, H.V. and J. S. Kuwabara** (1985). Trace metals. In; (Rand, G.M. and S.R. Petrocelli eds) Fundamentals of aquatic toxicology. Hemisphere Publishing Corp., Washington, pp.374-415.

**Lester, R.J.G.** (1971). The influence of *Schistocephalus* plerocercoids on the respiration of Gasterosteus and a possible resulting effect on the behavior of the fish. Can. J. Zool. 49:361-366.

**Lewis, M.A.** (1980). Selected heavy metals in sediments and biota from desert streams of the Gila River drainage (Arizona). In; (Eaton, J.G., P.R. Parrish, and A.C. Hendricks eds.) Aquatic toxicology, ASTM STP 707. American Society for Testing and Materials, pp.191-204.

**Lovett, R.J., W.H. Gutenmann, I.S. Pakkala, W.D. Youngs, D.J. Lisk, G.E. Burdick and E.J. Harris** (1972). A survey of the total cadmium content of 406 fish from 49 New York State fresh waters. J. Fish. Res. Bd. Can. 29:1283-1290.

**Lowe, T.P., T.W. May, W.G. Brumbaugh and D.A. Kane** (1985). National contaminant biomonitoring program: concentration of seven elements in freshwater fish, 1978-1981. Arch. Environ. Contam. Toxicol. 14:363-388.

**Lucas, H.F.Jr., D.N. Edgington, and P.J. Colby** (1970). Concentrations of trace elements in Great Lakes fishes. J. Fish. Res. Bd. Can. 27:677-684.

**Mackay, N.J., M.N. Kazacos, R.J. Williams and M.I. Leedow** (1975). Selenium and heavy metals in black marlin. Mar. Pollut. Bull. 6(4):57-60.

**Madigosky, S.R., X. Alvarez-Hernandez, and J. Glass** (1991). Lead, cadmium, and aluminium accumulation in the red swamp crayfish *Procambarus clarkii* G. collected from roadside drainage ditches in Louisiana. Arch. Environ. Contam. Toxicol. 20:253-258.

**Maher, W.A.** (1986). Trace metal concentrations in marine organisms from St. Vincent Gulf, south Australia. Water Air Soil Pollut. 29:77-84.

**Maitland, P.S.** (1965). The feeding relationships of salmon, trout, minnows, stone loach and three-spined stickleback in the River Endrick, Scotland. J. Anim. Ecol. 34:109-133.

**Mance, G., V.M. Brown, and J. Yates** (1984). Proposed environmental quality standards for list II substances in water-copper. Water Research Centre, UK. Technical report TR 210. August 1984, 53 pp.

**Mance, G.** (1987). Pollution Threat of Heavy Metals in Aquatic Environments. Elsevier Science Publishing Co., 372 pp.

**Mann, R.H.K. and E.R. Orr** (1969). A preliminary study of the feeding relationships of fish in a hard-water and a soft-water stream in southern England. J. Fish Biol. 1:31-44.

**Mann, R.H.K.** (1971). The populations, growth and production of fish in four small streams in Southern England. J. Anim. Ecol. 40:155-190.

**Manzer, J.I.** (1976). Distribution, food, and feeding of the threespine stickleback, *Gasterosteus aculeatus*, in Great Central Lake, Vancouver Island, with comments on competition for food with juvenile sockeye salmon, *Oncorhynchus nerka*. Can. Fish. Bull. 74(3):647-668.

**Marlborough, D.** (1963). A supplement to " The Fishes of the London Area". London Naturalists 42:62-70.

**Marlborough, D.** (1965). London fish since 1962. London Naturalists 44:70-73.

**Marlborough, D.** (1969). Further London fish records to 1968. London Naturalists 48:76-85.

**Marshell, R.C. and A.S. Inglis** (1986). Characterization of oligomer. In: Practical Protein Chemistry - A Handbook (A. Darbre ed.) John Wiley & Sons Ltd. P.4-11.

**Mason, C.F., MacDonald, S.M. and V.J. Aspen** (1982). Metals in freshwater fishes in the United Kingdom 1980-1981. London: The Vincent Wildlife Trust.

**Mason, C.F. and N.A-E. Barak** (1990). A catchment survey for heavy metals using the eel (Anguilla anguilla). Chemosphere 21(4/5):695-699.

**Mason, C.F.** (1987). A survey of mercury, lead and cadmium in muscle of British freshwater fish. Chemosphere 16(4):901-906.

**Mathis, B.J. and T.F. Cummings** (1973). Selected metals in sediments, water, and biota in the Illinois River. J. Wat. Poll. Contr. Fed. 45:1573-1583.

**Mathis, B.J. and N.R. Kevern** (1975). Distribution of mercury, cadmium, lead and thallium in a eutrophic lake. Hydrobiologia 46(2/3):207-222.

**Matthiessen, P. and A.E. Brafield** (1973). The effects of dissolved zinc on the gills of the stickleback *Gasterosteus aculeatus* (L.). J. Fish Biol.5:607-615.

**Matthiessen, P. and A.E. Brafield** (1977). Uptake and loss of dissolved zinc by the stickleback *Gasterosteus aculeatus* L. J. Fish Biol. 10:399-410.

**Mayes, R.A., A.W. McIntpsh and V.L. Anderson** (1977). Uptake of cadmium and lead by rooted Aquatic macrophyte (*Elodea candensis*). Ecology 58:1176-1180.

**McCarty, L.S., J.A.C Henry and A.H. Houston** (1978). Toxicity of cadmium to goldfish, *Carassius auratus*, in hard and soft water. J. Fish. Res. Bd. Can. 35:35-42.

**McFarlane, G.A. and W.G. Franzin** (1980). An examination of Cd, Cu, and Hg concentrations in livers of Northern pike, Esox lucius, and white sucker, *Catostomus commersoni*, from five lakes near a bass metal smelter at Flin Flon, Manitoba. Can. J. Fish. Aquat.. Sci. 37:1573-1578.

**McKim, J.M. and D.A. Benoit** (1971). Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Can. 28:655-662.

**McKim, J.M. and D.A. Benoit** (1974). Duration of toxicity tests for establishing 'no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd Can. 31:449-452.

**McLean, C., A.G. Miskiewicz, and E.A. Roberts** (1991). Effect of three primary treatment sewage outfalls on metal concentrations in

the fish *Cheilodactylus fuscus* collected along the coast of Sydney, Australia. Mar. Pllut. Bull. 22(3):134-140.

**McLean, R.O. and A.K. Jones** (1975). Studies of tolerance to heavy metals in the flora of the rivers Ystwyth and Clarach, Wales. Freshwat. Biol. 5:431-44.

**Meakins, R.H. and M. Walkey** (1975). The effects of parasitism by the plerocercoid of *Schistocephalus solidus* Muller 1776 (Pseudophyllidea) on the respiration of the three-spined stickleback *Gasterosteus aculeatus* L. J. Fish Biol. 7:817-824.

**Moriarty, F., H.M. Hanson, and P. Freestone** (1984). Limitations of body burden as an index of environmental contamination: Heavy metals in fish, *Cottus gobio* L. from the River Ecclesbourne, Derbyshire. Environ. Pollut. (ser. A) 34:297-320.

**Morris, O.P and G. Russle** (1973). Effect of chelation on toxicity of copper. Mar. Pollut. Bull. 4:159-160.

**Munkittrick, K.R., P.A. Miller, D.R. Barton and D.G. Dixon** (1991). Altered performance of whiter sucker populations in the Manitouwadge Chain of Lakes is associated with changes in benthic macroinvertebrate communities as a result of copper and zinc contamination. Ecotoxicol. Environ. Saf. 21:318-326.

**Murai, T., J.W. Andrews, and R.G. Smith, Jr.** (1981). Effects of dietary copper on channel catfish. Aquaculture 22:353-357.

**Murphy, B.R., G.J. Atchison and A.W. McIntosh** (1978). Cadmium and zinc content of fish from an industrially contaminated lake. J. Fish Biol. 13:327-335.

**Nichikawa, K. and K. Tabata** (1969). Studies on the toxicity of heavy metals to aquatic animals and the factors to decrease the toxicity - III. Bull. Tokai Reg. Fish. Res. Lab. 58: 233-241.

**Nicholson, M.D., N.W. Green and S.J. Wilson** (1991). Regression models for assessing trends in cadmium and PCBs in cod livers from the Oslofjord. Mar. Pollut. Bull. 22(3):77-81.

**Noel-Lambot, F., C. Gerday and A. Disteche** (1978). Distribution of Cd, Zn and Cu in liver and gills of the eel, *Anguilla anguilla* with special reference to metallothioneins. Comp. Biochem. Physiol. 61C:177-187.

**Norey, C.G., A. Cryer and J. Kay** (1990a). Cadmium uptake and sequestration in the pike (*Esox lucius*). Comp. Biochem. Physiol. 95C(2):217-221.

**Norey, C.G., A. Cryer and J. Kay** (1990b). A Comparison of cadmium-induced metallothionein gene expression and Me2+ distribution in the tissues of cadmium-sensitive (rainbow trout; *Salmo gairdneri*) and tolerant (stone loach; *Noemacheilus barbatulus*) species of freshwater fish. Comp. Biochem. Physiol. 97C(2):221-225.

**Norey, C.G., M.W. Brown, A. Cryer and J. Kay** (1990c). A comparison of the accumulation, tissue distribution and secretion of

cadmium in different species of freshwater fish. Comp. Biochem. Physiol. 96C(1):181-184.

**NRA** (1991). The quality of rivers, canals and estuaries in England and Wales. Rept. of the 1990 Survey Publ. NRA. 64 pp.

**Olafson, R.W., R.G. Sim and K.G. Boto** (1979). Isolation and chemical characterization of the heavy metal-binding protein metallothionein from marine invertebrates. Comp. Biochem. Physiol. 62B:407-416.

**Olsen, C.R., N.H. Cutshall, and I.L. Larsen** (1982). Pollutant-particle associations and dynamics in coastal marine environments: A review. Mar. Chem. 11:501.

**Oronsaye, J.A.O. and A.E. Brafield** (1984). The effect of dissolved cadmium on the chloride cells of the gills of the sticklebacks, *Gasterosteus aculeatus* L. J. Fish Biol. 25:253-258.

**Overnell, J. and M.I. Abdullah** (1988). Metallothionein and metal levels in flounder *Platichthys flesus* from four field sites and in flounder dosed with water-borne copper. Mar. Ecol. Prog. Ser. 46:71-74.

**Overnell, J. and R. McIntosh** (1988). The effects of supplementary dietary copper on copper and metallothionein levels in the fish, dab, *Limanda limanda*. Mar. Environ. Res. 26:237-247.

**Ozretic, B., M. Krajnovic-Ozretic, J. Santin, B. Medjugorac and M. Kras** (1990). As, Cd, Pb, and Hg in benthic animals from the Kvarner-Rijeka Bay region, Yugoslavia. Mar. Pollut. Bull. 21(12):595-598.

**Pagenkopf, G.K. R.C. Russo and R.U. Thorston** (1974). Effect on complexation on toxicity of copper to fishes. J. Fish. Res. Bd Can. 31:462-465.

**Pascoe, D. and D.L. Mattey** (1977). Studies on the toxicity of cadmium to three-spined stickleback (*Gasterosteus aculeatus* L.). J. Fish Biol. 11:207-215.

**Pascoe, D. and P. Cram** (1977). The effects of parasitism on the toxicity of cadmium to the three-spined stickleback, Gasterosteus aculeatus L. J. Fish Biol. 10:467-472.

**Pascoe, D., S.A. Evans and J. Woodworth** (1986). Heavy metal toxicity to fish and the influence of water hardness. Arch. Environ. Contam. Toxicol. 15:481-487.

**Pedon, J.D., J.H. Crothers, C.E. Waterfall and J. Beasley** (1973). Heavy metals in Somerset marine organisms. Mar. Pollut. Bull. 4(1):7-9.

**Pennycuick, L.** (1971a). Seasonal variations in the parasite infections in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L. Parasitology 63:373-388.

**Pennycuick, L.** (1971b). Quantitative effects of three species of parasites on a population of three-spined sticklebacks, *Gasterosteus aculeatus*. J. Zool., Lon. 165:143-162.

**Pennycuick, L.** (1971c). Differences in the parasite infections in three-spined sticklebacks (*Gasterosteus aculeatus* L.) of different sex, age and size. Parasitology 63:407-418.

**Pennycuick, L.** (1971d). Frequency distribution of parasites in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. Parasitology 63:389-406.

**Pesch, H.-J., G.-A. Meyer, F. Henschke, and W. Wunder** (1988). Cadmium content in organs of Salmonides from Wollershof and Gangfischen. Fisher & Teichwirt 39(10):290-295.

**Phillips, D.J.H.** (1977). The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - A review. Environ. Pollut. 13:281-317.

**Pickering, Q.H. and M.H. Gast** (1972). Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). J. Fish Res. Bd. Can. 29:1099-1106.

**Pickering, Q.H. and Henderson C.** (1966). The acute toxicity of some heavy metals to different species of warmwater fishes. Air Wat. Pollut. Int. J. 10:453-463.

**Portmann, J.E.** (1972). The levels of certain metals in fish from coastal waters around England and Wales. Aquaculture 1 :91-96.

**Preston, A** (1973). Cadmium in the marine environment of the United Kingdom. Mar. Pollut. Bull. 4:105.

**Radhakrishnaiah, K.** (1988). Accumulation of copper in the organs of freshwater fish, *Labeo rohita* (Hamilton), on exposure to lethal and sublethal concentrations of copper. J. Environ. Biol. 9(3 suppl.):319-326.

**Ramamoorthy, S. and K. Blumhagen** (1984). Uptake of Zn, Cd and Hg by fish in the presence of competing compartments. Can. J. Fish. Aquat. Sci. 41:750-756.

**Rainbow, P.S. and S.L. White** (1989). Comparative strategies of heavy metal accumulation by crustaceans: Zinc, copper and cadmium in a decapod, an amphipod and a barnacle. Hydrobiologia, 174(3):245-262.

**Rainbow, P.S., D.J.H. Phillips and M.H. Depledge** (1990). The significance of trace metal concentrations in marine invertebrates. A need for laboratory investigation of accumulation strategies. Mar. Pollut. Bull. 21(7):321-324.

**Rashid, M.A. and J.D. Leonard** (1973). Modification in the solubility and precipitation behaviour of various trace metals as a result of their interaction with sedimentary humic acids. Chem. Geol. 11:89-97.

**Reash, R.J. and T.M. Berra** (1986). Fecundity and trace-metal content of creek chubs from a metal-contaminated stream. Trans. Am. Fish. Soc. 115:346-351.

**Reichert, W.L. and D.A. Federighi and D.C. Malins** (1979). Uptake and metabolism of lead and cadmium in coho salmon (*Oncorhychus kisutch*). Comp. Biochem. Physiol. 63C:227-234.

**Roch, M. and J.A. McCarter** (1984). Metallothionein induction, growth, and survival of chinook salmon exposed to zinc, copper and cadmium. Bull. Environ. Contam. Toxicol. 32:478-485.

**Roch, M., J.A. McCarter, A.T. Matheson, M.J.R. Clark and R.W. Olafson** (1982). Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39:1596-1601.

**Roch, M., R.N. Nordin, A. Austin, C.J.P. McKean, J. Deniseger, R.D.Kathman, J.A. McCarter and M.J.R. Clark** (1985). The effects of heavy metal contamination on the aquatic biota of Buttle Lake and the Campbell River Drainage (Canada). Arch. Environ. Contam. Toxicol. 14:347-362.

**Rothery, E.** (1982). Analytical methods for graphite tube atomizers. Varian Techtron Pty. Ltd. Mulgrave, Victoria, Australia, January, 1982, 132 pp.

**Sangalang, G.B. and H.C. Freeman** (1979). Tissue uptake of cadmium in brook trout during chronic sublethal exposure. Arch. Environ. Contam. Toxicol. 8:77-84.

**Sankaraperumal, G., M.K. Rajan, and A. Mohandoss** (1990). Synergistic effects of cadmium and zinc on the erythrocytes and opercular activity of the fishes *Lepidocephalichthyes thermalis* and *Amblypharyngodon melettinus*. Environ. Ecol. 8(4):1213-1216.

**Sapunar, J., M. Jusic and D. Bazulic** (1989). Total mercury and cadmium in some cephalopods and fish from the Adricatic Sea. Mar. Pollut. Bull. 20(11):576-577.

**Sastry, K.V. and P.K. Gupta** (1979). The effect of cadmium on the digestive system of the teleost fish, *Heteropneustes fossilis*. Environ. Res. 19:221-230.

**Say, P.J., J.P.C. Harding and B.A. Whitton** (1981). Aquatic mosses as monitors of heavy metal contamination in the River Etherow, Great Britain. Environ. Pollut. Ser. B. 2:295-307.

**Schachter, M.M. and K.M. Boyer** (1980). Digestion of organic matrices with a single acid for trace element determination. Anal. Chem. 52(2):360-364.

**Schierup, H.-H. and V.J. Larsen** (1981). Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metal in *Phragmites australis* (Cav.) Trin. Aquat. Bot. 11:197-210.

**Shaikh, Z.A. and K. Hirayama** (1979). Metallothionein in the extracellular fluids as an index of cadmium toxicity. Environ. Hlth. Persp. 28:267-271.

**Sheehan, P.J., D.R. Miller, G.C. Butler, P. Bourdeau and J.M. Ridgeway** (1984). Effects of Pollutants at the Ecosystem Level. SCOPE 22, John Wiley & Sons. Chichester, 435 pp.

**Singh, N.K., M.C. Varma and J.S.D. Munshi** (1990). Accumulation of copper, zinc, lead, iron and cadmium in certain freshwater fishes of River Subernarekha. J. Freshwat. Biol. 2(3):189-193.

**Singh, J.G., I. Chang-Yen, V.A. Stoute and L. Chatergoon** (1991). Distribution of selected heavy metals in skin and muscle of five tropical marine fishes. Environ. Pollut. 69:203-215.

**Smith, B.P., E. Hejtmancik and B.J. Camp** (1976). Acute effects of cadmium on *Ictalurus punctatus* (catfish). Bull. Environ. Contam. Toxicol. 15:271-277.

**Solbe, J.E. de L.G.** (1973). The relation between water quality and the status of fish populations in Willow Brood. Water Treat. Examin. 22:41-61.

**Sprenger, M. and A. McIntosh** (1989). Relationship between concentration of aluminium, cadmium, lead and zinc in water, sediments, and aquatic macrophytes in six acidic lakes. Arch. Environ. Contam. Toxicol. 18:225-231.

**Steele, C.W., D.W. Owens and A.D. Scarfe** (1990). Attraction of zebrafish, *Brachydanio rerio*, to alanine and its suppression by copper. J. Fish Biol. 36:341-352.

**Stiff, M.J.** (1971a). Copper/Bicarbonate equilibria in solutions of bicarbonate ion at concentrations similar to those found in natural water. Wat. Res. 5:171-176.

**Stiff, M.J.** (1971b). The chemical states of copper in polluted fresh water and a scheme of analysis to differentiate them. Wat. Res. 5:585-599.

**Stromberg, P.C., J.G. Ferrante, S. Cater** (1983). Pathology of lethal and sublethal exposure of fathead minnows, Pimephales promelas, to cadmium: a model for aquatic toxicity assessment. J. Tox. Environ. Hlth 11:247-259.

**Szefer, P., K. Szefer and B. Skwarzec** (1990). Distribution of trace metals in some representative fauna of the Southern Baltic. Mar. Pollut. Bull. 21(2):60-62.

**Szefer,P. and J. Falandysz** (1985). Trace metals in muscle tissue of fish taken from the Southern Baltic. Z Lebensm Unters Forsch 181:217-220.

**Tabata, K. and K. Nishikawa** (1969). Studies on the toxicity of heavy metals to aquatic animals and the factors to decrease the toxicity - V. Bull. Tokai Reg. Fish. Res. Lab. 58:255-264.

**Takeda, H. and C. Shimizu** (1982). Purification of metallothionein from the liver of skipjack and its properties. Bull. Jpn Soc. Sci. Fish. 48(5):717-723.

**Taylor, D.** (1983). The significance of the accumulation of cadmium by aquatic organisms. Ecotoxicol. Environ. Saf. 7:33.

**Tessier, A., P.G.C. Campbell, j.C. Auclair, and M. Bisson** (1984). Relationship between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. Can. J. Fish. Aquat.. Sci. 41:1463-1472.

**Thomas, D.G., A. Cryer, J.F. de L.G Solbe and J. Kay** (1983). A comparison of the accumulation and protein binding of environmental cadmium in the gills, kidney and liver of rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 76C(2):241-246.

**Tsoukali-Papadopoulou, H., I. Kaniou-gregoriades, P. Epivatianos, and J.A. Stratis** (1989). Heavy metals in marine organisms of Geras Gulf, Lesvos, Greece. J. Environ. Sci. Hlth, Pt. A. A24(1):39-47.

**Ukegbu, A.A. and F.A. Huntingford** (1988). Diet composition and stomach fullness in three-spined sticklebacks from three Scottich populations. Scott. Nat.,1988:5-16.

**Uthe, J.F. and E.G. Bligh** (1971). Preliminary survey of heavy metal contamination of Canadian freshwater fish. J. Fish. Res. Bd Canada 28:786-788.

**Vas, P.** (1991). Trace metal levels in sharks from British and Atlantic Waters. Mar. Pollut. Bull. 22(2):67-72.

**Vijayram, K., P. Geraldine, T.S. Varadarajan, L. James, K. Periaswamy, G. John, and P. Loganathan** (1990). Vertebral deformities and decrease of mineral content in *Anabas testudineus* exposed to cadmium. Environ. Ecol. 8(2):672-674.

**Vinikour, W.S., R.M. Goldstein, and R.V. Anderson** (1980). Bioconcentration patterns of zinc, copper, cadmium and lead in selected fish species from the Fox River, Illinois. Bull. Environ. Contam. Toxicol. 24:727-734.

**Voyer, R.A., P.P. Yevich and C.A. Barszcz** (1975). Histological and toxicological responses of the mummichog (*Fundulus heteroclitus* (L.)) to combination of levels of cadmium and dissolved oxygen in freshwater. Wat. Res. 9:1069-1074.

**Walkey, M. and R.H. Meakins** (1970). An attempt to balance the energy budget of a host-parasite system. J. Fish Biol. 2:361-372.

**Weber, O.** (1985). Concentration of metal in fish from the River Rednitz. Z Lebensm Unters Forsch 180:463-466.

**Wharfe, J.R. and W.L.F. Van Den Broek** (1977). Heavy metals in macroinvertebrates and fish from the Lower Medway Estuary, Kent. Mar. Pollut. Bull. 8(2):31-34.

**Wheeler, A.** (1979). The Tidal Thames - The history of a river and its fishes. Routledge & Kegan Paul Ltd., London and Boston. 228 pp.

**Whitton, B.A.** (1970). Biology of Cladophora in freshwaters. Wat. Res. 4:457-476.

**Whitton, B.A., I.G. Burrows and M.G. Kelly** (1989). Use of *Cladophore glomerata* to monitor heavy metals in rivers. J. Appl. Phycol. 1(4):293-299.

**Wiener, J.G. and J.P. Giesy, Jr.** (1979). Concentrations of Cd, Cu, Mn, Pb, and Zn in fishes in highly organic softwater pond. J. Fish. Res. Bd. Can. 36:270-279.

**Williams, D.R. and J.P. Giesy** (1978). Relative importance of food and water sources to cadmium uptake by *Gambusia affinis* (Poecilliidae). Environ. Res. 16:326-332.

**Windell, J.T. and S.H. Bowen** (1978). Methods for study of fish diets based on analysis of stomach contents. In Methods for Assessment of fish production in fresh waters (T. Bagend ed.), Blackwell, pp.219-226.

**Winder, H., R. Stickney, R. Smith, D. White and F. Taylor** (1973). Arsenic, cadmium, copper, mercury and zinc in some species of North Atlantic finfish. J. Fish. Res. Bd. Can. 30:275-279.

**Winger, P.V., D.P. Schultz and W. W. Johnson** (1990). Environmental contaminant concentrations in biota from the Lower Savannah River, Georgia and South Carolina. Arch. Environ. Contam. Toxicol. 19:101-117.

**Winner, R.W.** (1984). The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat. Toxicol. 5:267-274.

**Woodworth, J. and D. Pascoe** (1983). Cadmium uptake and distribution in sticklebacks related to the concentration and method of exposure. Ecotoxicol. Environ. Saf. 7:525-530.

**Wootton, R.J.** (1976). The Biology of the Stickleback. Academic Press, London, New York & San Francisco. 387 pp.

**Wootton, R.J.** (1984). A Functional Biology of Stickleback. Croom Helm, London & Sydney. 265 pp.

**Yamamoto, Y., T.Ishii and S. Ikeda** (1978). Studies on copper metabolism in fishes - III. Existence of metallothionein-like protein in carp hepato-pancreas. Bull. Jpn Soc. Sci. Fish. 44(2):149-153.

**Young, L.B. and H.H. Harvey** (1991). Metal concentrations in Chironomids in relation to the geochemical characteristics of surficial sediments. Arch. Environ. Contam. Toxicol. 21:202-211.

**Zamojski, J., S. Smoczynski, K.A. Skibniewska and R. Amarowicz** (1986). Organochlorine insecticides and heavy metals in fish form Mutek Lake, N.E. Poland. Bull. Environ. Contam. Toxicol. 37:587-592.

# APPENDIX

# TABLE OF APPENDIX


**Appendix A.1** The graphite furnace programme of Cu for checking different ashing times at 900$^{o}$C of ashing temperature and the Cu absorbance values. ---------- 242

**Appendix A.2** The graphite furnace programme of Cu for checking different atomisation temperatures and the Cu absorbance values.-- 244

**Appendix A.3** The graphite furnace programme of Cd for checking the working range and the Cd absorbance values. ---------- 246

**Appendix A.4** The graphite furnace programme of Cd with 0.25% of $H_3PO_4$ for checking different ashing and atomisation temperatures and the Cu absorbance values. ---------- 247

**Appendix A.5** The original measurements of various standard materials. ---------- 248

**Appendix B.1** The original data of day-time water temperatures ($^{o}$C), pH values, conductivity (us/cm), total hardness (mg $CaCO_3$), and D.O. (%) at the various sampling sites in the River Wandle during Dec. 1987 and Jul. 1989. ---------- 249

**Appendix B.2** Concentrations of heavy metals in samples of sediment taken from the River Wandle during 4th May 1988 and 3rd Aug. 1988. ---------- 251

**Appendix B.3** Concentrations of copper in samples of water taken from the River Wandle during 12th Dec. 1988 and 6th Jul. 1989. ---- 252

**Appendix B.4** Concentrations of heavy metals in samples of 3-spined stickleback taken from the River Wandle during 4th May 1988 and 15th Mar. 1989. ---------- 254

**Appendix B.5** Concentration copper in samples of 3-spined stickleback taken from The Thames on 29th Jun. 1989. ---------- 255

**Appendix C.1** The original data of non-parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990. ---------- 256

**Appendix C.2** The original data of parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990. ---------- 261

**Appendix C.3** The growth data of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990. ---------- 264

**Appendix C.4** The ANOVA tables of the site effects on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- 266

**Appendix C.5** The ANOVA tables of the site effects on total length (mm), dry weight (g), total body burdens of Cu and Cd (ug), and concentrations of Cu and Cd (ug/g dw) in the cestode parasites in the parasitised sticklebacks at the three sites in the River Wandle.--267

**Appendix C.6** The ANOVA tables of the effects of age on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle. ---------- 268

**Appendix C.7** The ANOVA tables of the effects of host's age on total length (mm), dry weight (g), total body burdens of Cu and Cd (ug), and concentrations of Cu and Cd (ug/g dw) in the cestode parasites in the parasitised sticklebacks at the three sites in the River Wandle. ---------- 271

**Appendix C.8** The ANOVA tables of the seasonal effects on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the $0^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle. ---------- 273

**Appendix D.1** Copper and cadmium concentrations of water samples taken from the three sampling sites (A, B and E) in the River Wandle during each season (1989-1990). ---------- 275

**Appendix D.2** Copper and cadmium concentrations of floc samples taken from the three sampling sites (A, B and E) in the River Wandle during each season (1989-1990). ---------- 276

**Appendix D.3** Copper and cadmium concentrations of sediment samples taken from the three sampling sites (A, B and E) in the River Wandle during each season (1989-1990). ---------- 277

**Appendix D.4** Copper and cadmium concentrations of algae and aquatic plant samples taken from the three sampling sites (A, B and E) in the River Wandle during each season (1989-1990). ---------- 279

**Appendix D.5** Copper and cadmium concentrations of aquatic invertebrate samples taken from the three sampling sites (A, B and E) in the River Wandle during each season (1989-1990). --------- 284

**Appendix E.1** Original data of copper and cadmium contents in the tissues of sticklebacks. ---------- 290

**Appendix E.2** Copper and cadmium concentrations in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- 294

**Appendix E.3** Percentage of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- 296

**Appendix E.4** One-way ANOVA of Cu and Cd distributions in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- 298

**Appendix E.5** One-way ANOVA of Cu and Cd concentrations in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- 299

**Appendix E.6** One-way ANOVA of percentage of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. ---------- 300

**APPENDIX A.1** The graphite furnace programme of Cu for checking different ashing times at 900$^{o}$C of ashing temperature and the Cu absorbance values.

Copper Programme :

| Step | Temp ($^{o}$C) | Time (sec) | Gas | Flow rate (L/min) | Read |
|---|---|---|---|---|---|
| 1 | 75 | 5 | Nitrogen | 3 | |
| 2 | 90 | 50 | Nitrogen | 3 | |
| 3 | 120 | 10 | Nitrogen | 3 | |
| 4 | 120 | 5 | Nitrogen | 3 | |
| 5 | 700 | 15 | Nitrogen | 3 | |
| 6 | 900 | 5* | Nitrogen | 3 | |
| 7 | 900 | 1 | Nitrogen | 0 | |
| 8 | 2300 | 0 | Nitrogen | 0 | * |
| 9 | 2300 | 2 | Nitrogen | 0 | * |
| 10 | 2300 | 2 | Nitrogen | 3 | |
| 11 | 40 | 20 | Nitrogen | 3 | |

(*: indicates examining parameter)

ABSORBANCE (x 1000):

| Step 6 900$^{o}$C | | 2 sec | | | | 3 sec | | | | 9 sec | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reading | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% |
| Blank | 1 | 2 | 1.67 | 0.58 | 34.64 | 1 | 0.67 | 0.58 | 86.60 | 0 | 1.00 | 1.00 | 100.00 |
| | 2 | 1 | | | | 0 | | | | 1 | | | |
| | 3 | 2 | | | | 1 | | | | 2 | | | |
| Standard solution | 1 | 131 | 130.67 | 1.53 | 1.17 | 125 | 125.00 | 2.00 | 1.60 | 130 | 128.33 | 1.53 | 1.19 |
| (0.08 ng) | 2 | 132 | | | | 123 | | | | 127 | | | |
| | 3 | 129 | | | | 127 | | | | 128 | | | |
| Fish sample | 1 | 95 | 94.67 | 1.53 | 1.61 | 91 | 90.67 | 1.53 | 1.68 | 98 | 99.00 | 1.00 | 1.01 |
| | 2 | 96 | | | | 92 | | | | 100 | | | |
| | 3 | 93 | | | | 89 | | | | 99 | | | |
| Chironomid sample | 1 | 69 | 69.00 | 1.00 | 1.45 | 64 | 64.33 | 1.53 | 2.37 | 68 | 68.33 | 1.53 | 2.24 |
| | 2 | 70 | | | | 63 | | | | 67 | | | |
| | 3 | 68 | | | | 66 | | | | 70 | | | |
| Mollusc sample | 1 | 52 | 51.67 | 1.53 | 2.96 | 48 | 48.00 | 1.00 | 2.08 | 50 | 50.33 | 0.58 | 1.15 |
| | 2 | 53 | | | | 49 | | | | 51 | | | |
| | 3 | 50 | | | | 47 | | | | 50 | | | |

(continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gammarus sample | 1 | 149 | 149.00 | 1.00 | 0.67 | 146 | 146.33 | 1.53 | 1.04 | 147 | 147.33 | 2.52 | 1.71 |
| | 2 | 148 | | | | 145 | | | | 145 | | | |
| | 3 | 150 | | | | 148 | | | | 150 | | | |
| Leech sample | 1 | 110 | 110.00 | 2.00 | 1.82 | 102 | 101.67 | 1.53 | 1.50 | 105 | 105.00 | 2.00 | 1.90 |
| | 2 | 112 | | | | 103 | | | | 107 | | | |
| | 3 | 108 | | | | 100 | | | | 103 | | | |

| (continue) Step 6 900°C | | 12 sec | | | | 18 sec | | | | 30 sec | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reading | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% |
| Blank | 1 | 2 | 1.67 | 1.53 | 91.65 | 1 | 0.67 | 0.58 | 86.60 | 2 | 1.67 | 0.58 | 34.64 |
| | 2 | 3 | | | | 0 | | | | 1 | | | |
| | 3 | 0 | | | | 1 | | | | 2 | | | |
| Standard (0.08 ng) | 1 | 131 | 129.00 | 2.65 | 2.05 | 131 | 129.33 | 2.08 | 1.61 | 127 | 129.67 | 2.52 | 1.94 |
| | 2 | 126 | | | | 127 | | | | 130 | | | |
| | 3 | 130 | | | | 130 | | | | 132 | | | |
| Fish sample | 1 | 94 | 93.67 | 1.53 | 1.63 | 97 | 95.67 | 1.53 | 1.60 | 99 | 97.33 | 2.08 | 2.14 |
| | 2 | 95 | | | | 94 | | | | 98 | | | |
| | 3 | 92 | | | | 96 | | | | 95 | | | |
| Chironomid sample | 1 | 69 | 69.00 | 1.00 | 1.45 | 70 | 68.67 | 1.53 | 2.22 | 71 | 71.00 | 1.00 | 1.41 |
| | 2 | 68 | | | | 69 | | | | 70 | | | |
| | 3 | 70 | | | | 67 | | | | 72 | | | |
| Mollusc sample | 1 | 53 | 53.67 | 1.15 | 2.15 | 52 | 52.67 | 0.58 | 1.10 | 55 | 55.67 | 1.15 | 2.07 |
| | 2 | 55 | | | | 53 | | | | 55 | | | |
| | 3 | 53 | | | | 53 | | | | 57 | | | |
| Gammarus sample | 1 | 150 | 150.67 | 2.08 | 1.38 | 148 | 149.33 | 1.53 | 1.02 | 152 | 151.00 | 2.65 | 1.75 |
| | 2 | 149 | | | | 149 | | | | 153 | | | |
| | 3 | 153 | | | | 151 | | | | 148 | | | |
| Leech sample | 1 | 107 | 106.67 | 3.51 | 3.29 | 110 | 108.67 | 1.15 | 1.06 | 111 | 110.67 | 1.53 | 1.38 |
| | 2 | 103 | | | | 108 | | | | 109 | | | |
| | 3 | 110 | | | | 108 | | | | 112 | | | |

**APPENDIX A.2** The graphite furnace programme of Cu for checking different atomisation temperatures and the Cu absorbance values.

Copper Programme :

| Step | Temp ($^{o}$C) | Time (sec) | Gas | Flow rate (L/min) | Read | |
|---|---|---|---|---|---|---|
| 1 | 75 | 5 | Nitrogen | 3 | | |
| 2 | 90 | 50 | Nitrogen | 3 | | |
| 3 | 120 | 10 | Nitrogen | 3 | | |
| 4 | 120 | 5 | Nitrogen | 3 | | |
| 5 | 700 | 15 | Nitrogen | 3 | | |
| 6 | 900 | 5 | Nitrogen | 3 | | |
| 7 | 900 | 1 | Nitrogen | 0 | | |
| 8 | 2300* | 0 | Nitrogen | 0 | | * |
| 9 | 2300* | 2 | Nitrogen | 0 | | * |
| 10 | 2300* | 2 | Nitrogen | 3 | | |
| 11 | 40 | 20 | Nitrogen | 3 | | |

(*: indicates examining parameter)

ABSORBANCE (x 1000):

Step 8 0 sec 1800$^{o}$C

| | Reading | Abs. | Mean | S.S.D. | RSD% |
|---|---|---|---|---|---|
| Blank | 1 | 2 | 2.67 | 1.15 | 43.30 |
| | 2 | 4 | | | |
| | 3 | 2 | | | |
| Standard sol. | 1 | 98 | 98.67 | 4.04 | 4.10 |
| (0.08 ng) | 2 | 95 | | | |
| | 3 | 103 | | | |
| Fish sample | 1 | 75 | 77.33 | 2.52 | 3.25 |
| | 2 | 77 | | | |
| | 3 | 80 | | | |
| Chironomid | 1 | 53 | 55.67 | 2.52 | 4.52 |
| sample | 2 | 56 | | | |
| | 3 | 58 | | | |
| Mollusc sample | 1 | 51 | 53.33 | 2.08 | 3.90 |
| | 2 | 54 | | | |
| | 3 | 55 | | | |
| Gammarus | 1 | 130 | 130.33 | 3.51 | 2.69 |
| sample | 2 | 127 | | | |
| | 3 | 134 | | | |
| Leech sample | 1 | 94 | 93.33 | 2.08 | 2.23 |
| | 2 | 95 | | | |
| | 3 | 91 | | | |

Step 8 (continuted) 0 sec

| | Reading | 2000$^{o}$C Abs. | Mean | S.S.D. | RSD% | 2200$^{o}$C Abs. | Mean | S.S.D. | RSD% | 2400$^{o}$C Abs. | Mean | S.S.D. | RSD% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blank | 1 | 1 | 4.00 | 2.65 | 66.14 | 5 | 2.67 | 2.08 | 78.06 | 3 | 3.33 | 1.53 | 45.83 |
| | 2 | 5 | | | | 1 | | | | 5 | | | |
| | 3 | 6 | | | | 2 | | | | 2 | | | |
| Standard sol. | 1 | 126 | 128.00 | 2.00 | 1.56 | 131 | 129.67 | 1.53 | 1.18 | 135 | 135.33 | 1.53 | 1.13 |
| (0.08 ng) | 2 | 130 | | | | 130 | | | | 137 | | | |
| | 3 | 128 | | | | 128 | | | | 134 | | | |
| Fish sample | 1 | 94 | 94.00 | 3.00 | 3.19 | 93 | 94.67 | 2.08 | 2.20 | 97 | 98.67 | 1.53 | 1.55 |
| | 2 | 97 | | | | 94 | | | | 99 | | | |
| | 3 | 91 | | | | 97 | | | | 100 | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chironomid | 1 | 70 | 67.00 | 2.65 | 3.95 | 66 | 68.67 | 2.52 | 3.66 | 68 | 67.67 | 1.53 | 2.26 |
| sample | 2 | 66 | | | | 69 | | | | 66 | | | |
| | 3 | 65 | | | | 71 | | | | 69 | | | |
| Mollusc | 1 | 55 | 52.33 | 2.52 | 4.81 | 51 | 53.67 | 2.52 | 4.69 | 53 | 52.67 | 1.53 | 2.90 |
| sample | 2 | 50 | | | | 56 | | | | 51 | | | |
| | 3 | 52 | | | | 54 | | | | 54 | | | |
| Gammarus | 1 | 146 | 146.33 | 3.51 | 2.40 | 150 | 150.67 | 2.08 | 1.38 | 151 | 151.67 | 2.08 | 1.37 |
| sample | 2 | 150 | | | | 153 | | | | 154 | | | |
| | 3 | 143 | | | | 149 | | | | 150 | | | |
| Leech sample | 1 | 109 | 108.33 | 2.08 | 1.92 | 107 | 107.67 | 1.15 | 1.07 | 111 | 109.00 | 1.73 | 1.59 |
| | 2 | 110 | | | | 107 | | | | 108 | | | |
| | 3 | 106 | | | | 109 | | | | 108 | | | |

| Step 8 | 0 sec | 2600°C | | | | 2800°C | | | | 3000°C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (continue) | Reading | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% | Abs. | Mean | S.S.D. | RSD% |
| Blank | 1 | 2 | 1.67 | 1.53 | 91.65 | 5 | 3.33 | 2.08 | 62.45 | 4 | 4.33 | 2.52 | 58.08 |
| | 2 | 3 | | | | 4 | | | | 7 | | | |
| | 3 | 0 | | | | 1 | | | | 2 | | | |
| Standard sol. | 1 | 134 | 134.33 | 1.53 | 1.14 | 130 | 131.67 | 1.53 | 1.16 | 140 | 138.67 | 1.53 | 1.10 |
| (0.08 ng) | 2 | 136 | | | | 132 | | | | 139 | | | |
| | 3 | 133 | | | | 133 | | | | 137 | | | |
| Fish sample | 1 | 96 | 96.33 | 1.53 | 1.59 | 96 | 92.67 | 4.16 | 4.49 | 99 | 93.67 | 4.73 | 5.05 |
| | 2 | 98 | | | | 88 | | | | 92 | | | |
| | 3 | 95 | | | | 94 | | | | 90 | | | |
| Chironomid | 1 | 69 | 69.00 | 1.00 | 1.45 | 65 | 67.00 | 2.00 | 2.99 | 71 | 70.33 | 5.03 | 7.16 |
| sample | 2 | 68 | | | | 67 | | | | 65 | | | |
| | 3 | 70 | | | | 69 | | | | 75 | | | |
| Mollusc | 1 | 53 | 54.00 | 1.00 | 1.85 | 50 | 52.00 | 2.00 | 3.85 | 52 | 55.00 | 4.36 | 7.93 |
| sample | 2 | 55 | | | | 54 | | | | 53 | | | |
| | 3 | 54 | | | | 52 | | | | 60 | | | |
| Gammarus | 1 | 157 | 159.00 | 2.00 | 1.26 | 148 | 150.00 | 2.00 | 1.33 | 152 | 159.00 | 11.27 | 7.09 |
| sample | 2 | 159 | | | | 152 | | | | 153 | | | |
| | 3 | 161 | | | | 150 | | | | 172 | | | |
| Leech sample | 1 | 110 | 110.00 | 3.00 | 2.73 | 107 | 107.67 | 2.08 | 1.93 | 111 | 108.00 | 3.00 | 2.78 |
| | 2 | 113 | | | | 106 | | | | 105 | | | |
| | 3 | 107 | | | | 110 | | | | 108 | | | |

**APPENDIX A.3** The graphite furnace programme of Cd for checking the working range and the Cd absorbance values.

Cadmium Progarmme :

| Step | Temp ($^{O}$C) | Time (sec) | Gas | Flow rate (L/min) | Read |
|---|---|---|---|---|---|
| 1 | 75 | 5 | Nitrogen | 3 | |
| 2 | 90 | 50 | Nitrogen | 3 | |
| 3 | 120 | 10 | Nitrogen | 3 | |
| 4 | 120 | 5 | Nitrogen | 3 | |
| 5 | 300 | 1 | Nitrogen | 3 | |
| 6 | 300 | 20 | Nitrogen | 3 | |
| 7 | 300 | 1 | Nitrogen | 0 | |
| 8 | 1800 | 0 | Nitrogen | 0 | * |
| 9 | 1800 | 2 | Nitrogen | 0 | * |
| 10 | 1800 | 1 | Nitrogen | 3 | |
| 11 | 40 | 20 | Nitrogen | 3 | |

ABSORBANCE (x 1000):

PEAK HEIGHT MODE

Result 1:

| | Obs.Conc. (ng) | Abs.1 | Abs.2 | Abs.3 | Mean | S.D. | RSD% | Est.Abs |
|---|---|---|---|---|---|---|---|---|
| Blank | 0 | 2 | 3 | 2 | 2.33 | 0.58 | 24.74 | 12.11 |
| Standard 1 | 0.01 | 65 | 71 | 64 | 66.67 | 3.79 | 5.68 | 69.31 |
| Standard 2 | 0.02 | 140 | 140 | 131 | 137.00 | 5.20 | 3.79 | 126.5 |
| Standard 3 | 0.03 | 195 | 200 | 195 | 196.67 | 2.89 | 1.47 | 183.7 |
| Standard 4 | 0.04 | 239 | 246 | 244 | 243.00 | 3.61 | 1.48 | 240.9 |
| Standard 5 | 0.05 | 289 | 279 | 287 | 285.00 | 5.29 | 1.86 | 298.1 |

Regression Output:

| | | | |
|---|---|---|---|
| Constant | 12.11 | | |
| Std Err of Y Est | 11.8 | r= | |
| R Squared | 0.99 | | 0.995 |
| No. of Observations | 6 | | |
| Degrees of Freedom | 4 | | |
| X Coefficient(s) | 5720 | | |
| Std Err of Coef. | 282 | | |

Result 2:

| | Obs.Conc. (ng) | Abs.1 | Abs.2 | Abs.3 | Mean | S.D. | RSD% | Est.Abs |
|---|---|---|---|---|---|---|---|---|
| Blank | 0 | 0 | 1 | 0 | 0.33 | 0.58 | 173.21 | 3.698 |
| Standard 1 | 0.008 | 50 | 51 | 49 | 50.00 | 1.00 | 2.00 | 53.77 |
| Standard 2 | 0.016 | 110 | 105 | 115 | 110.00 | 5.00 | 4.55 | 103.8 |
| Standard 3 | 0.024 | 158 | 162 | 158 | 159.33 | 2.31 | 1.45 | 153.9 |
| Standard 4 | 0.032 | 197 | 211 | 212 | 206.67 | 8.39 | 4.06 | 204 |
| Standard 5 | 0.04 | 245 | 249 | 247 | 247.00 | 2.00 | 0.81 | 254.1 |

Regression Output:

| | | | |
|---|---|---|---|
| Constant | 3.698 | | |
| Std Err of Y Est | 6.12 | r= | |
| R Squared | 0.997 | | 0.998 |
| No. of Observations | 6 | | |
| Degrees of Freedom | 4 | | |
| X Coefficient(s) | 6260 | | |
| Std Err of Coef. | 182.9 | | |

**APPENDIX A.4** The graphite furnace programme of Cd with 0.25% of $H_3PO_4$ for checking different ashing and atomisation temperatures and the Cu absorbance values.

Cadmium Programme :

| Step | Temp (°C) | Time (sec) | Gas | Flow rate (L/min) | Read | |
|---|---|---|---|---|---|---|
| 1 | 75 | 5 | Nitrogen | 3 | | |
| 2 | 90 | 50 | Nitrogen | 3 | | |
| 3 | 120 | 10 | Nitrogen | 3 | | |
| 4 | 120 | 5 | Nitrogen | 3 | | |
| 5 | 450* | 10 | Nitrogen | 3 | | |
| 6 | 600* | 5 | Nitrogen | 3 | | |
| 7 | 600* | 1 | Nitrogen | 0 | | |
| 8 | 1800** | 0 | Nitrogen | 0 | | * |
| 9 | 1800** | 2 | Nitrogen | 0 | | * |
| 10 | 1800** | 1 | Nitrogen | 3 | | |
| 11 | 40 | 20 | Nitrogen | 3 | | |

(*, ** indicate examining parameter)

Operation Setting:

| Standard vol. | Blank vol. | Modifier vol. |
|---|---|---|
| 0 | 25 | 3 |
| 1 | 24 | 3 |
| 2 | 23 | 3 |
| 4 | 21 | 3 |
| 8 | 17 | 3 |
| 12 | 13 | 3 |
| Sample vol | | |
| 5/10/15/20 | 20/15/10/5 | 3 |

ABSORBANCE (x 1000):

* Checking the ashing temperature:

| | Temp. Settings | Readings Abs.1 | Abs.2 | Abs.3 | Mean | S.D. | RSD% | M+S.D. | M−S.D. |
|---|---|---|---|---|---|---|---|---|---|
| Standard | (1) | 224 | 224 | 229 | 225.67 | 2.89 | 1.28 | 229 | 223 |
| Solution | (2) | 248 | 243 | 241 | 244.00 | 3.61 | 1.48 | 248 | 240 |
| 0.02 ng | (3) | 247 | 245 | 248 | 246.67 | 1.53 | 0.62 | 248 | 245 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (1) | Step 5 | 450 | 10 sec | (2) | Step 5 | 600 | 10 sec | (3) | Step 5 | 600 |
| | Step 6 | 600 | 5 sec | | Step 6 | 600 | 5 sec | | Step 6 | 700 |
| | Step 7 | 600 | 1 sec | | Step 7 | 600 | 1 sec | | Step 7 | 700 |

** Checking the atomisation temperature:

| | Atomisation temperature | Readings Abs.1 | Abs.2 | Abs.3 | Mean | S.D. | RSD% | M+S.D. | M−S.D. |
|---|---|---|---|---|---|---|---|---|---|
| Standard | 1700 | 241 | 241 | 240 | 240.67 | 0.58 | 0.24 | 241 | 240 |
| Solution | 1900 | 245 | 247 | 243 | 245.00 | 2.00 | 0.82 | 247 | 243 |
| 0.02 ng | 2100 | 244 | 243 | 244 | 243.67 | 0.58 | 0.24 | 244 | 243 |

**APPENDIX A.5** The original measurements of various standard materials.

| Standard Material | No. | Cu concentration (ug/g dw) Method | Data | Mean | S.D. | Cd concentration (ug/g dw) Method | Data | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|
| Fish Flesh | | | | | | | | | |
| MA-A-2/TM | 1 | Flame | 4.0118 | 4.0151 | 0.2237 | GTA | 0.0732 | 0.0768 | 0.0081 |
| MA-A-2/TM | 2 | Flame | 4.1359 | | | GTA | 0.0801 | | |
| MA-A-2/TM | 3 | Flame | 3.9830 | | | GTA | 0.0861 | | |
| MA-A-2/TM | 4 | Flame | 3.7506 | | | GTA | 0.0834 | | |
| MA-A-2/TM | 5 | Flame | 4.1354 | | | GTA | 0.0749 | | |
| MA-A-2/TM | 6 | Flame | 4.2269 | | | GTA | 0.0846 | | |
| MA-A-2/TM | 7 | Flame | 4.2499 | | | GTA | 0.0669 | | |
| MA-A-2/TM | 8 | Flame | 3.6275 | | | GTA | 0.0648 | | |
| MA-A-2/TM | 1 | GTA | 3.8576 | 3.9221 | 0.1262 | | | | |
| MA-A-2/TM | 2 | GTA | 4.0344 | | | | | | |
| MA-A-2/TM | 3 | GTA | 3.8323 | | | | | | |
| MA-A-2/TM | 4 | GTA | 4.1805 | | | | | | |
| MA-A-2/TM | 5 | GTA | 3.8237 | | | | | | |
| MA-A-2/TM | 6 | GTA | 3.9416 | | | | | | |
| MA-A-2/TM | 7 | GTA | 3.8650 | | | | | | |
| MA-A-2/TM | 8 | GTA | 3.8413 | | | | | | |
| Copepoda | | | | | | | | | |
| MA-A-1/TM | 1 | Flame | 7.2737 | 7.2951 | 0.3353 | GTA | 1.0709 | 1.0910 | 0.0411 |
| MA-A-1/TM | 2 | Flame | 8.0438 | | | GTA | 1.0663 | | |
| MA-A-1/TM | 3 | Flame | 7.1326 | | | GTA | 1.0721 | | |
| MA-A-1/TM | 4 | Flame | 6.9999 | | | GTA | 1.1200 | | |
| MA-A-1/TM | 5 | Flame | 7.1340 | | | GTA | 1.1317 | | |
| MA-A-1/TM | 6 | Flame | 7.4402 | | | GTA | 1.0684 | | |
| MA-A-1/TM | 7 | Flame | 7.0391 | | | GTA | 1.0389 | | |
| MA-A-1/TM | 8 | Flame | 7.2975 | | | GTA | 1.1597 | | |
| Tea Leaves | | | | | | | | | |
| NIES No.7 | 1 | Flame | 6.7029 | 6.6903 | 0.1243 | GTA | 0.0317 | 0.0317 | 0.0053 |
| NIES No.7 | 2 | Flame | 6.5231 | | | GTA | 0.0368 | | |
| NIES No.7 | 3 | Flame | 6.7313 | | | GTA | 0.0256 | | |
| NIES No.7 | 4 | Flame | 6.6955 | | | GTA | 0.0263 | | |
| NIES No.7 | 5 | Flame | 6.6441 | | | GTA | 0.0334 | | |
| NIES No.7 | 6 | Flame | 6.8909 | | | GTA | 0.0289 | | |
| NIES No.7 | 7 | Flame | 6.7994 | | | GTA | 0.0300 | | |
| NIES No.7 | 8 | Flame | 6.5350 | | | GTA | 0.0412 | | |
| Lake Sediment | | | | | | | | | |
| SL-1 | 1 | Flame | 31.00 | 28.55 | 1.49 | Flame | N.D | | |
| SL-1 | 2 | Flame | 30.57 | | | Flame | N.D | | |
| SL-1 | 3 | Flame | 27.40 | | | Flame | N.D | | |
| SL-1 | 4 | Flame | 28.83 | | | Flame | N.D | | |
| SL-1 | 5 | Flame | 26.50 | | | Flame | N.D | | |
| SL-1 | 6 | Flame | 29.45 | | | Flame | N.D | | |
| SL-1 | 7 | Flame | 29.11 | | | Flame | N.D | | |
| SL-1 | 8 | Flame | 28.27 | | | Flame | N.D | | |
| SL-1 | 9 | Flame | 28.81 | | | Flame | N.D | | |
| SL-1 | 10 | Flame | 25.97 | | | Flame | N.D | | |
| SL-1 | 11 | Flame | 31.03 | | | Flame | N.D | | |
| SL-1 | 12 | Flame | 27.90 | | | Flame | 0.2403 | 0.3040 | 0.0561 |
| SL-1 | 13 | Flame | 27.15 | | | Flame | 0.3260 | | |
| SL-1 | 14 | Flame | 27.56 | | | Flame | 0.3458 | | |
| SL-1 | 15 | Flame | 28.28 | | | Flame | N.D | | |
| SL-1 | 16 | Flame | 28.98 | | | Flame | N.D | | |
| Pond Sediment | | | | | | | | | |
| NISE No.2 | 1 | Flame | 192.74 | 192.71 | 1.52 | Flame | N.D | | |
| NISE No.2 | 2 | Flame | 191.53 | | | Flame | N.D | | |
| NISE No.2 | 3 | Flame | 190.30 | | | Flame | N.D | | |
| NISE No.2 | 4 | Flame | 193.67 | | | Flame | 0.7173 | 0.8681 | 0.1308 |
| NISE No.2 | 5 | Flame | 193.91 | | | Flame | 0.9370 | | |
| NISE No.2 | 6 | Flame | 194.10 | | | Flame | 0.9500 | | |

**APPENDIX B.1** The original data of day-time water temperatures ($^{o}C$), pH values, conductivity (us/cm), total hardness (mg $CaCO_3$), and D.O. (%) at the various sampling sites in the River Wandle during Dec. 1987 and Jul. 1989.

Day Time Water Temperature ($^{o}C$) at the River Wandle

| Date | A+ | B | C | D | E | F | G | H | I | J | Min. | Max. | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec.'87 | | 8.0 | | 10.0 | | | | | | | 8.0 | 10.0 | 9.0 | 1.4 |
| Jan.'88 | | 9.5 | 9.7 | 10.8 | 9.8 | 12.1 | 11.0 | 10.7 | | | 9.5 | 12.1 | 10.5 | 0.9 |
| May. | | | 11.2 | 12.5 | 12.5 | | | | | | 11.2 | 12.5 | 12.1 | 0.8 |
| Jun. | 12.8 | 13.2 | 11.2 | 13.6 | 13.5 | 17.7 | 16.7 | 17.2 | 16.9 | | 11.2 | 17.7 | 14.8 | 2.4 |
| Aug. | 12.7 | 13.7 | 11.4 | 12.8 | 13.8 | 18.4 | 17.5 | 17.5 | 16.9 | | 11.4 | 18.4 | 15.0 | 2.6 |
| Sep. | 12.2 | 12.0 | 11.4 | 17.8 | 13.1 | 17.5 | 16.8 | 15.3 | 15.1 | | 11.4 | 17.8 | 14.6 | 2.5 |
| Oct. | 11.0 | 10.0 | 12.0 | 11.5 | 10.0 | 16.5 | 15.5 | 13.5 | 12.0 | | 10.0 | 16.5 | 12.4 | 2.3 |
| Dec. | 11.0 | 10.0 | 12.0 | 10.5 | 10.0 | 15.0 | 13.5 | 12.0 | | 9.0 | 9.0 | 15.0 | 11.4 | 1.9 |
| Mar.'89 | | 9.6 | 11.1 | 11.5 | 10.0 | | | 12.2 | 11.6 | | 9.6 | 12.2 | 11.0 | 1.0 |
| Apr. | | | 11.5 | 13.0 | | | | | | | 11.5 | 13.0 | 12.3 | 1.1 |
| May. | 13.5 | 15.1 | 11.9 | 10.6 | 16.5 | 17.8 | 17.8 | 19.2 | 18.5 | 18.4 | 10.6 | 19.2 | 15.9 | 3.0 |
| Jun. | 13.0 | 13.6 | 12.0 | 17.3 | 14.3 | 16.5 | 16.0 | 15.7 | 15.7 | 15.4 | 12.0 | 17.3 | 15.0 | 1.7 |
| Jul. | 16.1 | 15.8 | 13.3 | 16.6 | 14.8 | 16.2 | | 17.2 | | 16.6 | 13.3 | 17.2 | 15.8 | 1.2 |

pH values in the River Wandle

| DATE | A+ | B | C | D | E | F | G | H | I | J | Min. | Max. | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec.'87 | | 7.6 | 7.2 | 7.6 | | | | | | | 7.2 | 7.6 | 7.5 | 0.2 |
| Jan.'88 | | 7.5 | 7.4 | 7.6 | 7.6 | | | | | | 7.4 | 7.6 | 7.5 | 0.1 |
| Mar. | | | 7.3 | 7.5 | 7.4 | | 7.3 | | | | 7.3 | 7.5 | 7.4 | 0.1 |
| May. | | | 7.2 | 7.8 | 7.6 | | | | | | 7.2 | 7.8 | 7.5 | 0.3 |
| Jun. | 8.4 | 7.6 | 7.2 | 8.0 | 8.2 | 7.2 | 8.2 | 7.4 | 8.4 | | 7.2 | 8.4 | 7.8 | 0.5 |
| Aug. | 7.4 | 7.6 | 7.2 | 7.8 | 7.6 | 7.4 | 7.4 | 7.4 | 7.2 | | 7.2 | 7.8 | 7.4 | 0.2 |
| Sep. | 7.4 | 7.6 | 7.2 | 7.6 | 7.4 | 7.2 | 7.3 | 7.6 | 7.6 | | 7.2 | 7.6 | 7.4 | 0.2 |
| Oct. | 7.1 | 7.0 | 7.0 | 7.0 | 7.0 | 6.9 | 6.9 | 6.8 | 6.9 | | 6.8 | 7.1 | 7.0 | 0.1 |
| Mar.'89 | | 8.0 | 7.4 | 8.2 | 7.8 | | | 7.4 | 7.8 | | 7.4 | 8.2 | 7.8 | 0.3 |
| Apr. | | | 7.0 | 8.2 | | | | | | | 7.0 | 8.2 | 7.6 | 0.8 |
| May. | 6.9 | 7.5 | 6.9 | 7.9 | 7.8 | 7.3 | 7.0 | 7.5 | 7.5 | 7.6 | 6.9 | 7.9 | 7.4 | 0.4 |
| Jun. | 6.8 | 6.9 | 6.6 | 6.9 | 6.9 | 6.9 | 6.9 | 7.0 | 7.0 | 7.1 | 6.6 | 7.1 | 6.9 | 0.1 |
| Jul. | 7.1 | 7.2 | 7.1 | 7.3 | 7.4 | 7.4 | | 7.4 | | 7.4 | 7.1 | 7.4 | 7.3 | 0.1 |
| Min. | 6.8 | 6.9 | 6.6 | 6.9 | 6.9 | 6.9 | 6.9 | 6.8 | 6.9 | 7.1 | 6.6 | 7.1 | 6.9 | 0.1 |
| Max. | 8.4 | 8 | 7.4 | 8.2 | 8.2 | 7.4 | 8.2 | 7.6 | 8.4 | 7.6 | 7.4 | 8.4 | 7.8 | 0.8 |
| Mean | 7.3 | 7.4 | 7.1 | 7.6 | 7.5 | 7.2 | 7.3 | 7.3 | 7.5 | 7.4 | | | 7.4 | 0.3 |
| S.D. | 0.53 | 0.3 | 0.2 | 0.4 | 0.4 | 0.2 | 0.5 | 0.3 | 0.5 | 0.3 | | | 0.3 | 0.2 |

Conductivity (us/cm) at the River Wandle

| DATE | A+ | B | C | D | E | F | G | H | I | J | M+SD | M−SD | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec.'87 | | 647 | 664 | 617 | | | | | | | 617 | 664 | 643 | 24 |
| Jan.'88 | | 582 | 619 | 612 | 593 | 869 | 750 | 755 | | | 582 | 869 | 683 | 109 |
| Mar. | | | 390 | 370 | 270 | | 450 | | | | 270 | 450 | 370 | 75 |
| May. | | | 399 | 366 | 372 | | | | | | 366 | 399 | 379 | 18 |
| Jun. | 386 | 371 | 395 | 363 | 370 | 490 | 454 | 458 | 446 | | 363 | 490 | 415 | 47 |
| Aug. | 635 | 628 | 669 | 616 | 636 | 870 | 813 | 808 | 781 | | 616 | 870 | 717 | 99 |
| Sep. | 361 | 363 | 392 | 347 | 356 | 509 | 488 | 468 | 487 | | 347 | 509 | 419 | 67 |
| Oct. | 440 | 430 | 470 | 450 | 430 | 710 | 780 | 620 | 580 | | 430 | 780 | 546 | 133 |
| Dec. | 430 | 410 | 450 | 410 | 420 | 670 | 500 | | | 600 | 410 | 670 | 486 | 98 |
| Mar.'89 | | 590 | 650 | 535 | 435 | | | 726 | 755 | | 435 | 755 | 615 | 120 |
| Apr. | | | 460 | 450 | | | | | | | 450 | 460 | 455 | 7 |
| May. | 643 | 620 | 641 | 569 | 598 | 916 | 849 | 840 | 841 | 835 | 569 | 916 | 735 | 131 |
| Jun. | 648 | 636 | 642 | 514 | 566 | 807 | 753 | 748 | 762 | 768 | 514 | 807 | 684 | 97 |
| Jul. | 684 | 632 | 646 | 1422 | 1449 | 2390 | | 2260 | | 2240 | 632 | 2390 | 1465 | 762 |
| Min. | 361 | 363 | 390 | 347 | 270 | 490 | 450 | 458 | 446 | 600 | 270 | 399 | 370 | 7.07 |
| Max. | 684 | 636 | 669 | 1422 | 1449 | 2390 | 849 | 2260 | 841 | 2240 | 632 | 2390 | 1465 | 762 |
| Mean | 528.4 | 526 | 525 | 540 | 541 | 915 | 649 | 854 | 665 | 1111 | | | 613 | 136 |
| S.D. | 135.7 | 117 | 119 | 282 | 308 | 574 | 170 | 545 | 157 | 759 | | | 288 | 192 |

Total Hardness ($CaCO_3$ mg/L) in the River Wandle

| DATE | A+ | B | C | D | E | F | G | H | I | J | M+SD | M−SD | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oct. | 220 | 190 | 140 | 150 | 190 | 235 | 260 | 260 | 270 | | 140 | 270 | 213 | 48 |
| Dec.'88 | 275 | 240 | 260 | 240 | 230 | 210 | 230 | 230 | | 290 | | | | |
| Mar.'89 | | 240 | 260 | 190 | | 160 | | 250 | 235 | | 160 | 260 | 223 | 39 |
| Apr. | 220 | 230 | 283 | 240 | 230 | 240 | 260 | 260 | 270 | 300 | 220 | 300 | 253 | 26 |
| May. | 280 | 285 | 283 | 250 | 300 | 275 | 300 | 300 | 305 | 310 | 250 | 310 | 289 | 18 |
| Jun. | 275 | 250 | 275 | 245 | 230 | 255 | 270 | 270 | 275 | 280 | 230 | 280 | 263 | 17 |
| Jul. | 250 | 270 | 290 | 240 | 245 | 270 | | 280 | | 295 | 240 | 295 | 268 | 21 |

D.O. (%) in the River Wandle

| DATE | A+ | B | C | D | E | F | G | H | I | J | Min. | Max. | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jan.'88 | | 92 | 92 | 100 | 98 | | | | | | 92 | 100 | 96 | 4 |
| Jun.'89 | 78 | 92 | 86 | 120 | 80 | 68 | 72 | 74 | 78 | 74 | 68 | 120 | 82 | 15 |
| Jul. | 84 | 83 | 75 | 90 | 96 | 48 | | 72 | | 62 | 48 | 96 | 76 | 16 |
| Mean | 81 | 89 | 84 | 103 | 91 | 58 | 72 | 73 | 78 | 68 | | | 85 | 12 |
| S.D. | 4 | 5 | 9 | 15 | 10 | 14 | | 1 | | 8 | | | 10 | 6 |

# **APPENDIX B.2** Concentrations of heavy metals in samples of sediment taken from the River Wandle during 4th May 1988 and 3rd Aug. 1988.

Keys to sampling sites:
A: Waddon pond.
A+: Croydon Arm just below Waddon pond at Aldwick Rd..
B: Croydon Arm beside Church Rd. at Beddington Park.
C: Croydon Arm beside London Rd. at Manor Garden.
D: Carshalton Arm below Wandle Rail Bridge at Mill lane.
E: Junction of two Arms beside Hack Bridge.
F: Beddington sewage effluent channel at Goat road.
G: Below the junction of sewage effluent to River Wandle.
H: River Wandle beside Morden Cottage at Morden Hall Park.
I: River Wandle beside Steerforth street at Summertown.

| | | | COPPER | | | CADMIUM | | | CHROMIUM | | | LEAD | | | NICKEL | | | ZINC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CONCENTRATION ( ppm = ug/g dry wt.) | | | | | | | | | | | | | | | | | |
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. |
| 04/05/88 | A+ | 1 | 55.25 | 52.88 | 1.79 | 0.69 | 0.81 | 0.17 | 24.27 | 23.23 | 1.06 | 445.2 | 438.6 | 11.0 | 24.15 | 21.78 | 1.72 | 292.9 | 293.0 | 15.8 |
| 04/05/88 | A+ | 2 | 53.16 | | | 0.67 | | | 23.97 | | | 438.7 | | | 21.75 | | | 307.2 | | |
| 04/05/88 | A+ | 3 | 52.04 | | | 0.85 | | | 22.63 | | | 423.1 | | | 20.08 | | | 301.0 | | |
| 04/05/88 | A+ | 4 | 51.08 | | | 1.04 | | | 22.06 | | | 447.5 | | | 21.14 | | | 270.9 | | |
| 29/06/88 | A+ | 1 | 63.75 | 65.65 | 2.05 | 2.84 | 3.10 | 0.24 | 40.46 | 41.92 | 1.27 | 628.4 | 637.4 | 10.9 | 29.60 | 30.69 | 1.15 | 492.9 | 490.3 | 2.3 |
| 29/06/88 | A+ | 2 | 67.82 | | | 3.32 | | | 42.75 | | | 649.5 | | | 31.90 | | | 488.5 | | |
| 29/06/88 | A+ | 3 | 65.38 | | | 3.15 | | | 42.56 | | | 634.3 | | | 30.57 | | | 489.6 | | |
| 03/08/88 | A+ | 1 | 182.95 | 181.18 | 2.42 | 3.57 | 3.69 | 0.37 | 46.31 | 46.52 | 0.20 | 1117 | 1091.7 | 22.3 | 46.31 | 45.75 | 1.19 | 1111.4 | 1104.8 | 20.3 |
| 03/08/88 | A+ | 2 | 182.17 | | | 3.39 | | | 46.71 | | | 1074 | | | 44.38 | | | 1121.1 | | |
| 03/08/88 | A+ | 3 | 178.42 | | | 4.10 | | | 46.55 | | | 1083.8 | | | 46.55 | | | 1082.0 | | |
| 04/05/88 | B | 1 | 279.49 | 274.49 | 4.65 | 6.98 | 6.63 | 0.59 | 50.23 | 48.57 | 1.49 | 1164.8 | 1149.6 | 15.1 | 56.25 | 55.35 | 0.68 | 1139.8 | 1103.0 | 42.0 |
| 04/05/88 | B | 2 | 272.95 | | | 7.15 | | | 48.25 | | | 1158.8 | | | 55.04 | | | 1138.9 | | |
| 04/05/88 | B | 3 | 268.79 | | | 5.83 | | | 46.70 | | | 1131.3 | | | 54.67 | | | 1065.9 | | |
| 04/05/88 | B | 4 | 276.74 | | | 6.57 | | | 49.09 | | | 1143.6 | | | 55.44 | | | 1067.4 | | |
| 29/06/88 | B | 1 | 241.70 | 238.94 | 2.58 | 8.00 | 9.02 | 0.88 | 47.31 | 47.54 | 1.61 | 948.5 | 937.4 | 10.0 | 44.20 | 42.22 | 1.77 | 1151.1 | 1181.5 | 27.4 |
| 29/06/88 | B | 2 | 236.59 | | | 9.51 | | | 46.06 | | | 929.0 | | | 41.67 | | | 1204.2 | | |
| 29/06/88 | B | 3 | 238.51 | | | 9.56 | | | 49.25 | | | 934.6 | | | 40.78 | | | 1189.3 | | |
| 03/08/88 | B | 1 | 313.02 | 308.76 | 3.94 | 7.31 | 7.77 | 0.46 | 57.39 | 56.53 | 0.83 | 1371.2 | 1358.8 | 13.3 | 54.71 | 55.56 | 0.88 | 1439.9 | 1436.1 | 17.6 |
| 03/08/88 | B | 2 | 308.01 | | | 7.77 | | | 56.47 | | | 1360.4 | | | 56.47 | | | 1416.8 | | |
| 03/08/88 | B | 3 | 305.24 | | | 8.24 | | | 55.74 | | | 1344.8 | | | 55.50 | | | 1451.5 | | |
| 04/05/88 | C | 1 | 365.00 | 359.88 | 5.07 | 10.77 | 10.78 | 0.09 | 61.93 | 62.36 | 0.69 | 1640.6 | 1630.6 | 22.8 | 67.95 | 66.65 | 1.54 | 1273.9 | 1269.5 | 23.2 |
| 04/05/88 | C | 2 | 353.51 | | | 10.91 | | | 61.86 | | | 1658.1 | | | 67.56 | | | 1299.8 | | |
| 04/05/88 | C | 3 | 358.32 | | | 10.69 | | | 63.35 | | | 1612.5 | | | 64.50 | | | 1258.5 | | |
| 04/05/88 | C | 4 | 362.67 | | | 10.75 | | | 62.31 | | | 1611.4 | | | 66.60 | | | 1245.8 | | |
| 29/06/88 | C | 1 | 55.11 | 55.69 | 1.05 | 0.56 | 0.53 | 0.16 | 23.45 | 23.46 | 0.04 | 297.6 | 293.6 | 5.4 | 28.80 | 28.87 | 0.80 | 183.6 | 178.7 | 9.3 |
| 29/06/88 | C | 2 | 56.90 | | | 0.67 | | | 23.50 | | | 287.4 | | | 29.70 | | | 168.0 | | |
| 29/06/88 | C | 3 | 55.07 | | | 0.35 | | | 23.43 | | | 295.7 | | | 28.10 | | | 184.6 | | |
| 03/08/88 | C | 1 | 84.64 | 85.27 | 1.12 | 2.07 | 1.85 | 0.33 | 23.59 | 23.52 | 0.11 | 589.5 | 588.1 | 2.8 | 29.61 | 28.13 | 1.45 | 344.0 | 342.8 | 1.2 |
| 03/08/88 | C | 2 | 84.61 | | | 2.02 | | | 23.58 | | | 590.0 | | | 26.72 | | | 342.9 | | |
| 03/08/88 | C | 3 | 86.56 | | | 1.47 | | | 23.40 | | | 584.9 | | | 28.07 | | | 341.6 | | |
| 04/05/88 | D | 1 | 122.20 | 123.79 | 3.59 | 0.06 | 0.15 | 0.07 | 28.91 | 29.35 | 0.90 | 268.5 | 276.0 | 5.8 | 28.64 | 28.69 | 1.89 | 245.4 | 246.3 | 8.4 |
| 04/05/88 | D | 2 | 122.54 | | | 0.15 | | | 28.30 | | | 275.7 | | | 26.12 | | | 234.8 | | |
| 04/05/88 | D | 3 | 121.31 | | | 0.18 | | | 30.07 | | | 282.5 | | | 29.46 | | | 250.9 | | |
| 04/05/88 | D | 4 | 129.12 | | | 0.22 | | | 30.13 | | | 277.2 | | | 30.55 | | | 254.0 | | |
| 29/06/88 | D | 1 | 172.67 | 176.41 | 4.65 | 2.54 | 2.05 | 0.45 | 45.38 | 46.06 | 0.64 | 687.5 | 680.5 | 6.4 | 42.00 | 43.28 | 1.14 | 591.7 | 580.9 | 11.1 |
| 29/06/88 | D | 2 | 181.62 | | | 1.66 | | | 46.14 | | | 678.8 | | | 44.20 | | | 581.5 | | |
| 29/06/88 | D | 3 | 174.94 | | | 1.94 | | | 46.65 | | | 675.1 | | | 43.65 | | | 569.6 | | |
| 03/08/88 | D | 1 | 185.59 | 185.00 | 1.83 | 3.46 | 3.25 | 0.48 | 49.91 | 49.65 | 0.30 | 866.6 | 862.6 | 10.0 | 52.18 | 52.42 | 1.69 | 671.5 | 674.0 | 2.5 |
| 03/08/88 | D | 2 | 182.94 | | | 3.58 | | | 49.33 | | | 870.1 | | | 54.21 | | | 676.6 | | |
| 03/08/88 | D | 3 | 186.46 | | | 2.69 | | | 49.72 | | | 851.2 | | | 50.86 | | | 674.0 | | |
| 04/05/88 | E | 1 | 439.95 | 440.57 | 4.14 | 12.52 | 12.24 | 0.32 | 68.62 | 66.96 | 1.27 | 1797.0 | 1784.4 | 13.4 | 78.71 | 78.03 | 1.92 | 1414.6 | 1443.2 | 19.3 |
| 04/05/88 | E | 2 | 444.77 | | | 12.39 | | | 65.82 | | | 1775.5 | | | 80.15 | | | 1448.8 | | |
| 04/05/88 | E | 3 | 442.46 | | | 11.79 | | | 67.27 | | | 1770.4 | | | 77.67 | | | 1457.0 | | |
| 04/05/88 | E | 4 | 435.11 | | | 12.28 | | | 66.14 | | | 1794.6 | | | 75.59 | | | 1452.3 | | |
| 29/06/88 | E | 1 | 424.68 | 426.71 | 1.98 | 15.26 | 15.00 | 0.62 | 73.25 | 74.08 | 0.86 | 1444.6 | 1431.3 | 13.7 | 58.50 | 57.03 | 1.33 | 1147.3 | 1150.8 | 16.4 |
| 29/06/88 | E | 2 | 428.64 | | | 15.46 | | | 74.97 | | | 1417.3 | | | 55.90 | | | 1168.6 | | |
| 29/06/88 | E | 3 | 426.82 | | | 14.30 | | | 74.02 | | | 1431.9 | | | 56.70 | | | 1136.4 | | |
| 03/08/88 | E | 1 | 313.93 | 308.23 | 4.94 | 12.85 | 12.22 | 0.63 | 66.59 | 67.64 | 1.11 | 1498.3 | 1488.3 | 9.0 | 61.83 | 63.32 | 1.51 | 1312.8 | 1329.3 | 19.4 |
| 03/08/88 | E | 2 | 305.48 | | | 11.60 | | | 68.80 | | | 1480.9 | | | 63.30 | | | 1324.5 | | |
| 03/08/88 | E | 3 | 305.27 | | | 12.19 | | | 67.54 | | | 1485.8 | | | 64.84 | | | 1350.7 | | |
| 29/06/88 | F | 1 | 155.41 | 151.14 | 3.71 | 18.36 | 19.05 | 0.92 | 41.71 | 42.42 | 0.88 | 250.7 | 235.9 | 12.9 | 33.89 | 33.00 | 1.15 | 392.4 | 367.0 | 28.9 |
| 29/06/88 | F | 2 | 149.21 | | | 20.09 | | | 43.41 | | | 227.4 | | | 33.40 | | | 373.0 | | |
| 29/06/88 | F | 3 | 148.79 | | | 18.70 | | | 42.15 | | | 229.5 | | | 31.70 | | | 335.6 | | |
| 03/08/88 | F | 1 | 519.97 | 516.61 | 3.08 | 21.03 | 20.46 | 0.56 | 136.34 | 135.50 | 1.20 | 1361.6 | 1367.3 | 14.8 | 118.40 | 119.97 | 2.27 | 1746.0 | 1724.5 | 20.8 |
| 03/08/88 | F | 2 | 513.91 | | | 20.42 | | | 136.03 | | | 1356.1 | | | 118.94 | | | 1723.1 | | |
| 03/08/88 | F | 3 | 515.94 | | | 19.92 | | | 134.13 | | | 1384.1 | | | 122.58 | | | 1704.5 | | |
| 29/06/88 | G | 1 | 144.60 | 142.57 | 2.80 | 17.27 | 18.17 | 0.81 | 55.23 | 53.56 | 1.52 | 523.9 | 533.5 | 12.2 | 37.60 | 37.07 | 0.55 | 633.3 | 628.4 | 10.9 |
| 29/06/88 | G | 2 | 143.73 | | | 18.40 | | | 53.18 | | | 529.4 | | | 37.10 | | | 615.9 | | |
| 29/06/88 | G | 3 | 139.37 | | | 18.83 | | | 52.26 | | | 547.2 | | | 36.50 | | | 636.0 | | |
| 03/08/88 | G | 1 | 293.90 | 291.03 | 2.54 | 21.20 | 19.98 | 1.20 | 82.42 | 81.35 | 1.12 | 1131.4 | 1118.6 | 12.5 | 72.60 | 73.17 | 2.06 | 1270.7 | 1240.9 | 26.2 |
| 03/08/88 | G | 2 | 289.05 | | | 18.79 | | | 80.18 | | | 1118.0 | | | 71.45 | | | 1221.6 | | |
| 03/08/88 | G | 3 | 290.15 | | | 19.96 | | | 81.46 | | | 1106.4 | | | 75.46 | | | 1230.3 | | |
| 29/06/88 | H | 1 | 467.79 | 467.09 | 4.04 | 20.88 | 20.92 | 0.07 | 93.33 | 94.46 | 1.35 | 1319.4 | 1320.0 | 8.4 | 76.60 | 75.60 | 0.87 | 1444.5 | 1457.6 | 14.8 |
| 29/06/88 | H | 2 | 462.75 | | | 21.00 | | | 94.09 | | | 1311.9 | | | 75.20 | | | 1454.6 | | |
| 29/06/88 | H | 3 | 470.73 | | | 20.88 | | | 95.96 | | | 1328.6 | | | 75.00 | | | 1473.6 | | |
| 03/08/88 | H | 1 | 440.30 | 440.35 | 3.54 | 59.34 | 61.39 | 1.80 | 168.92 | 168.41 | 0.56 | 1329.2 | 1341.4 | 12.1 | 99.69 | 98.99 | 1.35 | 1811.7 | 1824.9 | 14.6 |
| 03/08/88 | H | 2 | 443.92 | | | 62.66 | | | 167.82 | | | 1353.4 | | | 97.44 | | | 1840.6 | | |
| 03/08/88 | H | 3 | 436.84 | | | 62.18 | | | 168.50 | | | 1341.7 | | | 99.85 | | | 1822.3 | | |
| 29/06/88 | I | 1 | 156.48 | 157.76 | 4.62 | 13.27 | 14.37 | 0.99 | 22.60 | 22.88 | 0.30 | 306.8 | 298.0 | 8.5 | 27.10 | 26.39 | 0.82 | 363.2 | 337.0 | 28.6 |
| 29/06/88 | I | 2 | 162.89 | | | 14.63 | | | 23.19 | | | 289.8 | | | 25.50 | | | 306.4 | | |
| 29/06/88 | I | 3 | 153.91 | | | 15.19 | | | 22.86 | | | 297.4 | | | 26.58 | | | 341.4 | | |

**APPENDIX B.3** Concentrations of copper in samples of water taken from the River Wandle during 12th Dec. 1988 and 6th Jul. 1989.

Keys to sampling sites:

A: Waddon pond.
A+: Croydon Arm just below Waddon pond at Aldwick Rd..
B: Croydon Arm beside Church Rd. at Beddington Park.
C: Croydon Arm beside London Rd. at Manor Garden.
D: Carshalton Arm below Wandle Rail Bridge at Mill lane.
E: Junction of two Arms beside Hack Bridge.
F: Beddington sewage effluent channel at Goat road.
G: Below the junction of sewage effluent to River Wandle.
H: River Wandle beside Morden Cottage at Morden Hall Park.
I: River Wandle beside Steerforth street at Summertowm.
J: Above the mouth of River Wandle to The Thames at Wandsworth.
K: River Graveney.

| | | | TOTAL COPPER Concentration ( ppb=ng/ml) | | | DISSOLVED COPPER | | |
|---|---|---|---|---|---|---|---|---|
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. |
| 06/07/89 | A | 1 | 2.8941 | 3.7004 | 1.1403 | 1.9592 | 2.4510 | 0.6955 |
| 06/07/89 | A | 2 | 4.5067 | | | 2.9428 | | |
| 12/12/88 | A+ | 1 | 8.5500 | 7.2300 | 1.2686 | | | |
| 12/12/88 | A+ | 2 | 7.1200 | | | | | |
| 12/12/88 | A+ | 3 | 6.0200 | | | | | |
| 08/06/89 | A+ | 1 | 7.7671 | 6.5685 | 1.6951 | | | |
| 08/06/89 | A+ | 2 | 5.3699 | | | | | |
| 12/12/88 | B | 1 | 10.3600 | 11.547 | 2.5886 | | | |
| 12/12/88 | B | 2 | 9.7656 | | | | | |
| 12/12/88 | B | 3 | 14.5167 | | | | | |
| 08/06/89 | B | 1 | 5.3775 | 4.6818 | 0.9839 | | | |
| 08/06/89 | B | 2 | 3.9860 | | | | | |
| 06/07/89 | B | 1 | 10.1687 | 9.5838 | 0.8272 | 5.8586 | 6.8765 | 1.4395 |
| 06/07/89 | B | 2 | 8.9988 | | | 7.8944 | | |
| 12/12/88 | C | 1 | 4.3900 | 3.2000 | 1.3584 | | | |
| 12/12/88 | C | 2 | 1.7200 | | | | | |
| 12/12/88 | C | 3 | 3.4900 | | | | | |
| 08/06/89 | C | 1 | 5.9312 | 4.4987 | 2.0259 | | | |
| 08/06/89 | C | 2 | 3.0662 | | | | | |
| 06/07/89 | C | 1 | 3.7478 | 4.4969 | 1.0593 | 3.3340 | 2.6623 | 0.9499 |
| 06/07/89 | C | 2 | 5.2459 | | | 1.9906 | | |
| 12/12/88 | D | 1 | 3.0600 | 2.0189 | 0.9379 | | | |
| 12/12/88 | D | 2 | 1.2400 | | | | | |
| 12/12/88 | D | 3 | 1.7567 | | | | | |
| 08/06/89 | D | 1 | 3.2792 | 2.9923 | 0.4057 | | | |
| 08/06/89 | D | 2 | 2.7054 | | | | | |
| 06/07/89 | D | 1 | 5.0548 | 5.1827 | 0.1809 | 4.2588 | 4.8030 | 0.7696 |
| 06/07/89 | D | 2 | 5.3106 | | | 5.3472 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12/12/88 | E | 1 | 4.1200 | 2.7867 | 1.1956 | | | |
| 12/12/88 | E | 2 | 2.4300 | | | | | |
| 12/12/88 | E | 3 | 1.8100 | | | | | |
| 08/06/89 | E | 1 | 2.5798 | 1.7961 | 1.1083 | | | |
| 08/06/89 | E | 2 | 1.0124 | | | | | |
| 06/07/89 | E | 1 | 4.7817 | 5.3334 | 0.6811 | 3.7339 | 4.3010 | 0.8890 |
| 06/07/89 | E | 2 | 6.3272 | | | 5.6091 | | |
| 06/07/89 | E | 3 | 5.0796 | | | 4.1089 | | |
| 06/07/89 | E | 4 | 5.1451 | | | 3.7522 | | |
| 12/12/88 | F | 1 | 12.3100 | 14.530 | 1.9679 | | | |
| 12/12/88 | F | 2 | 16.0600 | | | | | |
| 12/12/88 | F | 3 | 15.2200 | | | | | |
| 08/06/89 | F | 1 | 5.2609 | 4.8019 | 0.6491 | | | |
| 08/06/89 | F | 2 | 4.3429 | | | | | |
| 06/07/89 | F | 1 | 13.1973 | 12.197 | 1.4140 | 8.5582 | 7.2622 | 1.8328 |
| 06/07/89 | F | 2 | 11.1976 | | | 5.9662 | | |
| 12/12/88 | G | 1 | 10.0500 | 8.1700 | 1.6318 | | | |
| 12/12/88 | G | 2 | 7.1200 | | | | | |
| 12/12/88 | G | 3 | 7.3400 | | | | | |
| 08/06/89 | G | 1 | 5.5523 | 4.7435 | 1.1439 | | | |
| 08/06/89 | G | 2 | 3.9346 | | | | | |
| 12/12/88 | H | 1 | 6.8700 | 5.9000 | 1.0839 | | | |
| 12/12/88 | H | 2 | 6.1000 | | | | | |
| 12/12/88 | H | 3 | 4.7300 | | | | | |
| 08/06/89 | H | 1 | 7.2300 | 6.4932 | 1.0421 | | | |
| 08/06/89 | H | 2 | 5.7563 | | | | | |
| 06/07/89 | H | 1 | 9.3246 | 8.9253 | 0.5648 | 6.5335 | 5.8689 | 0.9398 |
| 06/07/89 | H | 2 | 8.5259 | | | 5.2044 | | |
| 08/06/89 | I | 1 | 3.9203 | 3.2019 | 1.0160 | | | |
| 08/06/89 | I | 2 | 2.4834 | | | | | |
| 08/06/89 | J | 1 | 4.4740 | 4.5889 | 0.1625 | | | |
| 08/06/89 | J | 2 | 4.7038 | | | | | |
| 06/07/89 | J | 1 | 10.6900 | 10.097 | 0.5762 | 6.6335 | 5.3595 | 1.8017 |
| 06/07/89 | J | 2 | 11.5049 | | | 4.0855 | | |
| 12/12/88 | K | 1 | 5.3200 | 5.3978 | 0.7497 | | | |
| 12/12/88 | K | 2 | 4.6900 | | | | | |
| 12/12/88 | K | 3 | 6.1833 | | | | | |

(end)

# **APPENDIX B.4** Concentrations of heavy metals in samples of 3-spined sticklebacks taken from the River Wandle during 4th May 1988 and 15th Mar. 1989.

Keys to sampling sites:
A: Waddon pond.
A+: Croydon Arm just below Waddon pond at Aldwick Rd..
B: Croydon Arm beside Church Rd. at Beddington Park.
C: Croydon Arm beside London Rd. at Manor Garden.
D: Carshalton Arm below Wandle Rail Bridge at Mill lane.
E: Junction of two Arms beside Hack Bridge.
F: Beddington sewage effluent channel at Goat road.
G: Below the junction of sewage effluent to River Wandle.
H: River Wandle beside Morden Cottage at Morden Hall Park.
I: River Wandle beside Steerforth street at Summertown.

| | | | COPPER | | | CADMIUM | | | CHROMIUM | | | LEAD | | | NICKEL | | | ZINC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CONCENTRATION ( ppm = ug/g dry wt.) | | | | | | | | | | | | | | | | |
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. | Data | Mean | S.D. |
| 04/05/88 | B | 1 | 5.31 | 16.88 | 9.90 | | 0.34 | 0.20 | 1.6867 | 1.37 | 0.44 | | | | N.D. | | | | | |
| 29/06/88 | B | 1 | 22.34 | | | | | | 1.0627 | | | 17.19 | | | | | | 158.13 | | |
| 15/03/89 | B | 1 | 30.91 | | | 0.5656 | | | | | | | | | | | | | | |
| 15/03/89 | B | 2 | 13.22 | | | 0.2322 | | | | | | | | | | | | | | |
| 15/03/89 | B | 3 | 12.63 | | | 0.2106 | | | | | | | | | | | | | | |
| 04/05/88 | C | 1 | 22.00 | 11.54 | 4.61 | | 0.96 | 0.28 | 2.0304 | 3.85 | 1.10 | | 21.71 | 4.93 | | | | | 301.85 | 96.50 |
| 04/05/88 | C | 2 | 9.26 | | | | | | 2.0464 | | | | | | | | | 201.91 | | |
| 29/06/88 | C | 1 | 8.04 | | | 1.0715 | | | 4.8219 | | | | | | | | | | | |
| 29/06/88 | C | 2 | 6.38 | | | 1.1253 | | | 4.5011 | | | | | | | | | | | |
| 29/06/88 | C | 3 | 10.61 | | | 1.4164 | | | 4.7214 | | | | | | | | | | | |
| 29/06/88 | C | 4 | 14.77 | | | 1.1148 | | | 5.5741 | | | | | | | | | | | |
| 29/06/88 | C | 5 | 10.01 | | | 0.7899 | | | 3.9494 | | | | | | | | | | | |
| 29/06/88 | C | 6 | 8.59 | | | 1.1211 | | | 3.7369 | | | | | | | | | | | |
| 29/06/88 | C | 7 | 8.70 | | | 0.9317 | | | 4.6584 | | | | | | | | | | | |
| 29/06/88 | C | 8 | 8.76 | | | 0.9217 | | | 3.4562 | | | | | | | | | | | |
| 29/06/88 | C | 9 | 8.85 | | | 1.0111 | | | 3.7917 | | | | | | | | | | | |
| 29/06/88 | C | 10 | 5.57 | | | 0.5862 | | | 2.9308 | | | | | | | | | | | |
| 29/06/88 | C | 1 | 9.27 | | | | | | | | | 25.96 | | | N.D. | | | 270.77 | | |
| 29/06/88 | C | 2 | 16.87 | | | | | | | | | 16.87 | | | N.D. | | | 216.47 | | |
| 29/06/88 | C | 3 | 10.95 | | | | | | | | | 21.89 | | | N.D. | | | 245.18 | | |
| 29/06/88 | C | 4 | 21.98 | | | | | | | | | 18.69 | | | N.D. | | | 428.67 | | |
| 29/06/88 | C | 5 | 14.56 | | | | | | | | | 29.12 | | | N.D. | | | 436.77 | | |
| 29/06/88 | C | 6 | 16.34 | | | | | | | | | 17.70 | | | N.D. | | | 313.18 | | |
| 15/03/89 | C | 1 | 12.38 | | | N.D. | | | | | | | | | | | | | | |
| 15/03/89 | C | 2 | 10.62 | | | N.D. | | | | | | | | | | | | | | |
| 15/03/89 | C | 3 | 7.80 | | | 0.4207 | | | | | | | | | | | | | | |
| 04/05/88 | D | 1 | | 15.11 | 8.63 | | | | 2.5458 | 2.49 | 0.39 | | 28.26 | 8.56 | | | | | 226.60 | 33.36 |
| 04/05/88 | D | 1 | 10.78 | | | | | | | | | | | | | | | | | |
| 04/05/88 | D | 2 | 28.52 | | | | | | 2.8520 | | | | | | | | | | | |
| 04/05/88 | D | 3 | 33.02 | | | | | | 2.0855 | | | | | | | | | 245.25 | | |
| 29/06/88 | D | 1 | 9.99 | | | | | | | | | 21.22 | | | | | | 249.63 | | |
| 29/06/88 | D | 2 | 9.66 | | | | | | | | | 22.54 | | | | | | 209.27 | | |
| 29/06/88 | D | 3 | 11.72 | | | | | | | | | 29.31 | | | | | | 175.85 | | |
| 29/06/88 | D | 4 | 16.64 | | | | | | | | | 39.95 | | | | | | 253.00 | | |
| 15/03/89 | D | 1 | 12.38 | | | N.D. | | | | | | | | | | | | | | |
| 15/03/89 | D | 2 | 10.62 | | | N.D. | | | | | | | | | | | | | | |
| 15/03/89 | D | 3 | 7.80 | | | N.D. | | | | | | | | | | | | | | |
| 04/05/88 | E | 1 | 43.77 | 19.29 | 13.54 | | | | 2.0199 | 2.47 | 0.64 | | 28.33 | 8.43 | | | | | 217.41 | 74.02 |
| 04/05/88 | E | 2 | 31.74 | | | | | | 2.9297 | | | | | | | | | | | |
| 29/06/88 | E | 1 | 8.68 | | | | | | | | | 23.15 | | | | | | 156.30 | | |
| 29/06/88 | E | 2 | 9.51 | | | | | | | | | 38.06 | | | | | | 299.72 | | |
| 29/06/88 | E | 3 | 10.40 | | | | | | | | | 23.78 | | | | | | 196.20 | | |
| 15/03/89 | E | 1 | 12.45 | | | N.D | | | | | | | | | | | | | | |
| 15/03/89 | E | 2 | 28.77 | | | 0.1969 | | | | | | | | | | | | | | |
| 15/03/89 | E | 3 | 9.01 | | | N.D. | | | | | | | | | | | | | | |
| 29/06/88 | G | 1 | 14.24 | 8.17 | 4.75 | | | | | | | 14.24 | 40.52 | 29.18 | | | | 332.28 | 243.78 | 74.52 |
| 29/06/88 | G | 2 | 3.66 | | | | | | | | | 19.28 | | | | | | 161.05 | | |
| 29/06/88 | G | 3 | 5.24 | | | | | | | | | 52.36 | | | | | | 272.25 | | |
| 29/06/88 | G | 4 | 9.52 | | | | | | | | | 76.19 | | | | | | 209.52 | | |
| 29/06/88 | H | 1 | 14.49 | 13.00 | 5.64 | | | | | | | 20.28 | 21.15 | 2.74 | | | | 333.24 | 234.84 | 71.52 |
| 29/06/88 | H | 2 | 8.46 | | | | | | | | | 20.30 | | | | | | 169.20 | | |
| 29/06/88 | H | 3 | 10.47 | | | | | | | | | 25.13 | | | | | | 238.69 | | |
| 29/06/88 | H | 4 | 7.87 | | | | | | | | | 18.88 | | | | | | 198.24 | | |
| 29/06/88 | H | 1 | 13.11 | | | 0.8279 | | | | | | | | | | | | | | |
| 29/06/88 | H | 2 | 12.00 | | | N.D. | | | | | | | | | | | | | | |
| 29/06/88 | H | 3 | 24.58 | | | 0.7736 | | | | | | | | | | | | | | |
| 29/06/88 | I | 1 | 54.32 | 21.79 | 19.1 | | | | | | | 18.55 | 34.11 | 22 | | | | 271.62 | 264.4 | 10.2 |
| 29/06/88 | I | 2 | 4.92 | | | | | | | | | 49.66 | | | | | | 257.17 | | |
| 29/06/88 | I | 1 | 21.15 | | | 0.7200 | | | | | | | | | | | | | | |
| 29/06/88 | I | 2 | 12.95 | | | N.D. | | | | | | | | | | | | | | |
| 29/06/88 | I | 3 | 15.62 | | | N.D. | | | | | | | | | | | | | | |

**APPENDIX B.5** Concentrations copper in samples of 3-spined sticklebacks taken from The Thames on 29th Jun. 1989.

Keys to sampling sites:
PB: Putney Bridge
BA: Banes
IW: Ilsewich
TD: Teddington Lock
WT: West Thurrode

| Date | Site | No | Copper Concentration ( ppb=ng/ml) Data | Mean | S.D. | Total Mean | S.D. |
|---|---|---|---|---|---|---|---|
| 29/06/89 | TD | 1 | 6.71 | 6.93 | 0.7448 | 9.311 | 3.809 |
| 29/06/89 | TD | 2 | 6.32 | | | | |
| 29/06/89 | TD | 3 | 7.76 | | | | |
| 29/06/89 | BA | 1 | 8.60 | 10.34 | 1.51 | | |
| 29/06/89 | BA | 2 | 11.24 | | | | |
| 29/06/89 | BA | 3 | 11.19 | | | | |
| 29/06/89 | PB | 1 | 9.87 | 7.47 | 2.60 | | |
| 29/06/89 | PB | 2 | 9.65 | | | | |
| 29/06/89 | PB | 3 | 7.04 | | | | |
| 29/06/89 | PB | 1 | 5.31 | | | | |
| 29/06/89 | PB | 2 | 3.60 | | | | |
| 29/06/89 | PB | 3 | 9.35 | | | | |
| 29/06/89 | IW | 1 | 6.77 | 13.04 | 6.8487 | | |
| 29/06/89 | IW | 2 | 12.01 | | | | |
| 29/06/89 | IW | 3 | 20.35 | | | | |
| 29/06/89 | WT | 1 | 11.24 | 10.61 | 3.5966 | | |
| 29/06/89 | WT | 2 | 13.85 | | | | |
| 29/06/89 | WT | 3 | 6.74 | | | | |

**APPENDIX C.1** The original data of non-parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990.

| Sampling Date | Site | No | Sex | Wet wt. (g) | Dry wt. (g) | TL (mm) | COPPER std conc (ug/g dw) | COPPER Total Cu (ug) | CADMIUM std conc (ug/g dw) | CADMIUM Total Cd (ug) |
|---|---|---|---|---|---|---|---|---|---|---|
| 21/07/89 | A | 1 | I | 0.3958 | 0.0847 | 35.5 | 3.6273 | 0.3072 | 0.059879 | 0.005072 |
| 21/07/89 | A | 2 | I | 0.6023 | 0.1498 | 39.0 | 2.2737 | 0.3406 | 0.062938 | 0.009428 |
| 21/07/89 | A | 3 | I | 0.4474 | 0.1000 | 36.0 | 2.2933 | 0.2293 | 0.062703 | 0.006270 |
| 21/07/89 | A | 4 | I | 0.5779 | 0.1380 | 37.0 | 7.6292 | 1.0528 | 0.037993 | 0.005243 |
| 21/07/89 | A | 5 | I | 0.5555 | 0.1383 | 36.0 | 5.7619 | 0.7969 | 0.042863 | 0.005928 |
| 21/07/89 | A | 6 | I | 0.4586 | 0.1011 | 34.5 | 2.2739 | 0.2299 | 0.067177 | 0.006792 |
| 21/07/89 | A | 7 | I | 0.3286 | 0.0689 | 33.5 | 4.9436 | 0.3406 | 0.051354 | 0.003538 |
| 21/07/89 | A | 8 | I | 0.3537 | 0.0753 | 32.5 | 11.4694 | 0.8636 | 0.076547 | 0.005764 |
| 21/07/89 | A | 9 | I | 0.3598 | 0.0805 | 32.0 | 2.7106 | 0.2182 | 0.048114 | 0.003873 |
| 21/07/89 | A | 11 | I | 0.0587 | 0.0103 | 19.0 | 7.4231 | 0.0765 | 0.069448 | 0.000715 |
| 21/07/89 | A | 12 | I | 0.0684 | 0.0117 | 20.5 | 3.4436 | 0.0403 | 0.094382 | 0.001104 |
| 21/07/89 | A | 13 | I | 0.1748 | 0.0123 | 21.5 | 5.2776 | 0.0649 | 0.148702 | 0.001829 |
| 21/07/89 | A | 14 | I | 0.0804 | 0.0150 | 21.5 | 6.8414 | 0.1026 | 0.143221 | 0.002148 |
| 21/07/89 | A | 15 | I | 0.0934 | 0.0185 | 22.5 | 1.5123 | 0.0280 | 0.159271 | 0.002947 |
| 21/07/89 | A | 16 | I | 0.1101 | 0.0217 | 23.0 | 8.5817 | 0.1862 | 0.201994 | 0.004383 |
| 21/07/89 | A | 17 | I | 0.1811 | 0.0312 | 26.5 | 5.4138 | 0.1689 | 0.084206 | 0.002627 |
| 21/07/89 | A | 18 | I | 0.2063 | 0.0445 | 28.0 | 11.3611 | 0.5056 | 0.087738 | 0.003904 |
| 21/07/89 | A | 19 | I | 0.2551 | 0.0592 | 29.0 | 2.9832 | 0.1766 | 0.066520 | 0.003938 |
| 21/07/89 | A | 20 | I | 0.2082 | 0.0544 | 29.5 | 5.7926 | 0.3151 | 0.042426 | 0.002308 |
| 21/07/89 | A | 21 | I | 0.1750 | 0.0385 | 26.5 | 7.4853 | 0.2882 | 0.117351 | 0.004518 |
| 21/07/89 | A | 22 | I | 0.0411 | 0.0063 | 17.0 | 4.3189 | 0.0272 | 0.074956 | 0.000472 |
| 21/07/89 | A | 23 | I | 0.1019 | 0.0193 | 23.0 | 7.5556 | 0.1458 | 0.170881 | 0.003298 |
| 21/07/89 | A | 24 | I | 0.1058 | 0.0182 | 21.5 | 5.0466 | 0.0918 | 0.066017 | 0.001202 |
| 21/07/89 | A | 25 | I | 0.0712 | 0.0134 | 20.5 | 4.2127 | 0.0565 | 0.053382 | 0.000715 |
| 12/10/89 | A | 1 | I | 0.3961 | 0.0956 | 35.0 | 12.9852 | 1.2414 | 0.124613 | 0.011913 |
| 12/10/89 | A | 2 | I | 0.2435 | 0.0561 | 30.0 | 7.3197 | 0.4106 | 0.270207 | 0.015159 |
| 12/10/89 | A | 3 | I | 0.2262 | 0.0442 | 29.0 | 13.9119 | 0.6149 | 0.274361 | 0.012127 |
| 12/10/89 | A | 4 | I | 0.2202 | 0.0470 | 28.5 | 9.3954 | 0.4416 | 0.261845 | 0.012307 |
| 12/10/89 | A | 5 | I | 0.2028 | 0.0517 | 28.0 | 20.3106 | 1.0501 | 0.249513 | 0.012900 |
| 12/10/89 | A | 6 | I | 0.3729 | 0.0847 | 34.0 | 12.7960 | 1.0838 | 0.126401 | 0.010706 |
| 12/10/89 | A | 7 | I | 0.1642 | 0.0373 | 26.5 | 11.0089 | 0.4106 | 0.353802 | 0.013197 |
| 12/10/89 | A | 8 | I | 0.1650 | 0.0438 | 25.0 | 5.8420 | 0.2559 | 0.205753 | 0.009012 |
| 12/10/89 | A | 9 | I | 0.1604 | 0.0446 | 27.0 | 12.5380 | 0.5592 | 0.112494 | 0.005017 |
| 12/10/89 | A | 10 | I | 0.1166 | 0.0268 | 24.0 | 12.7814 | 0.3425 | 0.305809 | 0.008196 |
| 12/10/89 | A | 11 | I | 0.1437 | 0.0388 | 25.0 | 11.0620 | 0.4292 | 0.225354 | 0.008744 |
| 12/10/89 | A | 12 | I | 0.1000 | 0.0247 | 23.0 | 12.3644 | 0.3054 | 0.341357 | 0.008432 |
| 12/10/89 | A | 14 | I | 0.9324 | 0.2273 | 46.0 | 2.8584 | 0.6497 | 0.085752 | 0.019491 |
| 12/10/89 | A | 15 | I | 0.8592 | 0.2202 | 45.0 | 3.4612 | 0.7621 | 0.076956 | 0.016946 |
| 12/10/89 | A | 16 | F | 0.6458 | 0.1506 | 42.0 | 3.3188 | 0.4998 | 0.109921 | 0.016554 |
| 12/10/89 | A | 17 | I | 0.4354 | 0.1053 | 36.0 | 8.2166 | 0.8652 | 0.098509 | 0.010373 |
| 12/10/89 | A | 18 | F | 0.4211 | 0.0928 | 35.0 | 10.2321 | 0.9495 | 0.178385 | 0.016554 |

(continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10/01/90 | A | 1 | M | 1.0619 | 0.3032 | 49.0 | 3.3117 | 1.0041 | 0.095712 | 0.029020 |
| 10/01/90 | A | 2 | I | 0.7945 | 0.2140 | 46.0 | 4.2234 | 0.9038 | 0.168351 | 0.036027 |
| 10/01/90 | A | 3 | I | 0.6603 | 0.1672 | 43.0 | 5.7055 | 0.9540 | 0.167577 | 0.028019 |
| 10/01/90 | A | 4 | I | 0.4559 | 0.1146 | 38.0 | 5.6985 | 0.6530 | 0.174613 | 0.020011 |
| 10/01/90 | A | 6 | I | 0.3828 | 0.1051 | 37.0 | 3.8429 | 0.4039 | 0.208145 | 0.021876 |
| 10/01/90 | A | 7 | I | 0.4097 | 0.0975 | 37.0 | 6.9561 | 0.6782 | 0.238412 | 0.023245 |
| 10/01/90 | A | 8 | I | 0.3417 | 0.0932 | 35.5 | 4.3152 | 0.4022 | 0.285487 | 0.026607 |
| 10/01/90 | A | 9 | I | 0.2604 | 0.0721 | 33.0 | 3.6874 | 0.2659 | 0.174107 | 0.012553 |
| 10/01/90 | A | 10 | I | 0.3016 | 0.0738 | 34.0 | 5.5650 | 0.4107 | 0.192876 | 0.014234 |
| 10/01/90 | A | 11 | I | 0.3301 | 0.0836 | 34.0 | 3.6897 | 0.3085 | 0.331677 | 0.027728 |
| 10/01/90 | A | 12 | I | 0.2367 | 0.0619 | 30.5 | 7.4607 | 0.4618 | 0.164814 | 0.010202 |
| 10/01/90 | A | 13 | I | 0.2015 | 0.0519 | 30.5 | 3.9406 | 0.2045 | 0.077457 | 0.004020 |
| 10/01/90 | A | 14 | I | 0.2073 | 0.0499 | 29.0 | 5.3279 | 0.2659 | 0.084411 | 0.004212 |
| 10/01/90 | A | 15 | I | 0.0968 | 0.0266 | 24.0 | 4.3414 | 0.1155 | 0.069869 | 0.001859 |
| 25/04/90 | A | 1 | F | 1.8250 | 0.3871 | 55.0 | 3.1729 | 1.2282 | 0.171718 | 0.066472 |
| 25/04/90 | A | 2 | F | 1.4548 | 0.3295 | 52.0 | 2.3789 | 0.7838 | 0.199569 | 0.065758 |
| 25/04/90 | A | 3 | F | 1.6209 | 0.3377 | 51.0 | 2.9193 | 0.9858 | 0.093189 | 0.031470 |
| 25/04/90 | A | 4 | F | 1.6258 | 0.3889 | 54.0 | 3.2621 | 1.2686 | 0.155084 | 0.060312 |
| 25/04/90 | A | 5 | F | 1.5007 | 0.3038 | 52.0 | 3.8434 | 1.1676 | 0.110313 | 0.033513 |
| 25/04/90 | A | 6 | F | 1.6277 | 0.3593 | 54.0 | 3.1935 | 1.1474 | 0.154901 | 0.055656 |
| 25/04/90 | A | 7 | F | 1.2496 | 0.2737 | 49.0 | 3.0114 | 0.8242 | 0.063192 | 0.017296 |
| 25/04/90 | A | 8 | F | 1.4016 | 0.2964 | 50.0 | 4.2120 | 1.2484 | 0.128677 | 0.038140 |
| 25/04/90 | A | 9 | F | 1.2689 | 0.2645 | 49.0 | 3.2689 | 0.8646 | 0.159957 | 0.042309 |
| 25/04/90 | A | 10 | F | 1.2215 | 0.2632 | 49.0 | 3.5921 | 0.9454 | 0.235981 | 0.062110 |
| 25/04/90 | A | 11 | F | 1.0905 | 0.2311 | 47.0 | 3.9162 | 0.9050 | 0.041018 | 0.009479 |
| 25/04/90 | A | 12 | F | 1.1573 | 0.2685 | 47.0 | 3.5212 | 0.9454 | 0.043068 | 0.011564 |
| 25/04/90 | A | 13 | M | 0.9836 | 0.2255 | 45.0 | 3.8343 | 0.8646 | 0.083708 | 0.018876 |
| 25/04/90 | A | 14 | M | 0.8848 | 0.2091 | 49.0 | 4.3282 | 0.9050 | 0.192368 | 0.040224 |
| 25/04/90 | A | 15 | I | 0.4253 | 0.0933 | 35.0 | 4.4206 | 0.4124 | 0.129709 | 0.012102 |
| 25/04/90 | A | 16 | M* | 2.6022 | 0.5823 | 62.0 | 3.4533 | 2.0109 | 0.108099 | 0.062946 |
| 25/04/90 | A | 17 | F* | 2.2220 | 0.4830 | 60.0 | 2.6285 | 1.2696 | 0.180658 | 0.087258 |
| 25/04/90 | A | 18 | F* | 2.0477 | 0.3997 | 54.0 | 4.9349 | 1.9725 | 0.246362 | 0.098471 |
| 25/04/90 | A | 19 | F* | 1.8315 | 0.3316 | 55.0 | 2.2630 | 0.7504 | 0.244261 | 0.080997 |
| 25/04/90 | A | 20 | F* | 1.7074 | 0.3478 | 53.5 | 1.9173 | 0.6668 | 0.207326 | 0.072108 |
| 21/07/89 | B | 1 | I | 0.5134 | 0.1130 | 35.0 | 19.2636 | 2.1768 | 0.396919 | 0.044852 |
| 21/07/89 | B | 2 | I | 0.3490 | 0.0852 | 31.5 | 44.3575 | 3.7793 | 0.459441 | 0.039144 |
| 21/07/89 | B | 3 | I | 0.3271 | 0.0799 | 32.5 | 29.4658 | 2.3543 | 0.211330 | 0.016885 |
| 21/07/89 | B | 4 | I | 0.3460 | 0.0844 | 33.0 | 60.6286 | 5.1171 | 0.233874 | 0.019739 |
| 21/07/89 | B | 5 | I | 0.3600 | 0.0852 | 32.0 | 39.9484 | 3.4036 | 0.461496 | 0.039319 |
| 21/07/89 | B | 6 | I | 0.3728 | 0.0830 | 34.0 | 26.6942 | 2.2156 | 0.511077 | 0.042419 |
| 21/07/89 | B | 7 | I | 0.2777 | 0.0672 | 30.5 | 35.4831 | 2.3845 | 0.290908 | 0.019549 |
| 21/07/89 | B | 8 | I | 0.2126 | 0.0457 | 27.5 | 43.8060 | 2.0019 | 0.326466 | 0.014919 |
| 21/07/89 | B | 10 | I | 0.1393 | 0.0283 | 23.5 | 63.1209 | 1.7863 | 0.448217 | 0.012685 |
| 21/07/89 | B | 11 | I | 0.0788 | 0.0154 | 21.0 | 44.3656 | 0.6832 | 0.678544 | 0.010450 |
| 21/07/89 | B | 12 | I | 0.0582 | 0.0111 | 19.0 | 54.2838 | 0.6026 | 0.811966 | 0.009013 |
| 21/07/89 | B | 13 | I | 0.0404 | 0.0076 | 18.0 | 10.4633 | 0.0795 | 0.555896 | 0.004225 |
| 21/07/89 | B | 14 | I | 0.0488 | 0.0094 | 18.5 | 18.8185 | 0.1769 | 0.571882 | 0.005376 |
| 21/07/89 | B | 15 | I | 0.0201 | 0.0040 | 14.0 | 16.7505 | 0.0670 | 0.594282 | 0.002377 |
| 21/07/89 | B | 16 | I | 0.0129 | 0.0026 | 13.0 | 18.5472 | 0.0482 | 0.464013 | 0.001206 |

(continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 12/10/89 | B | 1 | I | 0.1550 | 0.0366 | 24.0 | 37.2180 | 1.3622 | 0.451002 | 0.016507 |
| 12/10/89 | B | 2 | F | 1.0685 | 0.3344 | 47.0 | 8.6169 | 2.8815 | 0.289890 | 0.096939 |
| 12/10/89 | B | 3 | F | 0.8266 | 0.2213 | 42.0 | 15.1103 | 3.3439 | 0.465173 | 0.102943 |
| 12/10/89 | B | 4 | M | 0.8922 | 0.2408 | 45.0 | 15.2584 | 3.6742 | 0.421270 | 0.101442 |
| 12/10/89 | B | 5 | I | 0.8556 | 0.2540 | 42.0 | 11.6046 | 2.9476 | 0.358014 | 0.090936 |
| 12/10/89 | B | 6 | F | 0.6240 | 0.1760 | 40.0 | 14.4955 | 2.5512 | 0.329066 | 0.057916 |
| 12/10/89 | B | 7 | F | 0.5671 | 0.1652 | 38.0 | 22.2410 | 3.6742 | 0.460423 | 0.076062 |
| 12/10/89 | B | 8 | F | 0.6747 | 0.1936 | 39.0 | 35.0154 | 6.7790 | 0.433372 | 0.083901 |
| 12/10/89 | B | 9 | I | 0.4947 | 0.1361 | 36.0 | 29.9087 | 4.0706 | 0.420023 | 0.057165 |
| 12/10/89 | B | 10 | I | 0.3549 | 0.0904 | 33.0 | 29.5092 | 2.6676 | 0.371046 | 0.033543 |
| 12/10/89 | B | 11 | I | 0.3991 | 0.1038 | 34.0 | 28.1060 | 2.9174 | 0.425835 | 0.044202 |
| 12/10/89 | B | 12 | I | 0.4170 | 0.1194 | 34.0 | 40.6455 | 4.8531 | 0.305273 | 0.036450 |
| 12/10/89 | B | 13 | I | 0.3061 | 0.0835 | 30.0 | 94.0150 | 7.8503 | 0.429028 | 0.035824 |
| 12/10/89 | B | 14 | I | 0.2620 | 0.0730 | 31.0 | 92.1413 | 6.7263 | 0.379842 | 0.027728 |
| 12/10/89 | B | 15 | I | 0.1919 | 0.0454 | 27.0 | 57.3831 | 2.6052 | 0.421474 | 0.019135 |
| 12/10/89 | B | 16 | I | 0.2027 | 0.0468 | 29.0 | 55.8723 | 2.6148 | 0.377320 | 0.017659 |
| 12/10/89 | B | 17 | I | 0.2186 | 0.0531 | 29.0 | 44.0694 | 2.3401 | 0.410559 | 0.021801 |
| 12/10/89 | B | 18 | I | 0.1913 | 0.0484 | 28.0 | 40.5437 | 1.9623 | 0.488852 | 0.023660 |
| 12/10/89 | B | 19 | I | 0.2311 | 0.0574 | 29.0 | 63.5036 | 3.6451 | 0.501714 | 0.028798 |
| 12/10/89 | B | 20 | I | 0.2112 | 0.0535 | 28.0 | 46.5637 | 2.4912 | 0.395033 | 0.021134 |
| 12/10/89 | B | 21 | I | 0.1708 | 0.0455 | 27.0 | 39.9982 | 1.8199 | 0.508430 | 0.023134 |
| 12/10/89 | B | 22 | I | 0.1580 | 0.0388 | 26.0 | 48.1120 | 1.8667 | 0.518932 | 0.020135 |
| 12/10/89 | B | 23 | I | 0.1811 | 0.0483 | 27.0 | 38.6490 | 1.8667 | 0.492754 | 0.023800 |
| 12/10/89 | B | 24 | I | 0.1462 | 0.0329 | 25.0 | 58.6379 | 1.9292 | 0.672763 | 0.022134 |
| 12/10/89 | B | 25 | I | 0.0879 | 0.0229 | 23.0 | 47.4337 | 1.0862 | 0.660970 | 0.015136 |
| 10/01/90 | B | 1 | I | 0.8968 | 0.2279 | 50.0 | 26.4474 | 6.0274 | 0.368918 | 0.084076 |
| 10/01/90 | B | 2 | I | 0.8387 | 0.2481 | 48.0 | 14.4074 | 3.5745 | 0.242047 | 0.060052 |
| 10/01/90 | B | 3 | I | 0.5697 | 0.1594 | 42.0 | 10.3895 | 1.6561 | 0.376736 | 0.060052 |
| 10/01/90 | B | 4 | I | 0.6119 | 0.1738 | 41.0 | 15.6576 | 2.7213 | 0.299445 | 0.052044 |
| 10/01/90 | B | 5 | I | 0.4771 | 0.1277 | 40.0 | 22.9803 | 2.9346 | 0.399707 | 0.051043 |
| 10/01/90 | B | 6 | I | 0.5878 | 0.1602 | 42.0 | 37.6240 | 6.0274 | 0.649794 | 0.104097 |
| 10/01/90 | B | 7 | I | 0.4339 | 0.1261 | 39.0 | 21.5804 | 2.7213 | 0.595299 | 0.075067 |
| 10/01/90 | B | 8 | I | 0.3805 | 0.1092 | 36.0 | 17.0026 | 1.8567 | 0.510874 | 0.055787 |
| 10/01/90 | B | 9 | I | 0.4580 | 0.1181 | 37.0 | 19.1185 | 2.2579 | 0.559339 | 0.066058 |
| 10/01/90 | B | 10 | I | 0.5156 | 0.1426 | 37.0 | 29.1798 | 4.1610 | 0.470259 | 0.067059 |
| 10/01/90 | B | 11 | F | 1.0446 | 0.2429 | 48.0 | 27.8876 | 6.7739 | 0.523345 | 0.127121 |
| 10/01/90 | B | 12 | I | 0.4590 | 0.1245 | 39.0 | 13.1172 | 1.6331 | 0.501781 | 0.062472 |
| 10/01/90 | B | 13 | I | 0.3690 | 0.0891 | 36.0 | 28.6559 | 2.5532 | 0.600512 | 0.053506 |
| 10/01/90 | B | 14 | I | 0.3227 | 0.0953 | 34.0 | 14.8120 | 1.4116 | 0.455601 | 0.043419 |
| 10/01/90 | B | 15 | I | 0.3648 | 0.1023 | 37.0 | 14.2981 | 1.4627 | 0.402515 | 0.041177 |
| 10/01/90 | B | 16 | I | 0.3027 | 0.0921 | 33.0 | 22.1351 | 2.0386 | 0.447093 | 0.041177 |
| 10/01/90 | B | 17 | I | 0.2260 | 0.0627 | 29.0 | 26.4267 | 1.6570 | 0.656735 | 0.041177 |
| 10/01/90 | B | 18 | I | 0.2065 | 0.0510 | 31.5 | 30.0168 | 1.5309 | 0.653568 | 0.033332 |
| 10/01/90 | B | 19 | I | 0.2691 | 0.0714 | 33.0 | 30.9389 | 2.2090 | 0.686591 | 0.049023 |
| 10/01/90 | B | 20 | I | 0.2159 | 0.0498 | 30.5 | 38.7468 | 1.9296 | 0.669316 | 0.033332 |
| 10/01/90 | B | 21 | M* | 1.2690 | 0.3476 | 51.5 | 17.1463 | 5.9601 | 0.356513 | 0.123924 |
| 10/01/90 | B | 22 | M* | 1.1438 | 0.3218 | 51.0 | 19.1838 | 6.1733 | 0.270501 | 0.087047 |
| 25/04/90 | B | 1 | M | 1.1781 | 0.2991 | 47.0 | 10.7250 | 3.2078 | 0.291284 | 0.087123 |

(continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 21/07/89 | E | 1 | I | 0.9302 | 0.2275 | 44.0 | 28.5752 | 6.5009 | 0.209695 | 0.047706 |
| 21/07/89 | E | 2 | I | 0.8247 | 0.1602 | 43.0 | 41.8631 | 6.7065 | 0.233659 | 0.037432 |
| 21/07/89 | E | 3 | I | 1.0582 | 0.2166 | 46.0 | 35.2342 | 7.6317 | 0.238006 | 0.051552 |
| 21/07/89 | E | 4 | I | 0.5103 | 0.1007 | 38.0 | 39.0175 | 3.9291 | 0.167679 | 0.016885 |
| 21/07/89 | E | 5 | I | 0.4661 | 0.0888 | 38.0 | 46.8189 | 4.1575 | 0.164368 | 0.014596 |
| 21/07/89 | E | 6 | I | 0.5354 | 0.1215 | 35.0 | 28.0132 | 3.4036 | 0.218831 | 0.026588 |
| 21/07/89 | E | 7 | I | 0.3384 | 0.0751 | 31.0 | 43.4957 | 3.2665 | 0.201656 | 0.015144 |
| 21/07/89 | E | 8 | I | 0.3465 | 0.0762 | 31.5 | 68.3522 | 5.2084 | 0.233662 | 0.017805 |
| 21/07/89 | E | 9 | I | 0.0182 | 0.0036 | 15.0 | 10.1108 | 0.0364 | 0.497717 | 0.001792 |
| 21/07/89 | E | 10 | I | 0.0460 | 0.0099 | 18.0 | 12.6692 | 0.1254 | 0.435498 | 0.004311 |
| 21/07/89 | E | 11 | I | 0.0811 | 0.0163 | 20.5 | 18.3623 | 0.2993 | 0.366851 | 0.005980 |
| 21/07/89 | E | 12 | I | 0.1050 | 0.0216 | 23.5 | 16.8191 | 0.3633 | 0.328571 | 0.007097 |
| 21/07/89 | E | 13 | I | 0.2337 | 0.0594 | 29.0 | 41.1481 | 2.4442 | 0.205482 | 0.012206 |
| 21/07/89 | E | 14 | I | 0.1002 | 0.0198 | 21.5 | 14.1328 | 0.2798 | 0.293941 | 0.005820 |
| 21/07/89 | E | 15 | I | 0.1911 | 0.0472 | 26.5 | 17.4813 | 0.8251 | 0.249554 | 0.011779 |
| 21/07/89 | E | 16 | I | 0.1781 | 0.0388 | 26.6 | 37.7684 | 1.4654 | 0.273802 | 0.010624 |
| 21/07/89 | E | 17 | I | 0.1651 | 0.0349 | 25.5 | 27.3983 | 0.9562 | 0.208275 | 0.007269 |
| 21/07/89 | E | 18 | I | 0.0800 | 0.0172 | 20.0 | 20.0000 | 0.3440 | 0.295404 | 0.005081 |
| 21/07/89 | E | 19 | I | 0.1042 | 0.0220 | 22.0 | 22.4835 | 0.4946 | 0.264102 | 0.005810 |
| 21/07/89 | E | 20 | F | 4.1037 | 0.7882 | 67.0 | 20.1985 | 15.9205 | 0.484195 | 0.381643 |
| 21/07/89 | E | 21 | F | 2.3567 | 0.5589 | 57.0 | 14.3873 | 8.0411 | 0.234088 | 0.130832 |
| 21/07/89 | E | 22 | F | 1.1262 | 0.2229 | 55.0 | 15.6093 | 3.4793 | 0.321709 | 0.071709 |
| 21/07/89 | E | 23 | M | 0.9308 | 0.1963 | 52.0 | 29.1326 | 5.7187 | 0.356597 | 0.070000 |
| 21/07/89 | E | 24 | M | 1.7250 | 0.3398 | 44.0 | 20.1501 | 6.8470 | 0.239275 | 0.081306 |
| 21/07/89 | E | 25 | M | 0.9372 | 0.1965 | 45.0 | 32.0092 | 6.2898 | 0.256314 | 0.050366 |
| 12/10/89 | E | 1 | F | 1.5273 | 0.4224 | 53.0 | 13.7029 | 5.7881 | 0.252114 | 0.106493 |
| 12/10/89 | E | 2 | M | 1.4776 | 0.3764 | 50.0 | 19.8528 | 7.4726 | 0.292022 | 0.109917 |
| 12/10/89 | E | 3 | F | 1.2164 | 0.2782 | 49.0 | 16.0565 | 4.4669 | 0.291917 | 0.081211 |
| 12/10/89 | E | 4 | F | 1.0562 | 0.2770 | 45.0 | 14.2182 | 3.9384 | 0.203130 | 0.056267 |
| 12/10/89 | E | 5 | M | 0.9413 | 0.2028 | 41.0 | 29.1924 | 5.9202 | 0.20428 | 0.041428 |
| 12/10/89 | E | 6 | I | 0.6472 | 0.1617 | 40.0 | 33.8071 | 5.4666 | 0.326797 | 0.052843 |
| 12/10/89 | E | 7 | F | 0.8212 | 0.2206 | 43.0 | 20.4746 | 4.5167 | 0.249889 | 0.055126 |
| 12/10/89 | E | 8 | I | 0.5716 | 0.1311 | 38.0 | 45.4342 | 5.9564 | 0.297140 | 0.038955 |
| 12/10/89 | E | 9 | I | 0.5369 | 0.1278 | 37.0 | 57.5630 | 7.3566 | 0.333092 | 0.042569 |
| 12/10/89 | E | 10 | I | 0.3143 | 0.0760 | 31.0 | 37.0158 | 2.8132 | 0.449078 | 0.034130 |
| 12/10/89 | E | 11 | I | 0.2893 | 0.0673 | 31.0 | 38.0969 | 2.5639 | 0.304128 | 0.020468 |
| 12/10/89 | E | 12 | I | 0.3592 | 0.0820 | 34.0 | 43.4510 | 3.5630 | 0.282117 | 0.023134 |
| 12/10/89 | E | 13 | I | 0.3552 | 0.0940 | 33.0 | 41.3000 | 3.8822 | 0.264057 | 0.024821 |
| 12/10/89 | E | 14 | I | 0.2291 | 0.0538 | 29.0 | 27.2246 | 1.4650 | 0.389246 | 0.020945 |
| 12/10/89 | E | 15 | I | 0.2207 | 0.0546 | 30.0 | 29.1179 | 1.5898 | 0.454604 | 0.024821 |
| 12/10/89 | E | 16 | I | 0.1777 | 0.0387 | 28.0 | 43.4186 | 1.6803 | 0.450189 | 0.017422 |
| 12/10/89 | E | 17 | I | 0.1851 | 0.0422 | 27.0 | 37.5616 | 1.5851 | 0.481738 | 0.020329 |
| 12/10/89 | E | 18 | I | 0.1291 | 0.0285 | 25.0 | 30.7404 | 0.8761 | 0.712377 | 0.020303 |
| 12/10/89 | E | 19 | I | 0.1346 | 0.0343 | 24.0 | 27.5448 | 0.9448 | 0.751915 | 0.025791 |
| 12/10/89 | E | 20 | I | 0.0909 | 0.0202 | 24.0 | 30.0794 | 0.6076 | 0.852479 | 0.017220 |
| 12/10/89 | E | 21 | I | 0.0458 | 0.0102 | 18.0 | 30.7972 | 0.3141 | 0.423850 | 0.004323 |
| 12/10/89 | E | 22 | F* | 2.3228 | 0.7321 | 63.0 | 18.7589 | 13.7334 | 0.144158 | 0.105538 |
| 12/10/89 | E | 23 | M* | 2.1083 | 0.6530 | 60.0 | 15.4704 | 10.1022 | 0.135718 | 0.088624 |

(continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10/01/90 | E | 1 | I | 0.8733 | 0.2114 | 46.0 | 27.5027 | 5.8141 | 0.814412 | 0.172167 |
| 10/01/90 | E | 2 | F | 0.9935 | 0.2516 | 45.0 | 18.4457 | 4.6409 | 0.944091 | 0.237533 |
| 10/01/90 | E | 3 | F | 0.7904 | 0.2093 | 44.0 | 24.7214 | 5.1742 | 0.759925 | 0.159052 |
| 10/01/90 | E | 4 | I | 0.5397 | 0.1193 | 40.0 | 48.7349 | 5.8141 | 1.254962 | 0.149717 |
| 10/01/90 | E | 5 | I | 0.5120 | 0.1354 | 37.0 | 28.5118 | 3.8605 | 0.819666 | 0.110983 |
| 10/01/90 | E | 6 | I | 0.3504 | 0.0930 | 34.0 | 22.3581 | 2.0793 | 0.539301 | 0.050155 |
| 10/01/90 | E | 7 | I | 0.2747 | 0.0597 | 33.5 | 38.0870 | 2.2738 | 0.708510 | 0.042298 |
| 10/01/90 | E | 8 | I | 0.2742 | 0.0678 | 32.0 | 22.4897 | 1.5248 | 0.475091 | 0.032211 |
| 10/01/90 | E | 9 | I | 0.3204 | 0.0783 | 33.0 | 22.4673 | 1.7592 | 0.468636 | 0.036694 |
| 10/01/90 | E | 10 | I | 0.2875 | 0.0605 | 33.0 | 24.2116 | 1.4648 | 0.569466 | 0.034453 |
| 10/01/90 | E | 11 | I | 0.2654 | 0.0601 | 33.0 | 33.2971 | 2.0012 | 0.643211 | 0.038657 |
| 10/01/90 | E | 12 | I | 0.2216 | 0.0508 | 30.0 | 36.5081 | 1.8546 | 0.634079 | 0.032211 |
| 10/01/90 | E | 13 | I | 0.1909 | 0.0453 | 29.0 | 15.0847 | 0.6833 | 0.686323 | 0.031090 |
| 10/01/90 | E | 14 | I | 0.1828 | 0.0458 | 28.0 | 39.2288 | 1.7967 | 0.923538 | 0.042298 |
| 10/01/90 | E | 15 | I | 0.1201 | 0.0269 | 20.5 | 25.9649 | 0.6985 | 1.239106 | 0.033332 |
| 10/01/90 | E | 16 | I | 0.1212 | 0.0249 | 26.5 | 25.1763 | 0.6269 | 0.888529 | 0.022124 |
| 10/01/90 | E | 17 | M* | 2.1968 | 0.7156 | 62.0 | 17.2281 | 12.3284 | 0.105878 | 0.075766 |
| 10/01/90 | E | 18 | F* | 1.3659 | 0.3071 | 54.0 | 30.0417 | 9.2258 | 0.242880 | 0.074588 |
| 25/04/90 | E | 1 | I | 0.0558 | 0.0066 | 18.5 | 11.4013 | 0.0752 | 0.570303 | 0.003764 |
| 25/04/90 | E | 2 | M | 0.9196 | 0.2099 | 46.0 | 12.5881 | 2.6422 | 0.107307 | 0.022524 |
| 25/04/90 | E | 3 | M | 0.9509 | 0.2176 | 46.0 | 9.7290 | 2.1170 | 0.194433 | 0.042309 |
| 25/04/90 | E | 4 | F | 0.7613 | 0.1704 | 43.0 | 9.8159 | 1.6726 | 0.125966 | 0.021465 |
| 25/04/90 | E | 5 | M | 0.7075 | 0.1493 | 44.0 | 18.5093 | 2.7634 | 0.143768 | 0.021465 |
| 25/04/90 | E | 6 | M | 0.6883 | 0.1450 | 43.0 | 9.8637 | 1.4302 | 0.090648 | 0.013144 |
| 25/04/90 | E | 7 | M | 0.9160 | 0.2035 | 43.0 | 14.7707 | 3.0058 | 0.197662 | 0.040224 |
| 25/04/90 | E | 8 | F | 0.7156 | 0.1555 | 42.0 | 13.0948 | 2.0362 | 0.178249 | 0.027718 |
| 25/04/90 | E | 9 | M | 0.5898 | 0.1192 | 42.0 | 9.9650 | 1.1878 | 0.215045 | 0.025633 |
| 25/04/90 | E | 10 | I | 0.3804 | 0.0750 | 35.0 | 10.1501 | 0.7613 | 0.147234 | 0.011043 |
| 25/04/90 | E | 11 | I | 0.3999 | 0.0778 | 36.0 | 10.8073 | 0.8408 | 0.249102 | 0.019380 |
| 25/04/90 | E | 12 | I | 0.3448 | 0.0563 | 35.0 | 13.0866 | 0.7368 | 0.281185 | 0.015831 |
| 25/04/90 | E | 13 | I | 0.4061 | 0.0828 | 35.0 | 6.6071 | 0.5471 | 0.208886 | 0.017296 |

(end)

**APPENDIX C.2** The original data of parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990.

| | | | | | | | | | | | | | | COPPER | | | | CADMIUM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling Date | Site | No | Sex | Fish Wet wt. (g) | Para. Wet wt. (g) | Fish Dry wt. (g) | Para. Dry wt. (g) | Fish TL (mm) | Para No | Para TL (mm) | Fish std conc (ug/g dw) | Para. std conc (ug/g dw) | Fish Total Cu (ug) | Para. Total Cu (ug) | Fish std conc (ug/g dw) | Para. std conc (ug/g dw) | Fish Total Cd (ug) | Para. Total Cd (ug) |
| 21/07/89 | A | 1 | I | 0.3887 | 0.0175 | 0.0839 | 0.0069 | 33.5 | 1 | 20.0 | 8.5695 | 3.1627 | 0.7190 | 0.0218 | 0.068703 | 0.329087 | 0.005764 | 0.002271 |
| 12/10/89 | A | 1 | I | 0.2444 | 0.0406 | 0.0561 | 0.0122 | 30.5 | 1 | 27.0 | 10.9609 | 7.7361 | 0.6149 | 0.0944 | 0.163505 | 0.040984 | 0.009173 | 0.000500 |
| 12/10/89 | A | 2 | I | 0.1691 | 0.0075 | 0.0346 | 0.0021 | 27.0 | 1 | 22.0 | 17.9508 | 0.4762 | 0.6211 | 0.0010 | 0.150289 | 0.215839 | 0.005200 | 0.000453 |
| 12/10/89 | A | 3 | I | 0.1330 | 0.0564 | 0.0351 | 0.0171 | 24.0 | 3 | 48.0 | 0.7292 | 5.9143 | 0.2559 | 0.1011 | 0.014245 | 0.029240 | 0.000500 | 0.000500 |
| 12/10/89 | A | 4 | I | 0.1682 | 0.0682 | 0.0426 | 0.0216 | 29.0 | 1 | 24.0 | 7.8956 | 3.6913 | 0.3364 | 0.0797 | 0.083324 | 0.055070 | 0.003550 | 0.001190 |
| 12/10/89 | A | 5 | I | 0.0665 | 0.0316 | 0.0186 | 0.0108 | 22.0 | 1 | 25.0 | 19.7475 | 2.9032 | 0.3673 | 0.0314 | 0.080691 | 0.061086 | 0.001501 | 0.000660 |
| 12/10/89 | A | 6 | I | 0.3452 | 0.1019 | 0.0775 | 0.0295 | 35.0 | 1 | 35.0 | 4.9983 | 3.7250 | 0.3874 | 0.1099 | 0.069582 | 0.149610 | 0.005393 | 0.004414 |
| 12/10/89 | A | 7 | M | 0.4816 | 0.0815 | 0.1018 | 0.0207 | 38.0 | 2 | 68.0 | 6.6584 | 2.5598 | 0.6778 | 0.0530 | 0.185697 | 0.203753 | 0.018904 | 0.004218 |
| 12/10/89 | A | 8 | F | 0.4746 | 0.0868 | 0.1126 | 0.0217 | 36.0 | 1 | 42.0 | 7.6007 | 1.6962 | 0.8558 | 0.0368 | 0.141800 | 1.024555 | 0.015967 | 0.022233 |
| 12/10/89 | A | 9 | I | 0.3569 | 0.0369 | 0.0879 | 0.0097 | 34.0 | 1 | 39.0 | 4.5136 | 6.8854 | 0.3967 | 0.0668 | 0.061349 | 0.293502 | 0.005393 | 0.002847 |
| 12/10/89 | A | 10 | F | 1.0091 | 0.1281 | 0.2131 | 0.0342 | 47.0 | 1 | 25.0 | 2.7851 | 1.2406 | 0.5935 | 0.0424 | 0.048278 | 0.272191 | 0.010288 | 0.009309 |
| 12/10/89 | A | 11 | M | 0.4905 | 0.0591 | 0.1077 | 0.0119 | 37.0 | 1 | 31.0 | 11.9483 | 5.6125 | 1.2868 | 0.0668 | 0.099161 | 0.765807 | 0.010680 | 0.009113 |
| 10/01/90 | A | 1 | I | 0.3467 | 0.0290 | 0.0859 | 0.0070 | 35.0 | 1 | 23.0 | 2.4206 | 0.9557 | 0.2079 | 0.0067 | 0.231465 | 7.323442 | 0.019883 | 0.051264 |
| 10/01/90 | A | 2 | I | 0.4987 | 0.0411 | 0.1252 | 0.0124 | 40.0 | 1 | 28.0 | 3.2132 | 0.5983 | 0.4023 | 0.0074 | 0.215798 | 0.040323 | 0.027018 | 0.000500 |
| 10/01/90 | A | 3 | I | 0.5927 | 0.2088 | 0.1413 | 0.0616 | 43.0 | 2 | 57.0 | 4.2668 | 1.4782 | 0.6029 | 0.0911 | 0.148703 | 0.055642 | 0.021012 | 0.003428 |
| 10/01/90 | A | 4 | I | 0.2324 | 0.0803 | 0.0546 | 0.0219 | 34.0 | 1 | 30.0 | 5.0253 | 4.8581 | 0.2744 | 0.1064 | 0.120251 | 0.100217 | 0.006566 | 0.002195 |
| 10/01/90 | A | 5 | I | 0.2208 | 0.0891 | 0.0531 | 0.0263 | 33.0 | 1 | 29.0 | 4.3971 | 3.9806 | 0.2335 | 0.1047 | 0.016004 | 0.130326 | 0.000850 | 0.003428 |
| 10/01/90 | A | 6 | I | 0.2627 | 0.1114 | 0.0610 | 0.0308 | 34.0 | 2 | 66.0 | 6.9004 | 3.8670 | 0.4209 | 0.1191 | 0.067214 | 0.114924 | 0.004100 | 0.003540 |
| 10/01/90 | A | 7 | I | 0.2839 | 0.0711 | 0.0726 | 0.0193 | 33.5 | 2 | 68.0 | 4.8590 | 5.2771 | 0.3528 | 0.1018 | 0.030231 | 0.253086 | 0.002195 | 0.004885 |
| 10/01/90 | A | 8 | I | 0.2675 | 0.0599 | 0.0737 | 0.0194 | 34.0 | 1 | 25.0 | 4.3241 | 3.1126 | 0.3187 | 0.0604 | 0.043466 | 0.917667 | 0.003203 | 0.017803 |
| 25/04/90 | A | 1 | I | 0.1320 | 0.4504 | 0.0408 | 0.0854 | 24.0 | 1 | 40.0 | 2.1821 | 3.3963 | 0.0890 | 0.2900 | 15.559742 | 1.460420 | 0.634837 | 0.059590 |

(continued)

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21/07/89 | B | 1 | I | 0.2612 | 0.0813 | 0.0556 | 0.0187 | 30.0 | 1 | 25.0 | 23.2465 | 8.6413 | 1.2925 | 0.1616 | 0.142003 | 0.067361 | 0.007895 | 0.001260 |
| 21/07/89 | B | 2 | I | 0.2711 | 0.0594 | 0.0644 | 0.0115 | 29.0 | 1 | 27.0 | 38.9699 | 10.3017 | 2.5097 | 0.1185 | 0.231669 | 0.262234 | 0.014919 | 0.003016 |
| 21/07/89 | B | 3 | M | 2.1499 | 0.9328 | 0.4759 | 0.2828 | 59.0 | 3 | 143.0 | 23.9174 | 8.2800 | 11.3823 | 2.3416 | 0.330503 | 0.076260 | 0.157286 | 0.021566 |
| 21/07/89 | B | 4 | M | 1.7187 | 0.6053 | 0.4359 | 0.1801 | 53.0 | 2 | 105.0 | 27.1074 | 4.4334 | 11.8161 | 1.4912 | 0.227239 | 1.805770 | 0.099054 | 0.013735 |
| 21/07/89 | B | 5 | M | 1.6128 | 0.6212 | 0.4132 | 0.1798 | 57.0 | 3 | 114.0 | 39.2205 | 6.3956 | 16.2059 | 0.7971 | 0.383711 | 0.056160 | 0.158549 | 0.324678 |
| 21/07/89 | B | 6 | M | 2.5578 | 0.6460 | 0.6246 | 0.1789 | 59.0 | 4 | 167.0 | 48.0329 | 6.8133 | 30.0014 | 1.1442 | 0.366950 | 0.211158 | 0.229197 | 0.010047 |
| 21/07/89 | B | 7 | M | 1.8376 | 0.6740 | 0.4604 | 0.1934 | 56.0 | 3 | 142.0 | 23.2150 | 5.7135 | 10.6882 | 1.3177 | 0.252930 | 0.213924 | 0.116449 | 0.040838 |
| 21/07/89 | B | 8 | M | 1.2079 | 0.5067 | 0.2590 | 0.1547 | 52.0 | 1 | 62.0 | 32.5573 | 17.1549 | 8.4323 | 0.8839 | 0.230593 | 0.098404 | 0.059724 | 0.033094 |
| 21/07/89 | B | 9 | M | 1.6611 | 0.4101 | 0.4134 | 0.1156 | 54.0 | 2 | 75.0 | 26.4840 | 9.0166 | 10.9485 | 1.0424 | 0.175493 | 0.117874 | 0.072549 | 0.013626 |
| 21/07/89 | B | 10 | F | 3.1697 | 1.3940 | 0.6825 | 0.4112 | 70.0 | 3 | 168.0 | 26.4146 | 8.4796 | 18.0280 | 3.4868 | 0.077936 | 0.214998 | 0.053191 | 0.088407 |
| 21/07/89 | B | 11 | F | 2.9159 | 0.8142 | 0.5899 | 0.2431 | 61.0 | 2 | 103.0 | 13.1178 | 8.2757 | 7.7382 | 2.0118 | 0.175503 | 0.076216 | 0.103529 | 0.018528 |
| 21/07/89 | B | 12 | F | 3.3451 | 0.7019 | 0.6548 | 0.1968 | 62.0 | 2 | 114.0 | 42.6375 | 7.5774 | 27.9190 | 1.4912 | 0.410409 | 0.095084 | 0.268736 | 0.018713 |
| 21/07/89 | B | 13 | F | 3.4785 | 0.9220 | 0.7526 | 0.2620 | 71.0 | 2 | 103.0 | 19.1590 | 6.3540 | 14.4190 | 1.6648 | 0.230920 | 0.153758 | 0.173790 | 0.040285 |
| 21/07/89 | B | 14 | F | 2.0103 | 0.8929 | 0.5353 | 0.2625 | 62.0 | 3 | 147.0 | 29.7882 | 9.6472 | 15.9456 | 2.5324 | 0.092182 | 0.085333 | 0.049345 | 0.022400 |
| 21/07/89 | B | 15 | F | 2.0535 | 0.6385 | 0.5736 | 0.1865 | 59.0 | 2 | 79.0 | 20.9024 | 8.9263 | 11.9896 | 1.6648 | 0.159953 | 0.928257 | 0.091749 | 0.173120 |
| 21/07/89 | B | 16 | F | 2.0580 | 0.5146 | 0.3890 | 0.1393 | 57.0 | 2 | 91.0 | 24.7995 | 17.5566 | 9.6470 | 2.4456 | 0.385604 | 0.050948 | 0.150000 | 0.007097 |
| 12/10/89 | B | 1 | F | 0.6769 | 0.1651 | 0.1929 | 0.0551 | 43.0 | 1 | 36.0 | 31.7180 | 8.0277 | 6.1184 | 0.4423 | 0.490865 | 0.208038 | 0.094688 | 0.011463 |
| 12/10/89 | B | 2 | M | 0.7996 | 0.2276 | 0.1881 | 0.0661 | 44.0 | 1 | 38.0 | 57.4619 | 7.4005 | 10.8086 | 0.4892 | 0.782666 | 0.490169 | 0.147220 | 0.032400 |
| 12/10/89 | B | 3 | M | 0.5518 | 0.2947 | 0.1369 | 0.0925 | 40.0 | 2 | 73.0 | 73.1619 | 5.2377 | 10.0159 | 0.4845 | 0.708102 | 0.111227 | 0.096939 | 0.010288 |
| 12/10/89 | B | 4 | M | 0.4530 | 0.2172 | 0.1069 | 0.0672 | 39.0 | 1 | 38.0 | 20.1577 | 5.1879 | 2.1549 | 0.3486 | 0.275416 | 0.163294 | 0.029442 | 0.010973 |
| 12/10/89 | B | 5 | F | 0.5359 | 0.1960 | 0.1361 | 0.0595 | 39.0 | 1 | 39.0 | 45.9259 | 7.5915 | 6.2505 | 0.4517 | 0.388263 | 0.364764 | 0.052843 | 0.021703 |
| 12/10/89 | B | 6 | F | 0.3881 | 0.1572 | 0.0878 | 0.0450 | 37.0 | 1 | 34.0 | 108.0893 | 24.5379 | 9.4902 | 1.1042 | 0.515305 | 9.071523 | 0.045244 | 0.408219 |
| 12/10/89 | B | 7 | I | 0.3845 | 0.1371 | 0.0859 | 0.0408 | 35.0 | 1 | 31.5 | 186.8116 | 20.7409 | 16.0471 | 0.8462 | 1.029153 | 13.610476 | 0.088404 | 0.555307 |
| 12/10/89 | B | 8 | F | 0.2252 | 0.1084 | 0.0451 | 0.0295 | 33.0 | 1 | 29.0 | 227.1101 | 31.9651 | 10.2427 | 0.9430 | 3.858980 | 0.747533 | 0.174040 | 0.022052 |
| 12/10/89 | B | 9 | I | 0.2891 | 0.1123 | 0.0669 | 0.0347 | 33.0 | 1 | 24.0 | 58.3074 | 11.3767 | 3.9008 | 0.3948 | 0.645568 | 0.143670 | 0.043189 | 0.004985 |
| 12/10/89 | B | 10 | F | 0.1968 | 0.1034 | 0.0440 | 0.0271 | 30.0 | 2 | 52.0 | 201.0295 | 21.7068 | 8.8453 | 0.5883 | 1.183969 | 0.439592 | 0.052095 | 0.011913 |
| 12/10/89 | B | 11 | F | 0.2165 | 0.0858 | 0.0518 | 0.0238 | 30.0 | 1 | 39.0 | 162.4583 | 19.2969 | 8.4153 | 0.4593 | 0.846981 | 0.178765 | 0.043874 | 0.004255 |
| 12/10/89 | B | 12 | F | 0.2146 | 0.0742 | 0.0488 | 0.0214 | 29.0 | 1 | 25.0 | 236.3226 | 23.4701 | 11.5325 | 0.5023 | 1.432518 | 0.096371 | 0.069907 | 0.002062 |
| 12/10/89 | B | 13 | M* | 1.7288 | 0.3922 | 0.3724 | 0.1319 | 64.0 | 1 | 49.0 | 32.3548 | 16.6725 | 12.0489 | 2.1991 | 1.691770 | 0.632385 | 0.630015 | 0.083412 |
| 12/10/89 | B | 14 | M* | 1.5888 | 0.3735 | 0.3607 | 0.1256 | 56.0 | 1 | 43.0 | 28.1274 | 18.2515 | 10.1455 | 2.2924 | 0.494189 | 0.328649 | 0.178254 | 0.041278 |
| 12/10/89 | B | 15 | F* | 2.0109 | 0.6829 | 0.5454 | 0.2156 | 67.0 | 2 | 85.0 | 13.2139 | 9.3345 | 7.2068 | 2.0125 | 0.307394 | 0.681590 | 0.167653 | 0.146950 |
| 12/10/89 | B | 16 | F* | 2.0512 | 0.3088 | 0.4586 | 0.0992 | 61.0 | 1 | 38.0 | 54.1275 | 19.6606 | 24.8229 | 1.9503 | 0.471970 | 0.306520 | 0.216450 | 0.030410 |
| 10/01/90 | B | 1 | I | 0.4894 | 0.2289 | 0.1375 | 0.0636 | 39.0 | 2 | 77.0 | 14.2326 | 8.0919 | 1.9570 | 0.5146 | 0.407619 | 0.087375 | 0.056048 | 0.005557 |
| 10/01/90 | B | 2 | I | 0.3991 | 0.1635 | 0.0856 | 0.0411 | 37.0 | 1 | 45.0 | 21.6902 | 17.6212 | 1.8567 | 0.7242 | 0.502737 | 0.689704 | 0.043034 | 0.028347 |
| 10/01/90 | B | 3 | F* | 0.9435 | 0.1167 | 0.3171 | 0.0306 | 50.0 | 1 | 35.0 | 15.7107 | 10.5889 | 4.9819 | 0.3240 | 0.167490 | 0.232405 | 0.053110 | 0.007112 |

(continued)

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21/07/89 | E | 1 | I | 0.2151 | 0.0606 | 0.0434 | 0.0113 | 28.5 | 1 | 32.0 | 30.9067 | 23.9004 | 1.3414 | 0.2701 | 0.136519 | 0.273802 | 0.005925 | 0.003094 |
| 21/07/89 | E | 2 | I | 0.1497 | 0.0713 | 0.0329 | 0.0155 | 29.0 | 1 | 25.0 | 38.3029 | 13.2339 | 1.2602 | 0.2051 | 0.122456 | 0.208275 | 0.004029 | 0.003228 |
| 21/07/89 | E | 3 | I | 0.1738 | 0.0435 | 0.0383 | 0.0080 | 26.5 | 1 | 28.0 | 27.1321 | 13.4629 | 1.0392 | 0.1077 | 0.059491 | 0.295404 | 0.002279 | 0.002363 |
| 21/07/89 | E | 4 | I | 0.1233 | 0.0457 | 0.0276 | 0.0080 | 24.0 | 1 | 26.0 | 37.4873 | 19.1008 | 1.0347 | 0.1528 | 0.061417 | 0.264102 | 0.001695 | 0.002113 |
| 21/07/89 | E | 5 | F | 3.1425 | 1.2046 | 0.5884 | 0.3031 | 69.0 | 4 | 166.0 | 18.1767 | 8.4690 | 10.6952 | 2.5670 | 0.004054 | 1.118765 | 0.002385 | 0.339098 |
| 21/07/89 | E | 6 | F | 3.7001 | 0.2611 | 0.7594 | 0.0696 | 65.0 | 1 | 47.0 | 19.5447 | 19.0064 | 14.8422 | 1.3228 | 0.136515 | 0.154950 | 0.103670 | 0.010785 |
| 21/07/89 | E | 7 | F | 1.8919 | 0.3333 | 0.4691 | 0.0968 | 55.0 | 1 | 40.0 | 6.8866 | 5.9543 | 3.2305 | 0.5764 | 0.222010 | 0.155217 | 0.104145 | 0.015025 |
| 21/07/89 | E | 8 | F | 1.4990 | 0.3724 | 0.3348 | 0.1108 | 57.0 | 1 | 35.0 | 11.1354 | 8.1962 | 3.7281 | 0.9081 | 0.098417 | 0.060722 | 0.032950 | 0.006728 |
| 21/07/89 | E | 9 | F | 1.1438 | 0.2401 | 0.2768 | 0.0651 | 49.0 | 1 | 38.0 | 13.1691 | 7.5797 | 3.6452 | 0.4934 | 0.204867 | 0.148664 | 0.056707 | 0.009678 |
| 21/07/89 | E | 10 | M | 1.8065 | 0.6045 | 0.3796 | 0.1615 | 54.0 | 1 | 69.0 | 17.4686 | 9.2181 | 6.6311 | 1.4887 | 0.058804 | 0.093034 | 0.022322 | 0.015025 |
| 21/07/89 | E | 11 | M | 1.3954 | 0.2527 | 0.3348 | 0.0724 | 51.0 | 1 | 40.0 | 28.9722 | 11.3978 | 9.6999 | 0.8252 | 0.145870 | 0.064917 | 0.048837 | 0.004700 |
| 21/07/89 | E | 12 | M | 1.2691 | 0.1595 | 0.3015 | 0.0433 | 48.0 | 1 | 35.0 | 12.0902 | 5.6493 | 3.6452 | 0.2446 | 0.034460 | 0.227771 | 0.010390 | 0.009863 |
| 21/07/89 | E | 13 | M | 1.1855 | 0.3379 | 0.2576 | 0.1117 | 48.0 | 1 | 53.0 | 14.4726 | 8.1302 | 3.7281 | 0.9081 | 0.365675 | 0.238505 | 0.094198 | 0.026641 |
| 21/07/89 | E | 14 | M | 2.8542 | 1.2138 | 0.8858 | 0.0728 | 63.0 | 1 | 43.0 | 9.9205 | 7.9173 | 8.7875 | 0.5764 | 0.508015 | 0.333022 | 0.450000 | 0.024244 |
| 12/10/89 | E | 1 | M | 1.2578 | 0.2940 | 0.2974 | 0.0896 | 47.0 | 1 | 46.0 | 2.3422 | 1.4698 | 0.6966 | 0.1317 | 0.074049 | 0.322223 | 0.022022 | 0.028871 |
| 12/10/89 | E | 2 | I | 0.2775 | 0.0667 | 0.0695 | 0.0195 | 31.0 | 1 | 30.0 | 2.0808 | 6.0713 | 0.1446 | 0.1184 | 0.789364 | 0.025641 | 0.054861 | 0.000500 |
| 12/10/89 | E | 3 | I | 0.1997 | 0.0375 | 0.0461 | 0.0099 | 28.0 | 2 | 35.0 | 43.3084 | 14.6076 | 1.9965 | 0.1446 | 0.451494 | 0.269886 | 0.020814 | 0.002672 |
| 12/10/89 | E | 4 | I | 0.1342 | 0.0329 | 0.0283 | 0.0079 | 24.0 | 1 | 25.0 | 16.4405 | 0.1862 | 0.4653 | 0.0015 | 0.855315 | 2.435081 | 0.024205 | 0.019237 |
| 12/10/89 | E | 5 | F* | 2.5780 | 0.4110 | 0.9452 | 0.1354 | 65.0 | 1 | 41.0 | 13.8805 | 7.7065 | 13.1199 | 1.0435 | 0.062734 | 0.016634 | 0.059296 | 0.002252 |
| 10/01/90 | E | 1 | I | 0.2205 | 0.0317 | 0.0523 | 0.0063 | 30.0 | 2 | 40.0 | 13.2410 | 405.2767 | 0.6925 | 2.5532 | 0.582892 | 0.125288 | 0.030485 | 0.000789 |
| 25/04/90 | E | 1 | I | 0.4924 | 0.2073 | 0.0983 | 0.0441 | 40.0 | 2 | 73.0 | 31.4002 | 12.9581 | 3.0866 | 0.5715 | 0.197153 | 1.270130 | 0.019380 | 0.056010 |
| 25/04/90 | E | 2 | I | 0.5205 | 0.1917 | 0.0959 | 0.0314 | 38.0 | 2 | 88.0 | 11.5436 | 5.4345 | 1.1070 | 0.1706 | 0.245558 | 1.316130 | 0.023549 | 0.041330 |
| 25/04/90 | E | 3 | I | 0.4275 | 0.1767 | 0.0825 | 0.0342 | 36.0 | 1 | 56.0 | 9.9907 | 6.5236 | 0.8242 | 0.2231 | 0.254067 | 0.326300 | 0.020960 | 0.011160 |
| 25/04/90 | E | 4 | I | 0.3682 | 0.2390 | 0.0697 | 0.0462 | 35.0 | 3 | 79.0 | 10.7463 | 5.4027 | 0.7490 | 0.2496 | 0.083911 | 0.482110 | 0.005849 | 0.022270 |
| 25/04/90 | E | 5 | I | 0.3452 | 0.1368 | 0.0668 | 0.0246 | 36.0 | 1 | 40.0 | 10.1135 | 7.6907 | 0.6756 | 0.1892 | 0.087554 | 3.019180 | 0.005849 | 0.074270 |

(end)

**APPENDIX C.3** The growth data of non-parasitised and parasitised sticklebacks at the three sites in the River Wandle from 21st July 1989 to 25th April 1990.

Non-parasitised Stickleback

| | AGE (Year) | 0+ | 0+ 1/4 | 0+ 2/4 | 0+ 3/4 | 0+ | 1+ | 2+ | 3+ |
|---|---|---|---|---|---|---|---|---|---|
| ITEM | (Month) | 3 | 6 | 9 | 12 | | 12 | 24 | 36 |
| | Site A | | | | | | | | |
| | Fish No. | 12 | 10 | 10 | 11 | 43 | 17 | 13 | |
| | Mean TL | 21.920 | 26.600 | 33.850 | 49.090 | 32.730 | 42.470 | 41.460 | |
| | Absolute Growth | | 4.680 | 7.250 | 15.240 | | 9.740 | -1.010 | |
| | Abs. Growth Rate | | 1.560 | 2.417 | 5.080 | | 0.812 | -0.084 | |
| | Relative Growth | | 0.214 | 0.273 | 0.450 | | 0.298 | -0.024 | |
| | Rel. Growth Rate | | 0.071 | 0.091 | 0.150 | | 0.025 | -0.002 | |
| | Mean log DW | -1.791 | -1.395 | -1.111 | -0.568 | -1.228 | -0.800 | -0.813 | |
| | Mean DW | 0.016 | 0.040 | 0.077 | 0.270 | 0.059 | 0.158 | 0.154 | |
| | Absolute Growth | | 0.024 | 0.037 | 0.193 | | 0.099 | -0.005 | |
| | Abs. Growth Rate | | 0.008 | 0.012 | 0.064 | | 0.033 | -0.002 | |
| N | | | | | | | | | |
| O | Relative Growth | | 1.493 | 0.923 | 2.486 | | 1.677 | -0.029 | |
| N | Rel. Growth Rate | | 0.498 | 0.308 | 0.829 | | 0.140 | -0.002 | |
| | | | | | | | | | |
| l | Site B | | | | | | | | |
| | Fish No. | 6 | 15 | 17 | 1 | 39 | 22 | | |
| P | Mean TL | 21.250 | 27.730 | 36.290 | 47.000 | 30.960 | 39.730 | | |
| A | Absolute Growth | | 6.480 | 8.560 | 10.710 | | 8.770 | | |
| R | Abs. Growth Rate | | 2.160 | 2.853 | 3.570 | | 0.731 | | |
| A | | | | | | | | | |
| S | Relative Growth | | 0.305 | 0.309 | 0.295 | | 0.283 | | |
| I | Rel. Growth Rate | | 0.102 | 0.103 | 0.098 | | 0.024 | | |
| T | | | | | | | | | |
| I | Mean log DW | -1.800 | -1.311 | -0.990 | -0.524 | -1.226 | -0.804 | | |
| S | Mean DW | 0.016 | 0.049 | 0.102 | 0.299 | 0.059 | 0.157 | | |
| E | Absolute Growth | | 0.033 | 0.053 | 0.197 | | 0.098 | | |
| D | Abs. Growth Rate | | 0.011 | 0.018 | 0.066 | | 0.033 | | |
| | Relative Growth | | 2.086 | 1.094 | 1.922 | | 1.642 | | |
| | Rel. Growth Rate | | 0.695 | 0.365 | 0.641 | | 0.137 | | |
| | Site E | | | | | | | | |
| | Fish No. | 10 | 11 | 10 | 12 | 43 | 16 | 10 | 6 |
| | Mean TL | 21.900 | 28.730 | 32.250 | 40.830 | 31.340 | 38.840 | 49.000 | 59.000 |
| | Absolute Growth | | 6.830 | 3.520 | 8.580 | | 7.500 | 10.160 | 10.000 |
| | Abs. Growth Rate | | 2.277 | 1.173 | 2.860 | | 0.625 | 0.847 | 0.833 |
| | Relative Growth | | 0.312 | 0.123 | 0.266 | | 0.239 | 0.262 | 0.204 |
| | Rel. Growth Rate | | 0.104 | 0.041 | 0.089 | | 0.020 | 0.022 | 0.017 |
| | Mean log DW | -1.722 | -1.312 | -1.182 | -0.897 | -1.261 | -0.861 | -0.529 | -0.339 |
| | Mean DW | 0.019 | 0.049 | 0.066 | 0.127 | 0.055 | 0.138 | 0.296 | 0.458 |
| | Absolute Growth | | 0.030 | 0.017 | 0.061 | | 0.083 | 0.158 | 0.162 |
| | Abs. Growth Rate | | 0.010 | 0.006 | 0.020 | | 0.028 | 0.053 | 0.054 |
| | Relative Growth | | 1.573 | 0.349 | 0.926 | | 1.510 | 1.150 | 0.548 |
| | Rel. Growth Rate | | 0.524 | 0.116 | 0.309 | | 0.126 | 0.096 | 0.046 |

## Non-parasitised Stickleback

| ITEM | AGE (Year) | 0+ | 0+ 1/4 | 0+ 2/4 | 0+ 3/4 | 0+ | 1+ | 2+ | 3+ |
|---|---|---|---|---|---|---|---|---|---|
| PARASITISED | Site A | | | | | | | | |
| | Fish No. | | | | | 11 | 8 | 1 | |
| | Mean TL | | | | | 30.540 | 37.060 | 33.000 | |
| | Absolute Growth | | | | | | 6.520 | −4.060 | |
| | Abs. Growth Rate | | | | | | 0.543 | −0.338 | |
| | Relative Growth | | | | | | 0.213 | −0.110 | |
| | Rel. Growth Rate | | | | | | 0.018 | −0.009 | |
| | Mean log DW | | | | | −1.305 | −0.988 | −1.177 | |
| | Mean DW | | | | | 0.050 | 0.103 | 0.067 | |
| | Absolute Growth | | | | | | 0.053 | −0.036 | |
| | Abs. Growth Rate | | | | | | 0.018 | −0.012 | |
| | Relative Growth | | | | | | 1.076 | −0.352 | |
| | Rel. Growth Rate | | | | | | 0.090 | −0.029 | |
| | Site B | | | | | | | | |
| | Fish No. | | | | | 5 | 13 | 12 | 5 |
| | Mean TL | | | | | 33.000 | 39.080 | 57.580 | 66.600 |
| | Absolute Growth | | | | | | 6.080 | 18.500 | 9.020 |
| | Abs. Growth Rate | | | | | | 0.507 | 1.542 | 0.752 |
| | Relative Growth | | | | | | 0.184 | 0.473 | 0.157 |
| | Rel. Growth Rate | | | | | | 0.015 | 0.039 | 0.013 |
| | Mean log DW | | | | | −1.177 | −1.014 | −0.326 | −0.264 |
| | Mean DW | | | | | 0.067 | 0.097 | 0.472 | 0.544 |
| | Absolute Growth | | | | | | 0.030 | 0.375 | 0.073 |
| | Abs. Growth Rate | | | | | | 0.010 | 0.125 | 0.024 |
| | Relative Growth | | | | | | 0.454 | 3.872 | 0.154 |
| | Rel. Growth Rate | | | | | | 0.038 | 0.323 | 0.013 |
| | Site E | | | | | | | | |
| | Fish No. | | | | | 13 | 0 | 8 | 4 |
| | Mean TL | | | | | 31.230 | | 51.125 | 65.500 |
| | Absolute Growth | | | | | | | 19.895 | 14.375 |
| | Abs. Growth Rate | | | | | | | 1.658 | 1.198 |
| | Relative Growth | | | | | | | 0.637 | 0.281 |
| | Rel. Growth Rate | | | | | | | 0.053 | 0.023 |
| | Mean log DW | | | | | −1.276 | | −0.487 | −0.107 |
| | Mean DW | | | | | 0.053 | | 0.326 | 0.782 |
| | Absolute Growth | | | | | | | 0.273 | 0.456 |
| | Abs. Growth Rate | | | | | | | 0.091 | 0.152 |
| | Relative Growth | | | | | | | 5.153 | 1.399 |
| | Rel. Growth Rate | | | | | | | 0.429 | 0.117 |

**APPENDIX C.4** The ANOVA tables of the site effects on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| NON-PARASITISED STICKLEBACK | | | | | | |
| Total Length | Sum of Sq. Between Gr. | 2 | 419.66 | 209.83 | 1.816 | n.s. |
| | Sum of Sq. Within Gr. | 214 | 24729.96 | 115.56 | | |
| | Sum of Sq. of Total | 216 | 25149.62 | | | |
| Logarithmic Dry Weight | Sum of Sq. Between Gr. | 2 | 0.1642 | 0.0821 | 0.386 | n.s. |
| | Sum of Sq. Within Gr. | 214 | 45.5290 | 0.2128 | | |
| | Sum of Sq. of Total | 216 | 45.6932 | | | |
| Total Copper Burden | Sum of Sq. Between Gr. | 2 | 343.37 | 171.69 | 35.892 | **** |
| | Sum of Sq. Within Gr. | 214 | 1023.64 | 4.78 | | |
| | Sum of Sq. of Total | 216 | 1367.01 | | | |
| Logarithmic Total Cadmium Burden | Sum of Sq. Between Gr. | 2 | 10.5400 | 5.2700 | 23.543 | **** |
| | Sum of Sq. Within Gr. | 214 | 47.9032 | 0.2238 | | |
| | Sum of Sq. of Total | 216 | 58.4431 | | | |
| Logarithmic Copper Concentration | Sum of Sq. Between Gr. | 2 | 24.1800 | 12.0900 | 210.510 | **** |
| | Sum of Sq. Within Gr. | 214 | 12.2905 | 0.0574 | | |
| | Sum of Sq. of Total | 216 | 36.4705 | | | |
| Logarithmic Cadmium Concentration | Sum of Sq. Between Gr. | 2 | 11.6239 | 5.8120 | 105.427 | **** |
| | Sum of Sq. Within Gr. | 214 | 11.7974 | 0.0551 | | |
| | Sum of Sq. of Total | 216 | 23.4213 | | | |
| PARASITISED STICKLEBACK | | | | | | |
| Total Length | Sum of Sq. Between Gr. | 2 | 2948.29 | 1474.15 | 9.966 | **** |
| | Sum of Sq. Within Gr. | 78 | 11537.58 | 147.92 | | |
| | Sum of Sq. of Total | 80 | 14485.88 | | | |
| Logarithmic Dry Weight | Sum of Sq. Between Gr. | 2 | 3.5269 | 1.7634 | 7.867 | *** |
| | Sum of Sq. Within Gr. | 78 | 17.4853 | 0.2242 | | |
| | Sum of Sq. of Total | 80 | 21.0122 | | | |
| Total Copper Burden | Sum of Sq. Between Gr. | 2 | 1538.86 | 769.43 | 29.920 | **** |
| | Sum of Sq. Within Gr. | 78 | 2005.84 | 25.72 | | |
| | Sum of Sq. of Total | 80 | 3544.70 | | | |
| Logarithmic Total Cadmium Burden | Sum of Sq. Between Gr. | 2 | 17.1930 | 8.5965 | 29.658 | **** |
| | Sum of Sq. Within Gr. | 78 | 22.6084 | 0.2899 | | |
| | Sum of Sq. of Total | 80 | 22.6084 | | | |
| Logarithmic Copper Concentration | Sum of Sq. Between Gr. | 2 | 9.9508 | 4.9754 | 39.002 | **** |
| | Sum of Sq. Within Gr. | 78 | 9.9503 | 0.1276 | | |
| | Sum of Sq. of Total | 80 | 19.9011 | | | |
| Logarithmic Cadmium Concentration | Sum of Sq. Between Gr. | 2 | 5.4540 | 2.7270 | 18.845 | **** |
| | Sum of Sq. Within Gr. | 78 | 11.2873 | 0.1447 | | |
| | Sum of Sq. of Total | 80 | 16.7413 | | | |

(for significance abbreviations see Table 4.2)

**APPENDIX C.5** The ANOVA tables of the site effects on total length (mm), dry weight (g), total body burdens of Cu and Cd (ug), and concentrations of Cu and Cd (ug/g dw) in the cestode parasites in the parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| CESTODE PARASITISE | | | | | | |
| Total Length | Sum of Sq. Between Gr. | 2 | 17103.04 | 8551.52 | 7.284 | ** |
| | Sum of Sq. Within Gr. | 78 | 91566.91 | 1173.93 | | |
| | Sum of Sq. of Total | 80 | 108669.95 | | | |
| Dry Weight | Sum of Sq. Between Gr. | 2 | 0.137758 | 0.068879 | 12.610 | **** |
| | Sum of Sq. Within Gr. | 78 | 0.426044 | 0.005462 | | |
| | Sum of Sq. of Total | 80 | 0.563802 | | | |
| Total Copper Burden | Sum of Sq. Between Gr. | 2 | 17.2360 | 8.6180 | 20.941 | **** |
| | Sum of Sq. Within Gr. | 78 | 32.0999 | 0.4115 | | |
| | Sum of Sq. of Total | 80 | 49.3359 | | | |
| Total Cadmium Burden | Sum of Sq. Between Gr. | 2 | 0.004221 | 0.002111 | 3.537 | * |
| | Sum of Sq. Within Gr. | 78 | 0.046540 | 0.000597 | | |
| | Sum of Sq. of Total | 80 | 0.050761 | | | |
| Copper Concentration | Sum of Sq. Between Gr. | 2 | 1021.39 | 510.70 | 16.611 | **** |
| | Sum of Sq. Within Gr. | 78 | 2398.06 | 30.74 | | |
| | Sum of Sq. of Total | 80 | 3419.46 | | | |
| Cadmium Concentration | Sum of Sq. Between Gr. | 2 | 0.0249155 | 0.01246 | 0.147 | n.s. |
| | Sum of Sq. Within Gr. | 78 | 6.619621 | 0.08487 | | |
| | Sum of Sq. of Total | 80 | 6.6445365 | | | |

(for significance abbreviations see Table 4.2)

**APPENDIX C.6** The ANOVA tables of the effects of age on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the non-parasitised and parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| **NON-PARASITISED STICKLEBACK** | | | | | | |
| Total Length | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 1633.03 | 544.34 | 4.86 | ** |
| | Sum of Sq. Within Gr. | 71 | 7955.64 | 112.05 | | |
| | Sum of Sq. of Total | 74 | 9588.67 | | | |
| Site B | Sum of Sq. Between Gr. | 2 | 1904.38 | 952.19 | 19.51 | **** |
| | Sum of Sq. Within Gr. | 60 | 2929.06 | 48.82 | | |
| | Sum of Sq. of Total | 62 | 4833.44 | | | |
| Site E | Sum of Sq. Between Gr. | 4 | 6808.45 | 1702.11 | 35.99 | **** |
| | Sum of Sq. Within Gr. | 74 | 3499.41 | 42.29 | | |
| | Sum of Sq. of Total | 78 | 10307.86 | | | |
| Logarithmic Dry Weight | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 3.270 | 1.090 | 6.018 | ** |
| | Sum of Sq. Within Gr. | 71 | 12.860 | 0.181 | | |
| | Sum of Sq. of Total | 74 | 16.130 | | | |
| Site B | Sum of Sq. Between Gr. | 2 | 5.412 | 2.706 | 27.552 | **** |
| | Sum of Sq. Within Gr. | 60 | 5.893 | 0.098 | | |
| | Sum of Sq. of Total | 62 | 11.304 | | | |
| Site E | Sum of Sq. Between Gr. | 4 | 10.703 | 2.676 | 26.786 | *** |
| | Sum of Sq. Within Gr. | 74 | 7.392 | 0.100 | | |
| | Sum of Sq. of Total | 78 | 18.095 | | | |
| Total Copper Burden | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 2.536 | 0.845 | 5.248 | ** |
| | Sum of Sq. Within Gr. | 71 | 11.438 | 0.161 | | |
| | Sum of Sq. of Total | 74 | 13.974 | | | |
| Site B | Sum of Sq. Between Gr. | 2 | 53.122 | 26.561 | 11.188 | *** |
| | Sum of Sq. Within Gr. | 60 | 142.438 | 2.374 | | |
| | Sum of Sq. of Total | 62 | 195.560 | | | |
| Site E | Sum of Sq. Between Gr. | 4 | 594.422 | 148.606 | 50.058 | **** |
| | Sum of Sq. Within Gr. | 74 | 219.683 | 2.969 | | |
| | Sum of Sq. of Total | 78 | 814.105 | | | |
| Logarithmic Total Cadmium Burden | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 1.859 | 0.620 | 2.259 | + |
| | Sum of Sq. Within Gr. | 71 | 19.468 | 0.274 | | |
| | Sum of Sq. of Total | 74 | 21.327 | | | |
| Site B | Sum of Sq. Between Gr. | 2 | 5.808 | 2.904 | 31.717 | **** |
| | Sum of Sq. Within Gr. | 60 | 5.494 | 0.092 | | |
| | Sum of Sq. of Total | 62 | 11.302 | | | |

(continued)

| | | | | | |
|---|---|---|---|---|---|
| Site E | Sum of Sq. Between Gr. | 4 | 6.398 | 1.600 | 13.335 **** |
| | Sum of Sq. Within Gr. | 74 | 8.876 | 0.200 | |
| | Sum of Sq. of Total | 78 | 15.175 | | |
| Logarithmic Copper Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.275 | 0.092 | 1.632 n.s. |
| | Sum of Sq. Within Gr. | 71 | 3.981 | 0.056 | |
| | Sum of Sq. of Total | 74 | 4.256 | | |
| Site B | Sum of Sq. Between Gr. | 2 | 0.736 | 0.368 | 6.147 ** |
| | Sum of Sq. Within Gr. | 60 | 3.591 | 0.060 | |
| | Sum of Sq. of Total | 62 | 4.326 | | |
| Site E | Sum of Sq. Between Gr. | 4 | 0.605 | 0.151 | 3.606 ** |
| | Sum of Sq. Within Gr. | 74 | 3.103 | 0.042 | |
| | Sum of Sq. of Total | 78 | 3.708 | | |
| Logarithmic Cadmium Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.951 | 0.317 | 5.684 ** |
| | Sum of Sq. Within Gr. | 71 | 3.960 | 0.056 | |
| | Sum of Sq. of Total | 74 | 4.911 | | |
| Site B | Sum of Sq. Between Gr. | 2 | 0.438 | 0.219 | 11.718 *** |
| | Sum of Sq. Within Gr. | 60 | 1.122 | 0.019 | |
| | Sum of Sq. of Total | 62 | 1.561 | | |
| Site E | Sum of Sq. Between Gr. | 4 | 0.800 | 0.200 | 3.270 * |
| | Sum of Sq. Within Gr. | 74 | 4.526 | 0.061 | |
| | Sum of Sq. of Total | 78 | 5.326 | | |
| **PARASITISED STICKLEBACK** | | | | | |
| Total Length | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 470.05 | 156.68 | 9.74 *** |
| | Sum of Sq. Within Gr. | 17 | 273.45 | 16.09 | |
| | Sum of Sq. of Total | 20 | 743.50 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 4983.70 | 1661.23 | 51.24 **** |
| | Sum of Sq. Within Gr. | 31 | 1005.04 | 32.42 | |
| | Sum of Sq. of Total | 34 | 5988.74 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 4353.66 | 2176.83 | 106.03 **** |
| | Sum of Sq. Within Gr. | 22 | 451.68 | 20.53 | |
| | Sum of Sq. of Total | 24 | 4805.34 | | |
| Logarithmic Dry Weight | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.760 | 0.253 | 10.35 *** |
| | Sum of Sq. Within Gr. | 17 | 0.416 | 0.024 | |
| | Sum of Sq. of Total | 20 | 1.176 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 5.043 | 1.681 | 18.67 **** |
| | Sum of Sq. Within Gr. | 31 | 2.791 | 0.090 | |
| | Sum of Sq. of Total | 34 | 7.833 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 5.618 | 2.809 | 120.03 **** |
| | Sum of Sq. Within Gr. | 22 | 0.515 | 0.023 | |
| | Sum of Sq. of Total | 24 | 6.133 | | |

(continued)

| | | | | | |
|---|---|---|---|---|---|
| Total Copper Burden | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.591 | 0.197 | 3.964 * |
| | Sum of Sq. Within Gr. | 17 | 0.844 | 0.050 | |
| | Sum of Sq. of Total | 20 | 1.435 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 513.69 | 171.23 | 5.03 ** |
| | Sum of Sq. Within Gr. | 31 | 1055.97 | 34.06 | |
| | Sum of Sq. of Total | 34 | 1569.65 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 356.58 | 178.29 | 50.18 **** |
| | Sum of Sq. Within Gr. | 22 | 78.17 | 3.55 | |
| | Sum of Sq. of Total | 24 | 434.75 | | |
| Logarithmic Total Cadmium Burden | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 1.478 | 0.493 | 3.112 + |
| | Sum of Sq. Within Gr. | 17 | 2.691 | 0.158 | |
| | Sum of Sq. of Total | 20 | 4.168 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 2.094 | 0.698 | 0.029 * |
| | Sum of Sq. Within Gr. | 31 | 6.333 | 0.204 | |
| | Sum of Sq. of Total | 34 | 8.426 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 1.904 | 0.952 | 3.800 * |
| | Sum of Sq. Within Gr. | 22 | 5.512 | 0.251 | |
| | Sum of Sq. of Total | 24 | 7.416 | | |
| Logarithmic Copper Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.254 | 0.085 | 0.775 n.s. |
| | Sum of Sq. Within Gr. | 17 | 1.860 | 0.109 | |
| | Sum of Sq. of Total | 20 | 2.115 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 0.824 | 0.275 | 1.926 n.s. |
| | Sum of Sq. Within Gr. | 31 | 4.419 | 0.143 | |
| | Sum of Sq. of Total | 34 | 5.243 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 0.176 | 0.088 | 0.812 n.s. |
| | Sum of Sq. Within Gr. | 22 | 2.389 | 0.109 | |
| | Sum of Sq. of Total | 24 | 2.565 | | |
| Logarithmic Cadmium Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.383 | 0.128 | 1.114 n.s. |
| | Sum of Sq. Within Gr. | 17 | 1.949 | 0.115 | |
| | Sum of Sq. of Total | 20 | 2.332 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 1.016 | 0.339 | 4.531 n.s. |
| | Sum of Sq. Within Gr. | 31 | 2.318 | 0.075 | |
| | Sum of Sq. of Total | 34 | 3.334 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 1.203 | 0.602 | 2.957 + |
| | Sum of Sq. Within Gr. | 22 | 4.475 | 0.203 | |
| | Sum of Sq. of Total | 24 | 5.678 | | |

(end)

(for significance abbreviations see Table 4.2)

**APPENDIX C.7** The ANOVA tables of the effects of host's age on total length (mm), dry weight (g), total body burdens of Cu and Cd (ug), and concentrations of Cu and Cd (ug/g dw) in the cestode parasites in the parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| **CESTODE PARASITES** | | | | | | |
| Total Length | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 260.2 | 86.7 | 0.31 | n.s. |
| | Sum of Sq. Within Gr. | 17 | 4705.6 | 276.8 | | |
| | Sum of Sq. of Total | 20 | 4965.8 | | | |
| Site B | Sum of Sq. Between Gr. | 3 | 39903.3 | 13301.1 | 16.09 | **** |
| | Sum of Sq. Within Gr. | 31 | 25629.8 | 826.8 | | |
| | Sum of Sq. of Total | 34 | 65533.1 | | | |
| Site E | Sum of Sq. Between Gr. | 2 | 2988.2 | 1494.1 | 1.82 | n.s. |
| | Sum of Sq. Within Gr. | 22 | 18079.8 | 821.8 | | |
| | Sum of Sq. of Total | 24 | 21068.0 | | | |
| Dry Weight | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.00441 | 0.00147 | 8.57 | ** |
| | Sum of Sq. Within Gr. | 17 | 0.00292 | 0.00017 | | |
| | Sum of Sq. of Total | 20 | 0.00732 | | | |
| Site B | Sum of Sq. Between Gr. | 3 | 0.21321 | 0.07107 | 22.05 | **** |
| | Sum of Sq. Within Gr. | 31 | 0.09992 | 0.00322 | | |
| | Sum of Sq. of Total | 34 | 0.31314 | | | |
| Site E | Sum of Sq. Between Gr. | 2 | 0.05811 | 0.02905 | 13.46 | *** |
| | Sum of Sq. Within Gr. | 22 | 0.04747 | 0.00216 | | |
| | Sum of Sq. of Total | 24 | 0.10558 | | | |
| Total Copper Burden | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.0500 | 0.0167 | 10.85 | *** |
| | Sum of Sq. Within Gr. | 17 | 0.0261 | 0.0015 | | |
| | Sum of Sq. of Total | 20 | 0.0761 | | | |
| Site B | Sum of Sq. Between Gr. | 3 | 13.58 | 4.53 | 13.90 | **** |
| | Sum of Sq. Within Gr. | 31 | 10.10 | 0.33 | | |
| | Sum of Sq. of Total | 34 | 23.68 | | | |
| Site E | Sum of Sq. Between Gr. | 2 | 4.62 | 2.31 | 13.68 | *** |
| | Sum of Sq. Within Gr. | 22 | 3.72 | 0.17 | | |
| | Sum of Sq. of Total | 24 | 8.34 | | | |
| Total Cadmium Burden | | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.00293 | 0.00098 | 27.77 | **** |
| | Sum of Sq. Within Gr. | 17 | 0.00060 | 0.00004 | | |
| | Sum of Sq. of Total | 20 | 0.00353 | | | |

(continued)

| | | | | | |
|---|---|---|---|---|---|
| Site B | Sum of Sq. Between Gr. | 3 | 0.00516 | 0.00172 | 4.59 ** |
| | Sum of Sq. Within Gr. | 31 | 0.01162 | 0.00037 | |
| | Sum of Sq. of Total | 34 | 0.01678 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 0.00592 | 0.00296 | 3.21 + |
| | Sum of Sq. Within Gr. | 22 | 0.02030 | 0.00092 | |
| | Sum of Sq. of Total | 24 | 0.02622 | | |
| Copper Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 7.42 | 2.47 | 0.555 n.s. |
| | Sum of Sq. Within Gr. | 17 | 75.73 | 4.45 | |
| | Sum of Sq. of Total | 20 | 83.14 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 291.53 | 97.18 | 2.348 + |
| | Sum of Sq. Within Gr. | 31 | 1283.24 | 41.39 | |
| | Sum of Sq. of Total | 34 | 1574.77 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 54.55 | 27.27 | 0.875 n.s. |
| | Sum of Sq. Within Gr. | 22 | 685.60 | 31.16 | |
| | Sum of Sq. of Total | 24 | 740.15 | | |
| Cadmium Concentration | | | | | |
| Site A | Sum of Sq. Between Gr. | 3 | 0.423 | 0.108 | 1.141 n.s. |
| | Sum of Sq. Within Gr. | 16 | 1.516 | 0.095 | |
| | Sum of Sq. of Total | 19 | 1.860 | | |
| Site B | Sum of Sq. Between Gr. | 3 | 0.579 | 0.193 | 2.736 + |
| | Sum of Sq. Within Gr. | 31 | 2.188 | 0.071 | |
| | Sum of Sq. of Total | 34 | 2.768 | | |
| Site E | Sum of Sq. Between Gr. | 2 | 0.115 | 0.058 | 2.050 n.s. |
| | Sum of Sq. Within Gr. | 22 | 0.617 | 0.028 | |
| | Sum of Sq. of Total | 24 | 0.732 | | |

(end)

(for significance abbreviations see Table 4.2)

**APPENDIX C.8** The ANOVA tables of the seasonal effects on total length (mm), logarithmic dry weight (g), total Cu burden (ug), logarithmic total Cd burden (ug), and logarithmic concentrations of Cu and Cd (ug/g dw) in the $0^+$ age groups of non-parasitised sticklebacks at the three sites in the River Wandle.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| <u>Total Length</u> | | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 4735.92 | 1578.64 | 233.87 | **** |
| Site A | Sum of Sq. Within Gr. | 39 | 263.25 | 6.75 | | |
| | Sum of Sq. of Total | 42 | 4999.17 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 1462.85 | 487.62 | 40.80 | **** |
| Site B | Sum of Sq. Within Gr. | 35 | 418.34 | 11.95 | | |
| | Sum of Sq. of Total | 38 | 1881.34 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 2055.99 | 685.33 | 51.91 | **** |
| Site E | Sum of Sq. Within Gr. | 39 | 514.88 | 13.20 | | |
| | Sum of Sq. of Total | 42 | 2570.86 | | | |
| <u>Logarithmic Dry Weight</u> | | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 9.01 | 3.00 | 145.52 | **** |
| Site A | Sum of Sq. Within Gr. | 39 | 0.80 | 0.02 | | |
| | Sum of Sq. of Total | 42 | 9.82 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 3.53 | 1.18 | 33.57 | **** |
| Site B | Sum of Sq. Within Gr. | 35 | 1.23 | 0.04 | | |
| | Sum of Sq. of Total | 38 | 4.75 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 3.81 | 1.27 | 24.78 | **** |
| Site E | Sum of Sq. Within Gr. | 39 | 2.00 | 0.05 | | |
| | Sum of Sq. of Total | 42 | 5.81 | | | |
| <u>Total Copper Burden</u> | | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 4.15 | 1.38 | 56.38 | **** |
| Site A | Sum of Sq. Within Gr. | 39 | 0.96 | 0.02 | | |
| | Sum of Sq. of Total | 42 | 5.11 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 17.42 | 5.81 | 2.67 | + |
| Site B | Sum of Sq. Within Gr. | 35 | 76.27 | 2.18 | | |
| | Sum of Sq. of Total | 38 | 93.69 | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 14.02 | 4.67 | 6.57 | ** |
| Site E | Sum of Sq. Within Gr. | 39 | 27.77 | 0.71 | | |
| | Sum of Sq. of Total | 42 | 41.79 | | | |

(continued)

| | | | | | |
|---|---|---|---|---|---|
| Logarithmic Total Cadmium Burden | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 9.32 | 3.11 | 40.23 **** |
| Site A | Sum of Sq. Within Gr. | 39 | 3.01 | 0.08 | |
| | Sum of Sq. of Total | 42 | 12.34 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 3.19 | 1.06 | 57.18 **** |
| Site B | Sum of Sq. Within Gr. | 35 | 0.65 | 0.02 | |
| | Sum of Sq. of Total | 38 | 3.85 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 3.82 | 1.27 | 44.81 **** |
| Site E | Sum of Sq. Within Gr. | 39 | 1.11 | 0.03 | |
| | Sum of Sq. of Total | 42 | 4.93 | | |
| Logarithmic Copper Concentration | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 1.39 | 0.46 | 21.20 **** |
| Site A | Sum of Sq. Within Gr. | 39 | 0.85 | 0.02 | |
| | Sum of Sq. of Total | 42 | 2.24 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 1.30 | 0.43 | 12.78 **** |
| Site B | Sum of Sq. Within Gr. | 35 | 1.18 | 0.03 | |
| | Sum of Sq. of Total | 38 | 2.48 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 1.55 | 0.52 | 33.17 **** |
| Site E | Sum of Sq. Within Gr. | 39 | 0.61 | 0.02 | |
| | Sum of Sq. of Total | 42 | 2.16 | | |
| Logarithmic Cadmium Concentration | | | | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 1.06 | 0.35 | 8.43 *** |
| Site A | Sum of Sq. Within Gr. | 39 | 1.64 | 0.04 | |
| | Sum of Sq. of Total | 42 | 2.70 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 0.08 | 0.03 | 2.48 + |
| Site B | Sum of Sq. Within Gr. | 35 | 0.36 | 0.01 | |
| | Sum of Sq. of Total | 38 | 0.44 | | |
| 0+ age group | Sum of Sq. Between Gr. | 3 | 2.02 | 0.67 | 35.61 **** |
| Site E | Sum of Sq. Within Gr. | 39 | 0.74 | 0.02 | |
| | Sum of Sq. of Total | 42 | 2.76 | | |

(end)

(for significance abbreviations see Table 4.2)

**APPENDIX D.1** Concentrations of copper and cadmium in samples of water taken during each season (1989-1990) from the three sampling sites (A, B and E) in the River Wandle.

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

| | | | Total Copper Concentration (ppb=ng/ml) | | | Dissolved Copper | | | | | Total Cadmium Concentration (ppb=ng/ml) | | | Dissolved Cadmium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. | Site | No | Data | Mean | S.D. | Data | Mean | S.D. |
| 21/07/89 | A | 1 | 8.9152 | 6.2531 | 1.7195 | 2.2569 | 2.9364 | 0.8600 | A | 1 | | | | | | |
| 21/07/89 | A | 2 | 4.4264 | | | 2.7806 | | | A | 2 | | | | | | |
| 21/07/89 | A | 3 | 5.0623 | | | 4.6509 | | | A | 3 | | | | | | |
| 21/07/89 | A | 4 | 6.2219 | | | 2.7431 | | | A | 4 | | | | | | |
| 21/07/89 | A | 5 | 7.6434 | | | 2.5935 | | | A | 5 | | | | | | |
| 21/07/89 | A | 6 | 5.2494 | | | | | | A | 6 | | | | | | |
| 12/10/89 | A | 1 | 3.8253 | 4.1018 | 0.2102 | 4.2247 | 4.0570 | 0.2771 | A | 1 | | | | | | |
| 12/10/89 | A | 2 | 4.1018 | | | 3.7025 | | | A | 2 | | | | | | |
| 12/10/89 | A | 3 | 4.2861 | | | 3.8561 | | | A | 3 | | | | | | |
| 12/10/89 | A | 4 | 4.3783 | | | 4.1404 | | | A | 4 | | | | | | |
| 12/10/89 | A | 5 | 4.1018 | | | 4.4705 | | | A | 5 | | | | | | |
| 12/10/89 | A | 6 | 3.9175 | | | 3.9482 | | | A | 6 | | | | | | |
| 10/01/90 | A | 1 | 1.6962 | 1.6962 | 0.1118 | 1.4647 | 1.6769 | 0.1308 | A | 1 | | | | | | |
| 10/01/90 | A | 2 | 1.5033 | | | 1.6962 | | | A | 2 | | | | | | |
| 10/01/90 | A | 3 | 1.6962 | | | 1.5804 | | | A | 3 | | | | | | |
| 10/01/90 | A | 4 | 1.7347 | | | 1.7733 | | | A | 4 | | | | | | |
| 10/01/90 | A | 5 | 1.6962 | | | 1.7347 | | | A | 5 | | | | | | |
| 10/01/90 | A | 6 | 1.8505 | | | 1.8119 | | | A | 6 | | | | | | |
| 25/04/90 | A | 1 | 2.1361 | 2.4932 | 0.8510 | 1.6599 | 2.0816 | 0.7153 | A | 1 | 0.0287 | 0.0426 | 0.0164 | 0.0347 | 0.0376 | 0.0064 |
| 25/04/90 | A | 2 | 1.6939 | | | 1.6599 | | | A | 2 | 0.0311 | | | 0.0347 | | |
| 25/04/90 | A | 3 | 1.5238 | | | 1.3197 | | | A | 3 | 0.0323 | | | 0.0335 | | |
| 25/04/90 | A | 4 | 3.4626 | | | 3.2925 | | | A | 4 | 0.0528 | | | 0.0408 | | |
| 25/04/90 | A | 5 | 3.4626 | | | 2.4762 | | | A | 5 | 0.0708 | | | 0.0323 | | |
| 25/04/90 | A | 6 | 2.6803 | | | 2.0816 | | | A | 6 | 0.0396 | | | 0.0492 | | |
| 21/07/89 | B | 1 | 15.9601 | 17.4564 | 1.3557 | 14.1646 | 14.3641 | 0.3091 | B | 1 | | | | | | |
| 21/07/89 | B | 2 | 17.4564 | | | 14.2394 | | | B | 2 | | | | | | |
| 21/07/89 | B | 3 | 15.9601 | | | 14.2394 | | | B | 3 | | | | | | |
| 21/07/89 | B | 4 | 19.0274 | | | 14.0898 | | | B | 4 | | | | | | |
| 21/07/89 | B | 5 | 18.9526 | | | 14.9127 | | | B | 5 | | | | | | |
| 21/07/89 | B | 6 | 17.3816 | | | 14.5387 | | | B | 6 | | | | | | |
| 12/10/89 | B | 1 | 10.3072 | 9.9898 | 0.1633 | 7.7267 | 7.8189 | 0.1716 | B | 1 | | | | | | |
| 12/10/89 | B | 2 | 9.9078 | | | 7.7267 | | | B | 2 | | | | | | |
| 12/10/89 | B | 3 | 9.9693 | | | 7.6960 | | | B | 3 | | | | | | |
| 12/10/89 | B | 4 | 9.8771 | | | 7.8189 | | | B | 4 | | | | | | |
| 12/10/89 | B | 5 | 10.0000 | | | 8.1568 | | | B | 5 | | | | | | |
| 12/10/89 | B | 6 | 9.8771 | | | 7.7882 | | | B | 6 | | | | | | |
| 10/01/90 | B | 1 | 7.0972 | 8.6918 | 3.0440 | 5.7392 | 5.7392 | 0.2677 | B | 1 | | | | | | |
| 10/01/90 | B | 2 | 6.8272 | | | 5.5926 | | | B | 2 | | | | | | |
| 10/01/90 | B | 3 | 13.6942 | | | 5.7469 | | | B | 3 | | | | | | |
| 10/01/90 | B | 4 | 7.7145 | | | 5.3612 | | | B | 4 | | | | | | |
| 10/01/90 | B | 5 | 11.0708 | | | 5.8241 | | | B | 5 | | | | | | |
| 10/01/90 | B | 6 | 5.7469 | | | 6.1713 | | | B | 6 | | | | | | |
| 25/04/90 | B | 1 | 14.5510 | 20.7619 | 6.4778 | 14.1769 | 13.4966 | 6.1743 | B | 1 | 0.1081 | 0.0865 | 0.0184 | 0.0961 | 0.0859 | 0.0154 |
| 25/04/90 | B | 2 | 11.3878 | | | 6.5918 | | | B | 2 | 0.0829 | | | 0.0636 | | |
| 25/04/90 | B | 3 | 26.2177 | | | 14.4830 | | | B | 3 | 0.1021 | | | 0.0696 | | |
| 25/04/90 | B | 4 | 26.8980 | | | 13.4966 | | | B | 4 | 0.0937 | | | 0.0901 | | |
| 25/04/90 | B | 5 | 24.7551 | | | 24.1088 | | | B | 5 | 0.0720 | | | 0.0949 | | |
| 25/04/90 | B | 6 | 20.7619 | | | 8.1224 | | | B | 6 | 0.0600 | | | 0.1009 | | |
| 21/07/89 | E | 1 | 7.3815 | 7.6268 | 0.8092 | 5.2868 | 5.8791 | 0.3614 | E | 1 | | | | | | |
| 21/07/89 | E | 2 | 7.2693 | | | 5.9601 | | | E | 2 | | | | | | |
| 21/07/89 | E | 3 | 7.6434 | | | 5.6983 | | | E | 3 | | | | | | |
| 21/07/89 | E | 4 | 7.0449 | | | 5.9601 | | | E | 4 | | | | | | |
| 21/07/89 | E | 5 | 9.2269 | | | 5.9975 | | | E | 5 | | | | | | |
| 21/07/89 | E | 6 | 7.1945 | | | 6.3716 | | | E | 6 | | | | | | |
| 12/10/89 | E | 1 | 7.1123 | 7.3991 | 0.5069 | 5.9143 | 5.6890 | 0.2860 | E | 1 | | | | | | |
| 12/10/89 | E | 2 | 7.6346 | | | 5.4535 | | | E | 2 | | | | | | |
| 12/10/89 | E | 3 | 6.8973 | | | 5.7607 | | | E | 3 | | | | | | |
| 12/10/89 | E | 4 | 7.2045 | | | 6.0679 | | | E | 4 | | | | | | |
| 12/10/89 | E | 5 | 8.3104 | | | 5.2999 | | | E | 5 | | | | | | |
| 12/10/89 | E | 6 | 7.2352 | | | 5.6378 | | | E | 6 | | | | | | |
| 10/01/90 | E | 1 | 5.9013 | 6.0247 | 0.4229 | 3.8412 | 3.8412 | 0.2934 | E | 1 | | | | | | |
| 10/01/90 | E | 2 | 5.4769 | | | 4.3967 | | | E | 2 | | | | | | |
| 10/01/90 | E | 3 | 6.2485 | | | 3.8180 | | | E | 3 | | | | | | |
| 10/01/90 | E | 4 | 5.7855 | | | 3.7794 | | | E | 4 | | | | | | |
| 10/01/90 | E | 5 | 6.7114 | | | 3.5480 | | | E | 5 | | | | | | |
| 10/01/90 | E | 6 | 6.0247 | | | 3.6637 | | | E | 6 | | | | | | |
| 25/04/90 | E | 1 | 6.4898 | 6.4048 | 1.1115 | 5.5374 | 5.4410 | 0.2897 | E | 1 | 0.0540 | 0.0586 | 0.0298 | 0.0468 | 0.0420 | 0.0027 |
| 25/04/90 | E | 2 | 6.2177 | | | 5.2993 | | | E | 2 | 0.0552 | | | 0.0420 | | |
| 25/04/90 | E | 3 | 6.0136 | | | 5.9796 | | | E | 3 | 0.0540 | | | 0.0396 | | |
| 25/04/90 | E | 4 | 5.3333 | | | 5.3333 | | | E | 4 | 0.0359 | | | 0.0408 | | |
| 25/04/90 | E | 5 | 8.5306 | | | 5.1633 | | | E | 5 | 0.1165 | | | 0.0432 | | |
| 25/04/90 | E | 6 | 5.8435 | | | 5.3333 | | | E | 6 | 0.0359 | | | 0.0396 | | |

**APPENDIX D.2** Concentrations of copper and cadmium in samples of floc taken during each season (1989-1990) from the three sampling sites (A, B and E) in the River Wandle.

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

| | | | COPPER Concentration (ug/g dry wt.) | | | CADMIUM Concnetration (ug/g dry wt.) | | |
|---|---|---|---|---|---|---|---|---|
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. |
| 21/07/89 | A | 1 | 444.04 | 431.92 | 7.25 | 33.5218 | 33.0971 | 0.3866 |
| 21/07/89 | A | 2 | 431.10 | | | 33.1504 | | |
| 21/07/89 | A | 3 | 436.60 | | | 33.5737 | | |
| 21/07/89 | A | 4 | 428.03 | | | 32.8560 | | |
| 21/07/89 | A | 5 | 424.33 | | | 32.6302 | | |
| 21/07/89 | A | 6 | 427.41 | | | 32.8503 | | |
| 12/10/89 | A | 1 | 335.05 | 325.42 | 6.07 | 20.6085 | 21.0175 | 0.5369 |
| 12/10/89 | A | 2 | 317.64 | | | 20.8324 | | |
| 12/10/89 | A | 3 | 324.48 | | | 20.9625 | | |
| 12/10/89 | A | 4 | 321.42 | | | 20.4140 | | |
| 12/10/89 | A | 5 | 324.78 | | | 21.8538 | | |
| 12/10/89 | A | 6 | 329.12 | | | 21.4338 | | |
| 10/01/90 | A | 1 | 73.16 | 71.49 | 1.88 | 2.8115 | 2.6872 | 0.1802 |
| 10/01/90 | A | 2 | 73.46 | | | 2.4689 | | |
| 10/01/90 | A | 3 | 71.89 | | | 2.8380 | | |
| 10/01/90 | A | 4 | 71.88 | | | 2.4719 | | |
| 10/01/90 | A | 5 | 68.58 | | | 2.8601 | | |
| 10/01/90 | A | 6 | 69.99 | | | 2.6728 | | |
| 25/04/90 | A | 1 | 40.53 | 38.88 | 0.96 | 1.4374 | 1.2506 | 0.2677 |
| 25/04/90 | A | 2 | 39.13 | | | 1.5378 | | |
| 25/04/90 | A | 3 | 39.20 | | | 1.4243 | | |
| 25/04/90 | A | 4 | 38.09 | | | 0.9282 | | |
| 25/04/90 | A | 5 | 38.24 | | | 0.9238 | | |
| 25/04/90 | A | 6 | 38.08 | | | 1.2524 | | |
| 21/07/89 | B | 1 | 688.49 | 684.61 | 18.96 | 15.4914 | 15.8178 | 0.4763 |
| 21/07/89 | B | 2 | 706.89 | | | 16.0717 | | |
| 21/07/89 | B | 3 | 697.35 | | | 16.3624 | | |
| 21/07/89 | B | 4 | 691.96 | | | 16.0904 | | |
| 21/07/89 | B | 5 | 661.68 | | | 15.0484 | | |
| 21/07/89 | B | 6 | 661.29 | | | 15.8424 | | |
| 12/10/89 | B | 1 | 763.17 | 758.03 | 9.14 | 15.9170 | 16.7034 | 0.6328 |
| 12/10/89 | B | 2 | 746.19 | | | 16.9135 | | |
| 12/10/89 | B | 3 | 763.55 | | | 16.3424 | | |
| 12/10/89 | B | 4 | 752.32 | | | 16.9921 | | |
| 12/10/89 | B | 5 | 752.39 | | | 17.7043 | | |
| 12/10/89 | B | 6 | 770.53 | | | 16.3509 | | |
| 10/01/90 | B | 1 | 413.51 | 414.87 | 4.56 | 13.8762 | 13.9888 | 0.3177 |
| 10/01/90 | B | 2 | 423.27 | | | 14.1917 | | |
| 10/01/90 | B | 3 | 411.72 | | | 13.6906 | | |
| 10/01/90 | B | 4 | 410.33 | | | 13.6442 | | |
| 10/01/90 | B | 5 | 415.74 | | | 14.4777 | | |
| 10/01/90 | B | 6 | 414.63 | | | 14.0524 | | |
| 25/04/90 | B | 1 | 304.48 | 307.44 | 2.73 | 9.3977 | 9.1817 | 0.5132 |
| 25/04/90 | B | 2 | 310.35 | | | 8.9971 | | |
| 25/04/90 | B | 3 | 304.15 | | | 8.3734 | | |
| 25/04/90 | B | 4 | 306.86 | | | 9.2063 | | |
| 25/04/90 | B | 5 | 308.63 | | | 9.1713 | | |
| 25/04/90 | B | 6 | 310.18 | | | 9.9446 | | |
| 21/07/89 | E | 1 | 365.56 | 366.88 | 5.31 | 13.6823 | 13.6134 | 0.4355 |
| 21/07/89 | E | 2 | 376.83 | | | 13.9832 | | |
| 21/07/89 | E | 3 | 368.38 | | | 12.9248 | | |
| 21/07/89 | E | 4 | 363.57 | | | 14.0137 | | |
| 21/07/89 | E | 5 | 362.11 | | | 13.2543 | | |
| 21/07/89 | E | 6 | 364.80 | | | 13.8220 | | |
| 12/10/89 | E | 1 | 519.84 | 516.98 | 2.97 | 14.9424 | 14.9202 | 0.2999 |
| 12/10/89 | E | 2 | 520.67 | | | 15.0164 | | |
| 12/10/89 | E | 3 | 516.93 | | | 14.7232 | | |
| 12/10/89 | E | 4 | 513.75 | | | 14.4484 | | |
| 12/10/89 | E | 5 | 513.55 | | | 15.0785 | | |
| 12/10/89 | E | 6 | 517.13 | | | 15.3121 | | |
| 10/01/90 | E | 1 | 390.62 | 392.28 | 4.63 | 16.3902 | 15.6678 | 0.3696 |
| 10/01/90 | E | 2 | 384.19 | | | 15.6779 | | |
| 10/01/90 | E | 3 | 396.77 | | | 15.5328 | | |
| 10/01/90 | E | 4 | 392.23 | | | 15.5743 | | |
| 10/01/90 | E | 5 | 396.45 | | | 15.4749 | | |
| 10/01/90 | E | 6 | 393.42 | | | 15.3566 | | |
| 25/04/90 | E | 1 | 377.87 | 371.03 | 5.53 | 15.1171 | 14.0695 | 0.5366 |
| 25/04/90 | E | 2 | 374.66 | | | 13.5673 | | |
| 25/04/90 | E | 3 | 366.71 | | | 13.8570 | | |
| 25/04/90 | E | 4 | 375.36 | | | 13.8917 | | |
| 25/04/90 | E | 5 | 365.27 | | | 14.0142 | | |
| 25/04/90 | E | 6 | 366.30 | | | 13.9697 | | |

**APPENDIX D.3** Concentrations of copper and cadmium in samples of sediment taken during each season (1989-1990) from the three sampling sites (A, B and E) in the River Wandle.

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

| | | | COPPER | | | CADMIUM | | |
|---|---|---|---|---|---|---|---|---|
| | | | Concentration (ug/g dry wt.) | | | Concentration (ug/g dry wt.) | | |
| Date | Site | No | Data | Mean | S.D. | Data | Mean | S.D. |
| 21/07/89 | A | 1 | 50.70 | 50.31 | 1.36 | 1.9707 | 2.4577 | 0.3596 |
| 21/07/89 | A | 2 | 51.67 | | | 2.7325 | | |
| 21/07/89 | A | 3 | 48.34 | | | 2.5246 | | |
| 21/07/89 | A | 4 | 51.58 | | | 2.0500 | | |
| 21/07/89 | A | 5 | 50.56 | | | 2.8051 | | |
| 21/07/89 | A | 6 | 48.99 | | | 2.6634 | | |
| 12/10/89 | A | 1 | 52.39 | 53.08 | 0.82 | 0.8527 | 0.8227 | 0.0390 |
| 12/10/89 | A | 2 | 53.79 | | | 0.8112 | | |
| 12/10/89 | A | 3 | 53.45 | | | 0.8113 | | |
| 12/10/89 | A | 4 | 53.06 | | | 0.7900 | | |
| 12/10/89 | A | 5 | 53.94 | | | 0.8856 | | |
| 12/10/89 | A | 6 | 51.87 | | | 0.7852 | | |
| 10/01/90 | A | 1 | 59.26 | 57.81 | 1.12 | 2.3813 | 2.5358 | 0.1492 |
| 10/01/90 | A | 2 | 56.35 | | | 2.4597 | | |
| 10/01/90 | A | 3 | 58.11 | | | 2.8044 | | |
| 10/01/90 | A | 4 | 56.57 | | | 2.4518 | | |
| 10/01/90 | A | 5 | 58.23 | | | 2.5327 | | |
| 10/01/90 | A | 6 | 58.34 | | | 2.5847 | | |
| 25/04/90 | A | 1 | 58.61 | 58.52 | 0.97 | 1.6414 | 1.6352 | 0.0129 |
| 25/04/90 | A | 2 | 57.46 | | | 1.6194 | | |
| 25/04/90 | A | 3 | 60.23 | | | 1.6549 | | |
| 25/04/90 | A | 4 | 58.19 | | | 1.6400 | | |
| 25/04/90 | A | 5 | 57.85 | | | 1.6303 | | |
| 25/04/90 | A | 6 | 58.77 | | | 1.6250 | | |
| 21/07/89 | B | 1 | 267.34 | 276.68 | 6.75 | 6.2866 | 6.1331 | 0.4158 |
| 21/07/89 | B | 2 | 272.51 | | | 5.8527 | | |
| 21/07/89 | B | 3 | 286.98 | | | 6.2637 | | |
| 21/07/89 | B | 4 | 280.05 | | | 5.5715 | | |
| 21/07/89 | B | 5 | 274.90 | | | 6.0420 | | |
| 21/07/89 | B | 6 | 278.28 | | | 6.7823 | | |
| 12/10/89 | B | 1 | 301.28 | 302.17 | 8.35 | 8.6723 | 8.5529 | 0.3837 |
| 12/10/89 | B | 2 | 309.10 | | | 8.8874 | | |
| 12/10/89 | B | 3 | 305.39 | | | 8.8748 | | |
| 12/10/89 | B | 4 | 310.78 | | | 7.9212 | | |
| 12/10/89 | B | 5 | 298.52 | | | 8.2610 | | |
| 12/10/89 | B | 6 | 287.96 | | | 8.7010 | | |
| 10/01/90 | B | 1 | 283.26 | 281.71 | 1.68 | 9.8937 | 9.9046 | 0.5344 |
| 10/01/90 | B | 2 | 282.09 | | | 9.7580 | | |
| 10/01/90 | B | 3 | 278.44 | | | 10.1892 | | |
| 10/01/90 | B | 4 | 282.36 | | | 10.4631 | | |
| 10/01/90 | B | 5 | 281.74 | | | 8.9379 | | |
| 10/01/90 | B | 6 | 282.36 | | | 10.1857 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 25/04/90 | B | 1 | 436.99 | 440.73 | 6.96 | 14.7804 | 14.4359 | 0.3617 |
| 25/04/90 | B | 2 | 435.83 | | | 14.9361 | | |
| 25/04/90 | B | 3 | 434.18 | | | 14.4079 | | |
| 25/04/90 | B | 4 | 453.24 | | | 14.3669 | | |
| 25/04/90 | B | 5 | 441.17 | | | 14.0645 | | |
| 25/04/90 | B | 6 | 442.97 | | | 14.0597 | | |
| 21/07/89 | E | 1 | 402.50 | 401.60 | 3.72 | 11.9857 | 12.8628 | 0.6205 |
| 21/07/89 | E | 2 | 407.33 | | | 13.5674 | | |
| 21/07/89 | E | 3 | 399.08 | | | 12.7635 | | |
| 21/07/89 | E | 4 | 403.63 | | | 13.5480 | | |
| 21/07/89 | E | 5 | 400.35 | | | 12.8734 | | |
| 21/07/89 | E | 6 | 396.74 | | | 12.4389 | | |
| 21/07/89 | E | 7 | 414.20 | 408.83 | 4.88 | 12.1000 | 11.9000 | 0.2000 |
| 21/07/89 | E | 8 | 407.63 | | | 11.9000 | | |
| 21/07/89 | E | 9 | 404.67 | | | 11.7000 | | |
| 21/07/89 | E | 10 | 406.15 | 402.43 | 3.88 | 13.8037 | 13.7223 | 0.1578 |
| 21/07/89 | E | 11 | 402.73 | | | 13.8228 | | |
| 21/07/89 | E | 12 | 398.41 | | | 13.5405 | | |
| 21/07/89 | E | 13 | 405.21 | 408.45 | 3.26 | 14.5500 | 14.5600 | 0.0265 |
| 21/07/89 | E | 14 | 408.42 | | | 14.5400 | | |
| 21/07/89 | E | 15 | 411.73 | | | 14.5900 | | |
| 12/10/89 | E | 1 | 342.99 | 348.83 | 4.95 | 10.5661 | 10.2829 | 0.1986 |
| 12/10/89 | E | 2 | 350.45 | | | 10.4844 | | |
| 12/10/89 | E | 3 | 343.93 | | | 10.1159 | | |
| 12/10/89 | E | 4 | 351.76 | | | 10.1588 | | |
| 12/10/89 | E | 5 | 356.04 | | | 10.1014 | | |
| 12/10/89 | E | 6 | 347.80 | | | 10.2709 | | |
| 10/01/90 | E | 1 | 364.89 | 362.09 | 3.90 | 12.8644 | 12.5141 | 0.4097 |
| 10/01/90 | E | 2 | 359.05 | | | 12.6807 | | |
| 10/01/90 | E | 3 | 357.78 | | | 12.0542 | | |
| 10/01/90 | E | 4 | 361.83 | | | 12.0566 | | |
| 10/01/90 | E | 5 | 368.29 | | | 12.4080 | | |
| 10/01/90 | E | 6 | 360.70 | | | 13.0210 | | |
| 25/04/90 | E | 1 | 421.16 | 421.03 | 1.88 | 15.2954 | 15.2719 | 0.1371 |
| 25/04/90 | E | 2 | 421.16 | | | 15.2954 | | |
| 25/04/90 | E | 3 | 419.84 | | | 15.1140 | | |
| 25/04/90 | E | 4 | 423.44 | | | 15.1192 | | |
| 25/04/90 | E | 5 | 418.13 | | | 15.3335 | | |
| 25/04/90 | E | 6 | 422.43 | | | 15.4739 | | |

(end)

**APPENDIX D.4** Concentrations of copper and cadmium in samples of algae and aquatic plants taken during each season (1989-1990) from the three sampling sites (A, B and E) in the River Wandle.

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

| | | | COPPER Concentration (ug/g dry wt.) | | | CADMIUM Concentration (ug/g dry wt.) | | |
|---|---|---|---|---|---|---|---|---|
| Site | Date | No | Data | Mean | S.D. | Data | Mean | S.D. |
| *Spirogyoa sp.* | | | | | | | | |
| A | 21/07/89 | 1 | 3.8319 | 4.4603 | 0.7036 | 0.32435 | 0.35660 | 0.02623 |
| A | 21/07/89 | 2 | 5.1855 | | | 0.34234 | | |
| A | 21/07/89 | 3 | 5.3465 | | | 0.39809 | | |
| A | 21/07/89 | 4 | 4.5089 | | | 0.34150 | | |
| A | 21/07/89 | 5 | 4.2874 | | | 0.36190 | | |
| A | 21/07/89 | 6 | 3.6017 | | | 0.37140 | | |
| B | 12/10/89 | 1 | 29.0904 | | | | | |
| E | 21/07/89 | 1 | 18.0388 | 17.6477 | 0.4137 | 1.38787 | 1.21865 | 0.09784 |
| E | 21/07/89 | 2 | 17.6893 | | | 1.13315 | | |
| E | 21/07/89 | 3 | 17.8773 | | | 1.27866 | | |
| E | 21/07/89 | 4 | 18.0023 | | | 1.19679 | | |
| E | 21/07/89 | 5 | 17.0867 | | | 1.17217 | | |
| E | 21/07/89 | 6 | 17.1917 | | | 1.14330 | | |
| E | 12/10/89 | 1 | 9.5607 | 9.8303 | 0.4536 | 0.56910 | 0.58277 | 0.09237 |
| E | 12/10/89 | 2 | 10.7124 | | | 0.62120 | | |
| E | 12/10/89 | 3 | 9.4600 | | | 0.73842 | | |
| E | 12/10/89 | 4 | 9.7284 | | | 0.58336 | | |
| E | 12/10/89 | 5 | 9.8622 | | | 0.47828 | | |
| E | 12/10/89 | 6 | 9.6583 | | | 0.50628 | | |
| *Cladophora sp.* | | | | | | | | |
| B | 21/07/89 | 1 | 27.5332 | 26.8086 | 1.9336 | 3.95759 | 3.07099 | 0.54504 |
| B | 21/07/89 | 2 | 30.5222 | | | 3.78468 | | |
| B | 21/07/89 | 3 | 28.1654 | | | 2.83961 | | |
| B | 21/07/89 | 4 | 27.1744 | | | 2.39393 | | |
| B | 21/07/89 | 5 | 25.1785 | | | 2.95220 | | |
| B | 21/07/89 | 6 | 28.4544 | | | 3.58106 | | |
| B | 21/07/89 | 7 | 26.1765 | | | 2.87035 | | |
| B | 21/07/89 | 8 | 24.7106 | | | 3.06000 | | |
| B | 21/07/89 | 9 | 25.8763 | | | 2.92837 | | |
| B | 21/07/89 | 10 | 24.2939 | | | 2.34209 | | |
| B | 12/10/89 | 1 | 47.5668 | 47.4665 | 2.3158 | 3.95451 | 4.14663 | 0.49305 |
| B | 12/10/89 | 2 | 49.3549 | | | 4.67263 | | |
| B | 12/10/89 | 3 | 50.1337 | | | 4.86432 | | |
| B | 12/10/89 | 4 | 46.3363 | | | 3.90632 | | |
| B | 12/10/89 | 5 | 47.7811 | | | 3.74074 | | |
| B | 12/10/89 | 6 | 43.6261 | | | 3.74126 | | |
| B | 10/01/90 | 1 | 145.1399 | 146.3954 | 2.5965 | 3.96533 | 4.11390 | 0.18770 |
| B | 10/01/90 | 2 | 148.1955 | | | 4.29220 | | |
| B | 10/01/90 | 3 | 145.8954 | | | 3.95165 | | |
| B | 10/01/90 | 4 | 142.0306 | | | 4.36218 | | |
| B | 10/01/90 | 5 | 148.9158 | | | 4.17354 | | |
| B | 10/01/90 | 6 | 148.1951 | | | 3.93851 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| B | 25/04/90 | 1 | 27.7876 | 26.6701 | 0.7268 | 1.66818 | 1.67414 | 0.02957 |
| B | 25/04/90 | 2 | 26.9727 | | | 1.70583 | | |
| B | 25/04/90 | 3 | 26.4178 | | | 1.67877 | | |
| B | 25/04/90 | 4 | 27.0018 | | | 1.64282 | | |
| B | 25/04/90 | 5 | 25.8825 | | | 1.64049 | | |
| B | 25/04/90 | 6 | 25.9583 | | | 1.70876 | | |
| E | 21/07/89 | 1 | 45.4464 | 46.5069 | 1.0649 | 2.73075 | 2.27904 | 0.33486 |
| E | 21/07/89 | 2 | 47.8612 | | | 2.37660 | | |
| E | 21/07/89 | 3 | 47.7257 | | | 1.74852 | | |
| E | 21/07/89 | 4 | 46.0853 | | | 2.20160 | | |
| E | 21/07/89 | 5 | 46.4378 | | | 2.13820 | | |
| E | 21/07/89 | 6 | 45.4853 | | | 2.47855 | | |
| E | 12/10/89 | 1 | 46.8415 | 48.1225 | 1.7766 | 9.34676 | 9.57590 | 0.25586 |
| E | 12/10/89 | 2 | 49.1557 | | | 9.86510 | | |
| E | 12/10/89 | 3 | 49.4570 | | | 9.89797 | | |
| E | 12/10/89 | 4 | 45.6618 | | | 9.28773 | | |
| E | 12/10/89 | 5 | 47.3443 | | | 9.52688 | | |
| E | 12/10/89 | 6 | 50.2748 | | | 9.53097 | | |
| E | 25/04/90 | 1 | 47.7970 | 47.2951 | 2.4995 | 1.54529 | 1.55455 | 0.04964 |
| E | 25/04/90 | 2 | 49.2767 | | | 1.60488 | | |
| E | 25/04/90 | 3 | 47.7148 | | | 1.60818 | | |
| E | 25/04/90 | 4 | 43.7948 | | | 1.56190 | | |
| E | 25/04/90 | 5 | 44.9053 | | | 1.47556 | | |
| E | 25/04/90 | 6 | 50.2818 | | | 1.53150 | | |
| *Rhynchostegium riparioides* | | | | | | | | |
| B | 12/10/89 | 1 | 217.4043 | 211.8837 | 6.7053 | 13.80499 | 12.87753 | 0.73979 |
| B | 12/10/89 | 2 | 209.4454 | | | 12.26450 | | |
| B | 12/10/89 | 3 | 217.1877 | | | 13.14248 | | |
| B | 12/10/89 | 4 | 203.4976 | | | 12.29816 | | |
| E | 21/07/89 | 1 | 118.0631 | 126.9110 | 7.3798 | 2.54749 | 2.72511 | 0.18950 |
| E | 21/07/89 | 2 | 119.2623 | | | 2.74288 | | |
| E | 21/07/89 | 3 | 136.6815 | | | 2.99938 | | |
| E | 21/07/89 | 4 | 132.7839 | | | 2.81814 | | |
| E | 21/07/89 | 5 | 128.9501 | | | 2.76680 | | |
| E | 21/07/89 | 6 | 125.7249 | | | 2.47596 | | |
| E | 12/10/89 | 1 | 98.5642 | 94.2103 | 2.9786 | 2.84434 | 2.77401 | 0.10463 |
| E | 12/10/89 | 2 | 90.4266 | | | 2.72841 | | |
| E | 12/10/89 | 3 | 95.0280 | | | 2.94530 | | |
| E | 12/10/89 | 4 | 91.7735 | | | 2.67311 | | |
| E | 12/10/89 | 5 | 96.1367 | | | 2.76927 | | |
| E | 12/10/89 | 6 | 93.3329 | | | 2.68364 | | |
| E | 10/01/90 | 1 | 100.5258 | 109.6935 | 4.6135 | 4.15157 | 4.28836 | 0.10841 |
| E | 10/01/90 | 2 | 111.4930 | | | 4.32968 | | |
| E | 10/01/90 | 3 | 111.8547 | | | 4.15162 | | |
| E | 10/01/90 | 4 | 110.0183 | | | 4.39903 | | |
| E | 10/01/90 | 5 | 111.0292 | | | 4.35155 | | |
| E | 10/01/90 | 6 | 113.2398 | | | 4.34672 | | |
| E | 25/04/90 | 1 | 60.3130 | 57.7806 | 2.3907 | 1.05806 | 0.97156 | 0.10287 |
| E | 25/04/90 | 2 | 55.8792 | | | 1.09358 | | |
| E | 25/04/90 | 3 | 55.5991 | | | 0.87085 | | |
| E | 25/04/90 | 4 | 61.0747 | | | 1.04056 | | |
| E | 25/04/90 | 5 | 57.7505 | | | 0.88309 | | |
| E | 25/04/90 | 6 | 56.0668 | | | 0.88320 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Callitriche sp.* | | | | | | | | |
| B | 21/07/89 | 1 | 56.1710 | 57.0160 | 2.4125 | 4.67944 | 4.55952 | 0.19557 |
| B | 21/07/89 | 2 | 56.8261 | | | 4.73139 | | |
| B | 21/07/89 | 3 | 54.0236 | | | 4.19160 | | |
| B | 21/07/89 | 4 | 59.6083 | | | 4.66570 | | |
| B | 21/07/89 | 5 | 60.1469 | | | 4.53380 | | |
| B | 21/07/89 | 6 | 55.3204 | | | 4.55523 | | |
| B | 12/10/89 | 1 | 76.5851 | 79.7148 | 2.5615 | 7.31365 | 7.48704 | 0.22490 |
| B | 12/10/89 | 2 | 81.9399 | | | 7.71067 | | |
| B | 12/10/89 | 3 | 81.6718 | | | 7.64955 | | |
| B | 12/10/89 | 4 | 78.6623 | | | 7.27430 | | |
| B | 10/01/90 | 1 | 96.9863 | 98.9223 | 3.2475 | 10.78696 | 10.92119 | 0.28164 |
| B | 10/01/90 | 2 | 96.7704 | | | 10.78150 | | |
| B | 10/01/90 | 3 | 103.2436 | | | 11.34023 | | |
| B | 10/01/90 | 4 | 102.7859 | | | 10.90122 | | |
| B | 10/01/90 | 5 | 95.7881 | | | 11.15464 | | |
| B | 10/01/90 | 6 | 97.9598 | | | 10.56260 | | |
| B | 25/04/90 | 1 | 61.5933 | 60.3992 | 1.5555 | 6.89007 | 6.63205 | 0.29503 |
| B | 25/04/90 | 2 | 62.6701 | | | 6.75235 | | |
| B | 25/04/90 | 3 | 59.8836 | | | 6.20082 | | |
| B | 25/04/90 | 4 | 58.3695 | | | 6.45919 | | |
| B | 25/04/90 | 5 | 60.5333 | | | 6.98246 | | |
| B | 25/04/90 | 6 | 59.3453 | | | 6.50740 | | |
| E | 12/10/89 | 1 | 103.6844 | 96.8235 | 3.6050 | 5.91020 | 5.80961 | 0.19488 |
| E | 12/10/89 | 2 | 97.1718 | | | 6.06918 | | |
| E | 12/10/89 | 3 | 95.9786 | | | 5.60492 | | |
| E | 12/10/89 | 4 | 93.1598 | | | 5.80522 | | |
| E | 12/10/89 | 5 | 95.4364 | | | 5.90590 | | |
| E | 12/10/89 | 6 | 95.5100 | | | 5.56223 | | |
| E | 10/01/90 | 1 | 109.0833 | 114.1443 | 3.2547 | 8.83351 | 9.01436 | 0.18175 |
| E | 10/01/90 | 2 | 117.3584 | | | 8.80024 | | |
| E | 10/01/90 | 3 | 111.3610 | | | 9.08725 | | |
| E | 10/01/90 | 4 | 116.8676 | | | 9.29261 | | |
| E | 10/01/90 | 5 | 115.2382 | | | 8.99813 | | |
| E | 10/01/90 | 6 | 114.9574 | | | 9.07443 | | |
| E | 25/04/90 | 1 | 39.9984 | 37.6285 | 1.4802 | 1.96022 | 1.93213 | 0.14958 |
| E | 25/04/90 | 2 | 36.0840 | | | 1.99011 | | |
| E | 25/04/90 | 3 | 38.2381 | | | 1.92764 | | |
| E | 25/04/90 | 4 | 36.9805 | | | 2.13069 | | |
| E | 25/04/90 | 5 | 36.2728 | | | 1.67156 | | |
| E | 25/04/90 | 6 | 38.1971 | | | 1.91253 | | |
| *Elodea canadensis* | | | | | | | | |
| E | 21/07/89 | 1 | 61.7969 | 61.5771 | 2.4267 | 1.74728 | 1.59268 | 0.17196 |
| E | 21/07/89 | 2 | 60.6189 | | | 1.40156 | | |
| E | 21/07/89 | 3 | 62.5083 | | | 1.82031 | | |
| E | 21/07/89 | 4 | 62.8637 | | | 1.40949 | | |
| E | 21/07/89 | 5 | 64.3631 | | | 1.55696 | | |
| E | 21/07/89 | 6 | 57.3118 | | | 1.62047 | | |
| E | 12/10/89 | 1 | 99.1539 | 100.0885 | 2.2654 | 5.54711 | 5.65069 | 0.19920 |
| E | 12/10/89 | 2 | 100.9204 | | | 5.69453 | | |
| E | 12/10/89 | 3 | 97.2081 | | | 5.74071 | | |
| E | 12/10/89 | 4 | 103.3431 | | | 5.89777 | | |
| E | 12/10/89 | 5 | 99.8172 | | | 5.37334 | | |
| E | 10/01/90 | 1 | 106.0325 | 104.7116 | 1.6254 | 7.29203 | 7.35895 | 0.22800 |
| E | 10/01/90 | 2 | 103.5880 | | | 7.30836 | | |
| E | 10/01/90 | 3 | 104.4178 | | | 7.16209 | | |
| E | 10/01/90 | 4 | 102.7255 | | | 7.43972 | | |
| E | 10/01/90 | 5 | 104.3380 | | | 7.17549 | | |
| E | 10/01/90 | 6 | 107.1677 | | | 7.77601 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| E | 25/04/90 | 1 | 59.1733 | 56.9785 | 2.6944 | 7.29943 | 7.41840 | 0.18983 |
| E | 25/04/90 | 2 | 57.9885 | | | 7.65315 | | |
| E | 25/04/90 | 3 | 58.9540 | | | 7.41840 | | |
| E | 25/04/90 | 4 | 52.7163 | | | 7.28700 | | |
| E | 25/04/90 | 5 | 58.5159 | | | 7.64245 | | |
| E | 25/04/90 | 6 | 54.5230 | | | 7.20995 | | |
| *Ranunculus fluitans* | | | | | | | | |
| E | 21/07/89 | 1 | 41.4633 | 40.7173 | 1.4718 | 3.26897 | 2.92988 | 0.30570 |
| E | 21/07/89 | 2 | 42.4857 | | | 3.23139 | | |
| E | 21/07/89 | 3 | 39.5844 | | | 2.78763 | | |
| E | 21/07/89 | 4 | 40.7189 | | | 2.80278 | | |
| E | 21/07/89 | 5 | 41.5922 | | | 3.02359 | | |
| E | 21/07/89 | 6 | 38.4593 | | | 2.46492 | | |
| E | 12/10/89 | 1 | 52.2930 | 52.6099 | 1.6422 | 12.30243 | 13.00500 | 0.52755 |
| E | 12/10/89 | 2 | 54.9193 | | | 13.56711 | | |
| E | 12/10/89 | 3 | 50.2271 | | | 13.08730 | | |
| E | 12/10/89 | 4 | 51.5399 | | | 12.71926 | | |
| E | 12/10/89 | 5 | 53.6249 | | | 12.71087 | | |
| E | 12/10/89 | 6 | 53.0548 | | | 13.64300 | | |
| E | 10/01/90 | 1 | 61.4304 | 62.4069 | 1.5760 | 3.51133 | 3.51959 | 0.13524 |
| E | 10/01/90 | 2 | 64.2277 | | | 3.68663 | | |
| E | 10/01/90 | 3 | 62.2830 | | | 3.36324 | | |
| E | 10/01/90 | 4 | 60.8939 | | | 3.39474 | | |
| E | 10/01/90 | 5 | 64.4664 | | | 3.67011 | | |
| E | 10/01/90 | 6 | 61.1403 | | | 3.49147 | | |
| E | 25/04/90 | 1 | 41.9236 | 40.8866 | 1.4810 | 10.12540 | 9.92186 | 0.28356 |
| E | 25/04/90 | 2 | 42.8470 | | | 10.19675 | | |
| E | 25/04/90 | 3 | 40.4203 | | | 9.68245 | | |
| E | 25/04/90 | 4 | 39.7501 | | | 9.53261 | | |
| E | 25/04/90 | 5 | 38.8537 | | | 10.17713 | | |
| E | 25/04/90 | 6 | 41.5246 | | | 9.81680 | | |
| *Potamogeton erispus* | | | | | | | | |
| B | 10/01/90 | 1 | 153.7706 | | | 4.6332 | | |
| E | 12/10/89 | 1 | 43.2518 | 44.4292 | 1.1149 | 2.3144 | 2.5056 | 0.2064 |
| E | 12/10/89 | 2 | 44.4627 | | | 2.7254 | | |
| E | 12/10/89 | 3 | 45.9197 | | | 2.6373 | | |
| E | 12/10/89 | 4 | 44.0826 | | | 2.3455 | | |
| E | 10/01/90 | 1 | 87.0677 | 82.9919 | 4.0758 | 2.2564 | 2.6135 | 0.3265 |
| E | 10/01/90 | 2 | 78.9161 | | | 2.8967 | | |
| E | 10/01/90 | 3 | 82.9919 | | | 2.6873 | | |
| E | 25/04/90 | 1 | 67.1457 | 66.7852 | 2.7453 | 2.9754 | 2.6949 | 0.3067 |
| E | 25/04/90 | 2 | 65.9483 | | | 2.6819 | | |
| E | 25/04/90 | 3 | 71.0856 | | | 2.9568 | | |
| E | 25/04/90 | 4 | 64.0471 | | | 2.2890 | | |
| E | 25/04/90 | 5 | 63.9757 | | | 2.9075 | | |
| E | 25/04/90 | 6 | 68.5087 | | | 2.3589 | | |
| *Apium nodiflorum* (Water celery, leaves) | | | | | | | | |
| E | 12/10/89 | 1 | 36.8605 | 32.1858 | 3.0668 | 3.00988 | 2.97353 | 0.23522 |
| E | 12/10/89 | 2 | 31.1636 | | | 2.81609 | | |
| E | 12/10/89 | 3 | 28.3526 | | | 2.76427 | | |
| E | 12/10/89 | 4 | 32.3592 | | | 3.35876 | | |
| E | 12/10/89 | 5 | 32.1931 | | | 2.91864 | | |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| E | 10/01/90 | 1 | 71.9329 | 67.9028 | 2.8603 | 5.80208 | 5.52036 | 0.19967 |
| E | 10/01/90 | 2 | 64.3434 | | | 5.47219 | | |
| E | 10/01/90 | 3 | 68.8653 | | | 5.71070 | | |
| E | 10/01/90 | 4 | 69.9140 | | | 5.47885 | | |
| E | 10/01/90 | 5 | 65.4418 | | | 5.27178 | | |
| E | 10/01/90 | 6 | 66.9195 | | | 5.38656 | | |
| E | 25/04/90 | 1 | 28.0668 | 29.4053 | 1.2300 | 2.19219 | 2.32703 | 0.10363 |
| E | 25/04/90 | 2 | 28.2552 | | | 2.35120 | | |
| E | 25/04/90 | 3 | 31.4454 | | | 2.44342 | | |
| E | 25/04/90 | 4 | 29.1853 | | | 2.23276 | | |
| E | 25/04/90 | 5 | 29.8289 | | | 2.30581 | | |
| E | 25/04/90 | 6 | 29.6503 | | | 2.43680 | | |
| *Apium nodiflorum* (Water celery, roots) | | | | | | | | |
| E | 12/10/89 | 1 | 57.4557 | 56.7056 | 1.3315 | 7.92638 | 7.28115 | 0.74849 |
| E | 12/10/89 | 2 | 58.1328 | | | 7.55992 | | |
| E | 12/10/89 | 3 | 56.4100 | | | 6.85548 | | |
| E | 12/10/89 | 4 | 56.9126 | | | 6.18300 | | |
| E | 12/10/89 | 5 | 54.6170 | | | 7.88099 | | |
| E | 10/01/90 | 1 | 76.7767 | 74.1925 | 2.5387 | 11.58463 | 10.85150 | 0.86032 |
| E | 10/01/90 | 2 | 74.4110 | | | 11.02264 | | |
| E | 10/01/90 | 3 | 73.6990 | | | 11.09667 | | |
| E | 10/01/90 | 4 | 75.4923 | | | 11.35413 | | |
| E | 10/01/90 | 5 | 69.4679 | | | 9.17207 | | |
| E | 10/01/90 | 6 | 75.3079 | | | 10.87884 | | |
| E | 25/04/90 | 1 | 97.0525 | 94.4389 | 3.1461 | 16.06260 | 16.16569 | 0.63676 |
| E | 25/04/90 | 2 | 93.8979 | | | 16.59802 | | |
| E | 25/04/90 | 3 | 91.3242 | | | 15.90893 | | |
| E | 25/04/90 | 4 | 98.5283 | | | 16.92098 | | |
| E | 25/04/90 | 5 | 90.5384 | | | 15.09958 | | |
| E | 25/04/90 | 6 | 95.2923 | | | 16.40400 | | |

**(end)**

**APPENDIX D.5** Concentrations of copper and cadmium in samples of aquatic invertebrates taken during each season (1989-1990) from the three sampling sites (A, B and E) in the River Wandle.

Keys of sampling sites:
A: Waddon pond.
B: Croydon Arm beside Church Rd. at Beddington Park.
E: Junction of two Arms beside Hack Bridge.

| Site | Date | No | Total Dry Wt | Ind. No. | Ind. Dry Wt | COPPER Concentration (ug/g dry wt.) Data | Mean | S.D. | CADMIUM Concentration (ug/g dry wt.) Data | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Haemopsis sanguisuga* ( horse leech ) | | | | | | | | | | | |
| B | 21/07/89 | 1 | 0.8397 | 1 | 0.83970 | 88.4313 | 108.6088 | 16.7717 | 1.833479 | 1.287876 | 0.539772 |
| B | 21/07/89 | 2 | 0.3640 | 1 | 0.36400 | 105.2661 | | | 1.673178 | | |
| B | 21/07/89 | 3 | 0.4939 | 1 | 0.49390 | 133.9939 | | | 0.707714 | | |
| B | 21/07/89 | 4 | 0.3457 | 1 | 0.34570 | 102.1666 | | | 0.711769 | | |
| B | 21/07/89 | 5 | 0.1797 | 1 | 0.17970 | 113.1862 | | | 1.513238 | | |
| B | 12/10/89 | 1 | 0.2069 | 1 | 0.20690 | 86.5702 | 64.8103 | 18.8448 | 1.696599 | 1.283291 | 0.364683 |
| B | 12/10/89 | 2 | 0.1256 | 1 | 0.12560 | 53.8493 | | | 1.146469 | | |
| B | 12/10/89 | 3 | 0.0337 | 1 | 0.03370 | 54.0114 | | | 1.006806 | | |
| B | 10/01/90 | 1 | 0.0917 | 1 | 0.09170 | 59.4319 | | | 1.305246 | | |
| B | 25/04/90 | 1 | 0.4994 | 1 | 0.49940 | 73.4636 | 66.4085 | 18.2199 | 1.753717 | 1.340060 | 0.315129 |
| B | 25/04/90 | 2 | 0.3360 | 1 | 0.33600 | 68.1403 | | | 0.991505 | | |
| B | 25/04/90 | 3 | 0.2221 | 1 | 0.22210 | 42.9200 | | | 1.269297 | | |
| B | 25/04/90 | 4 | 0.4183 | 1 | 0.41830 | 78.3174 | | | 1.676797 | | |
| B | 25/04/90 | 5 | 0.3240 | 1 | 0.32400 | 92.0421 | | | 1.028228 | | |
| B | 25/04/90 | 6 | 0.2410 | 2 | 0.12050 | 74.3731 | | | 0.982096 | | |
| B | 25/04/90 | 7 | 0.2319 | 2 | 0.11595 | 64.8344 | | | 1.482978 | | |
| B | 25/04/90 | 8 | 0.1593 | 3 | 0.05310 | 37.1773 | | | 1.535861 | | |
| E | 25/04/90 | 1 | 0.4021 | 1 | 0.40210 | 32.1676 | 42.3038 | 14.3348 | 2.433551 | 1.652630 | 1.104389 |
| E | 25/04/90 | 2 | 0.2130 | 1 | 0.21300 | 52.4400 | | | 0.871709 | | |
| *Theromyzon tessulatum* | | | | | | | | | | | |
| A | 25/04/90 | 1 | 0.0012 | 1 | 0.00120 | 4.4050 | | | 0.025813 | | |
| E | 21/07/89 | 1 | 0.0154 | 1 | 0.01540 | 43.4720 | 47.1286 | 3.2587 | 3.436496 | 3.901754 | 0.822752 |
| E | 21/07/89 | 2 | 0.0133 | 2 | 0.00665 | 50.0106 | | | 4.401700 | | |
| E | 21/07/89 | 3 | 0.0086 | 2 | 0.00430 | 49.7388 | | | 4.768647 | | |
| E | 12/10/89 | 1 | 0.0170 | 1 | 0.01700 | 45.2932 | 37.6390 | 8.2165 | 3.000172 | 3.240565 | 0.447227 |
| E | 12/10/89 | 2 | 0.0187 | 1 | 0.01870 | 46.4844 | | | 3.326484 | | |
| E | 12/10/89 | 3 | 0.0205 | 1 | 0.02050 | 35.2756 | | | 3.111562 | | |
| E | 12/10/89 | 4 | 0.0119 | 1 | 0.01190 | 32.1692 | | | 3.964563 | | |
| E | 12/10/89 | 5 | 0.0112 | 1 | 0.01120 | 25.3160 | | | 2.800042 | | |
| E | 10/01/90 | 1 | 0.0247 | 1 | 0.02470 | 41.2954 | 36.4332 | 6.8763 | 5.131161 | 4.774516 | 0.504372 |
| E | 10/01/90 | 2 | 0.0121 | 1 | 0.01210 | 31.5709 | | | 4.417872 | | |
| E | 25/04/90 | 1 | 0.0178 | 1 | 0.01780 | 41.6253 | | | 6.547314 | | |
| *Erpobdella octoculata* | | | | | | | | | | | |
| E | 21/07/89 | 1 | 0.0065 | 2 | 0.00325 | 53.9116 | 44.7930 | 7.4347 | 0.965986 | 0.763978 | 0.200670 |
| E | 21/07/89 | 2 | 0.0197 | 5 | 0.00394 | 40.1002 | | | 0.941345 | | |
| E | 21/07/89 | 3 | 0.0320 | 1 | 0.03200 | 51.7566 | | | 0.744706 | | |
| E | 21/07/89 | 4 | 0.0177 | 2 | 0.00885 | 37.9631 | | | 0.475301 | | |
| E | 21/07/89 | 5 | 0.0166 | 2 | 0.00830 | 40.2336 | | | 0.692553 | | |
| E | 12/10/89 | 1 | 0.0134 | 1 | 0.01340 | 68.5169 | 64.0622 | 6.1087 | 2.532423 | 2.075274 | 0.256592 |
| E | 12/10/89 | 2 | 0.0145 | 1 | 0.01450 | 57.8949 | | | 1.883275 | | |
| E | 12/10/89 | 3 | 0.0097 | 1 | 0.00970 | 59.9343 | | | 1.971261 | | |
| E | 12/10/89 | 4 | 0.0133 | 2 | 0.00665 | 58.6399 | | | 1.994575 | | |
| E | 12/10/89 | 5 | 0.0126 | 2 | 0.00630 | 66.6250 | | | 1.857879 | | |
| E | 12/10/89 | 6 | 0.0099 | 2 | 0.00495 | 72.7623 | | | 2.212230 | | |

(continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 10/01/90 | 1 | 0.0238 | 1 | 0.02380 | 67.0520 | 59.5915 | 8.6107 | 1.232512 | 1.336065 | 0.112600 |
| E | 10/01/90 | 2 | 0.0164 | 1 | 0.01640 | 56.2651 | | | 1.268393 | | |
| E | 10/01/90 | 3 | 0.0076 | 1 | 0.00760 | 48.9465 | | | 1.358138 | | |
| E | 10/01/90 | 4 | 0.0101 | 2 | 0.00505 | 66.1024 | | | 1.485216 | | |
| E | 25/04/90 | 1 | 0.1167 | 7 | 0.01667 | 78.8064 | 67.7613 | 7.1914 | 1.402111 | 1.214647 | 0.169110 |
| E | 25/04/90 | 2 | 0.1492 | 8 | 0.01865 | 61.6401 | | | 1.238015 | | |
| E | 25/04/90 | 3 | 0.1432 | 8 | 0.01790 | 70.3790 | | | 1.267234 | | |
| E | 25/04/90 | 4 | 0.1286 | 9 | 0.01429 | 71.3286 | | | 0.963362 | | |
| E | 25/04/90 | 5 | 0.1430 | 9 | 0.01589 | 65.1933 | | | 1.064844 | | |
| E | 25/04/90 | 6 | 0.1156 | 10 | 0.01156 | 59.2205 | | | 1.352315 | | |
| *Lymnaea peregra* ( pond snail ) | | | | | | | | | | | |
| A | 21/07/89 | 1 | 0.0711 | 3 | 0.02370 | 85.1927 | 84.6188 | 4.3409 | 0.553993 | 0.647586 | 0.143893 |
| A | 21/07/89 | 2 | 0.0682 | 3 | 0.02273 | 88.6442 | | | 0.575489 | | |
| A | 21/07/89 | 3 | 0.0813 | 5 | 0.01626 | 80.0195 | | | 0.813275 | | |
| A | 12/10/89 | 1 | 0.1276 | 5 | 0.02552 | 52.4356 | 49.9928 | 2.6335 | 0.520326 | 0.581608 | 0.045231 |
| A | 12/10/89 | 2 | 0.1051 | 5 | 0.02102 | 52.1054 | | | 0.578236 | | |
| A | 12/10/89 | 3 | 0.0978 | 10 | 0.00978 | 47.7126 | | | 0.625779 | | |
| A | 12/10/89 | 4 | 0.0844 | 12 | 0.00703 | 47.7177 | | | 0.602091 | | |
| A | 10/01/90 | 1 | 0.0279 | 1 | 0.02790 | 49.7616 | 51.4236 | 4.8767 | 1.193790 | 1.351249 | 0.341855 |
| A | 10/01/90 | 2 | 0.0326 | 1 | 0.03260 | 49.5525 | | | 1.146460 | | |
| A | 10/01/90 | 3 | 0.0261 | 1 | 0.02610 | 47.3299 | | | 0.997044 | | |
| A | 10/01/90 | 4 | 0.0186 | 1 | 0.01860 | 59.8751 | | | 1.805951 | | |
| A | 10/01/90 | 5 | 0.0162 | 3 | 0.00540 | 50.5990 | | | 1.613000 | | |
| A | 25/04/90 | 1 | 0.1631 | 3 | 0.05437 | 33.3447 | 30.7205 | 3.9254 | 0.205872 | 0.272433 | 0.062026 |
| A | 25/04/90 | 2 | 0.1640 | 4 | 0.04100 | 25.5811 | | | 0.313236 | | |
| A | 25/04/90 | 3 | 0.1670 | 4 | 0.04175 | 31.3253 | | | 0.203007 | | |
| A | 25/04/90 | 4 | 0.1529 | 4 | 0.03822 | 35.2981 | | | 0.363556 | | |
| A | 25/04/90 | 5 | 0.1750 | 5 | 0.03500 | 32.4583 | | | 0.272322 | | |
| A | 25/04/90 | 6 | 0.1547 | 8 | 0.01934 | 26.3154 | | | 0.276603 | | |
| B | 21/07/89 | 1 | 0.1075 | 9 | 0.01194 | 368.5917 | 344.7122 | 17.1806 | 2.529571 | 2.067828 | 0.414038 |
| B | 21/07/89 | 2 | 0.1334 | 12 | 0.01112 | 339.1683 | | | 2.038448 | | |
| B | 21/07/89 | 3 | 0.1193 | 13 | 0.00918 | 341.4894 | | | 1.845669 | | |
| B | 21/07/89 | 4 | 0.1453 | 29 | 0.00501 | 322.0702 | | | 1.515405 | | |
| B | 21/07/89 | 5 | 0.1343 | 23 | 0.00584 | 352.2411 | | | 2.410048 | | |
| B | 12/10/89 | 1 | 0.0539 | 4 | 0.01347 | 219.9157 | 228.7612 | 13.1062 | 3.996621 | 3.584756 | 0.356952 |
| B | 12/10/89 | 2 | 0.0577 | 4 | 0.01442 | 222.5496 | | | 3.392617 | | |
| B | 12/10/89 | 3 | 0.0633 | 7 | 0.00904 | 243.8184 | | | 3.365031 | | |
| B | 10/01/90 | 1 | 0.2605 | 10 | 0.02605 | 225.1131 | 221.2725 | 17.3507 | 3.016177 | 3.113152 | 0.268036 |
| B | 10/01/90 | 2 | 0.1951 | 5 | 0.03902 | 230.6400 | | | 2.839846 | | |
| B | 10/01/90 | 3 | 0.2131 | 5 | 0.04262 | 260.6817 | | | 2.871746 | | |
| B | 10/01/90 | 4 | 0.1898 | 5 | 0.03796 | 231.5201 | | | 2.766578 | | |
| B | 10/01/90 | 5 | 0.1839 | 8 | 0.02299 | 217.8759 | | | 3.642654 | | |
| B | 10/01/90 | 6 | 0.2085 | 17 | 0.01226 | 210.7555 | | | 3.351758 | | |
| B | 10/01/90 | 7 | 0.2163 | 17 | 0.01272 | 212.9135 | | | 3.230891 | | |
| B | 10/01/90 | 8 | 0.1980 | 10 | 0.01980 | 216.6019 | | | 3.237003 | | |
| B | 10/01/90 | 9 | 0.2017 | 11 | 0.01834 | 210.0124 | | | 3.177623 | | |
| B | 10/01/90 | 10 | 0.2235 | 8 | 0.02794 | 196.6108 | | | 2.997244 | | |
| B | 10/01/90 | 11 | 0.1731 | 4 | 0.04328 | 171.5506 | 153.1689 | 15.9387 | 1.360607 | 1.597594 | 0.405639 |
| B | 10/01/90 | 12 | 0.1300 | 4 | 0.03250 | 143.1871 | | | 1.366201 | | |
| B | 10/01/90 | 13 | 0.1140 | 5 | 0.02280 | 144.7688 | | | 2.065975 | | |
| B | 25/04/90 | 1 | 0.1976 | 3 | 0.06587 | 468.7151 | 383.3056 | 59.0451 | 2.675492 | 2.483705 | 0.384755 |
| B | 25/04/90 | 2 | 0.1908 | 3 | 0.06360 | 468.7903 | | | 2.055213 | | |
| B | 25/04/90 | 3 | 0.2118 | 5 | 0.04236 | 423.6443 | | | 2.129950 | | |
| B | 25/04/90 | 4 | 0.2209 | 6 | 0.03682 | 340.7724 | | | 2.584734 | | |
| B | 25/04/90 | 5 | 0.2267 | 6 | 0.03778 | 404.9066 | | | 3.116240 | | |
| B | 25/04/90 | 6 | 0.1889 | 6 | 0.03148 | 383.1995 | | | 3.022593 | | |
| B | 25/04/90 | 7 | 0.1906 | 6 | 0.03177 | 384.1142 | | | 2.640220 | | |
| B | 25/04/90 | 8 | 0.1715 | 6 | 0.02858 | 320.9615 | | | 2.286499 | | |
| B | 25/04/90 | 9 | 0.1324 | 6 | 0.02207 | 337.7839 | | | 2.070673 | | |
| B | 25/04/90 | 10 | 0.0954 | 7 | 0.01363 | 300.1678 | | | 2.255433 | | |

(continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 21/07/89 | 1 | 0.2186 | 5 | 0.04372 | 214.0750 | 150.5435 | 26.5718 | 1.125611 | 1.352401 | 0.334685 |
| E | 21/07/89 | 2 | 0.3622 | 10 | 0.03622 | 155.9586 | | | 1.470801 | | |
| E | 21/07/89 | 3 | 0.3326 | 10 | 0.03326 | 151.6268 | | | 1.673187 | | |
| E | 21/07/89 | 4 | 0.4190 | 15 | 0.02793 | 128.0705 | | | 1.698624 | | |
| E | 21/07/89 | 5 | 0.4013 | 15 | 0.02675 | 133.7192 | | | 1.515680 | | |
| E | 21/07/89 | 6 | 0.3249 | 15 | 0.02166 | 126.6343 | | | 1.394339 | | |
| E | 21/07/89 | 7 | 0.3309 | 20 | 0.01655 | 148.7448 | | | 1.603601 | | |
| E | 21/07/89 | 8 | 0.2131 | 22 | 0.00969 | 158.1837 | | | 0.869510 | | |
| E | 21/07/89 | 9 | 0.2369 | 22 | 0.01077 | 137.8790 | | | 0.820254 | | |
| E | 12/10/89 | 1 | 0.1622 | 6 | 0.02703 | 645.8595 | 624.5126 | 28.8448 | 1.525964 | 1.846921 | 0.226833 |
| E | 12/10/89 | 2 | 0.1653 | 6 | 0.02755 | 613.9325 | | | 1.497347 | | |
| E | 12/10/89 | 3 | 0.1737 | 7 | 0.02481 | 598.4582 | | | 1.822230 | | |
| E | 12/10/89 | 4 | 0.1756 | 10 | 0.01756 | 602.5287 | | | 2.195508 | | |
| E | 12/10/89 | 5 | 0.1692 | 10 | 0.01692 | 654.5054 | | | 1.870694 | | |
| E | 12/10/89 | 6 | 0.1809 | 10 | 0.01809 | 663.3586 | | | 1.940444 | | |
| E | 12/10/89 | 7 | 0.2007 | 14 | 0.01434 | 630.7624 | | | 2.092856 | | |
| E | 12/10/89 | 8 | 0.2050 | 14 | 0.01464 | 630.5406 | | | 1.712324 | | |
| E | 12/10/89 | 9 | 0.2233 | 25 | 0.00893 | 636.5994 | | | 1.803779 | | |
| E | 12/10/89 | 10 | 0.1834 | 24 | 0.00764 | 568.5807 | | | 2.008063 | | |
| E | 10/01/90 | 1 | 0.2370 | 4 | 0.05925 | 194.3173 | 188.7169 | 22.3973 | 1.971229 | 1.924693 | 0.233408 |
| E | 10/01/90 | 2 | 0.2347 | 4 | 0.05868 | 160.2491 | | | 1.620404 | | |
| E | 10/01/90 | 3 | 0.2540 | 5 | 0.05080 | 164.6923 | | | 1.953303 | | |
| E | 10/01/90 | 4 | 0.2068 | 5 | 0.04136 | 164.0076 | | | 1.853256 | | |
| E | 10/01/90 | 5 | 0.2579 | 5 | 0.05158 | 209.2604 | | | 2.148329 | | |
| E | 10/01/90 | 6 | 0.2203 | 6 | 0.03672 | 197.0715 | | | 1.463430 | | |
| E | 10/01/90 | 7 | 0.2397 | 7 | 0.03424 | 174.5174 | | | 1.949025 | | |
| E | 10/01/90 | 8 | 0.2328 | 9 | 0.02587 | 218.2226 | | | 2.006793 | | |
| E | 10/01/90 | 9 | 0.2449 | 10 | 0.02449 | 185.8943 | | | 2.025884 | | |
| E | 10/01/90 | 10 | 0.2200 | 14 | 0.01571 | 218.9367 | | | 2.255279 | | |
| E | 25/04/90 | 1 | 0.2394 | 2 | 0.11970 | 198.8840 | 221.6556 | 23.1255 | 1.268385 | 1.383862 | 0.228859 |
| E | 25/04/90 | 2 | 0.4000 | 5 | 0.08000 | 197.4816 | | | 1.054072 | | |
| E | 25/04/90 | 3 | 0.4178 | 5 | 0.08356 | 205.8684 | | | 1.447675 | | |
| E | 25/04/90 | 4 | 0.3898 | 5 | 0.07796 | 217.1412 | | | 1.549182 | | |
| E | 25/04/90 | 5 | 0.3265 | 6 | 0.05442 | 231.8209 | | | 1.645013 | | |
| E | 25/04/90 | 6 | 0.3434 | 6 | 0.05723 | 256.4831 | | | 1.761324 | | |
| E | 25/04/90 | 7 | 0.3283 | 6 | 0.05472 | 209.3279 | | | 1.342140 | | |
| E | 25/04/90 | 8 | 0.2705 | 6 | 0.04508 | 201.9668 | | | 1.449666 | | |
| E | 25/04/90 | 9 | 0.2418 | 6 | 0.04030 | 241.3070 | | | 1.133818 | | |
| E | 25/04/90 | 10 | 0.2309 | 6 | 0.03848 | 256.2747 | | | 1.187341 | | |
| *Lymnaea stagnalis* (great pond snail) | | | | | | | | | | | |
| E | 10/01/90 | 1 | 0.1111 | 1 | 0.11110 | 123.0336 | 113.1509 | 6.9278 | 1.863107 | 2.059552 | 0.211731 |
| E | 10/01/90 | 2 | 0.1684 | 3 | 0.05613 | 108.1901 | | | 2.258366 | | |
| E | 10/01/90 | 3 | 0.1399 | 4 | 0.03498 | 112.8802 | | | 1.890484 | | |
| E | 10/01/90 | 4 | 0.1188 | 7 | 0.01697 | 108.4996 | | | 2.226251 | | |
| E | 25/04/90 | 1 | 0.1455 | 1 | 0.14550 | 116.2799 | 145.4285 | 23.9478 | 1.478820 | 1.210777 | 0.180725 |
| E | 25/04/90 | 2 | 0.1741 | 1 | 0.17410 | 163.9740 | | | 1.151184 | | |
| E | 25/04/90 | 3 | 0.1710 | 1 | 0.17100 | 135.3889 | | | 1.085812 | | |
| E | 25/04/90 | 4 | 0.2432 | 2 | 0.12160 | 166.0710 | | | 1.127291 | | |
| *Physa fontinalis* | | | | | | | | | | | |
| B | 21/07/89 | 1 | 0.0398 | 8 | 0.00497 | 2048.3483 | | | 6.182378 | | |
| B | 12/10/89 | 1 | 0.0761 | 10 | 0.00761 | 1495.7783 | 1515.9445 | 55.7344 | 3.025742 | 3.024788 | 0.402377 |
| B | 12/10/89 | 2 | 0.0823 | 10 | 0.00823 | 1473.1001 | | | 3.426688 | | |
| B | 12/10/89 | 3 | 0.0615 | 10 | 0.00615 | 1578.9551 | | | 2.621936 | | |
| B | 10/01/90 | 1 | 0.0343 | 5 | 0.00686 | 1760.3758 | | | 3.237569 | | |
| B | 25/04/90 | 1 | 0.0205 | 1 | 0.02050 | 1063.9535 | 1106.1296 | 50.9742 | 5.669001 | 5.527921 | 0.573427 |
| B | 25/04/90 | 2 | 0.0192 | 1 | 0.01920 | 1141.7100 | | | 4.681018 | | |
| B | 25/04/90 | 3 | 0.0222 | 2 | 0.01110 | 1158.0513 | | | 5.891175 | | |
| B | 25/04/90 | 4 | 0.0207 | 3 | 0.00690 | 1060.8036 | | | 5.870491 | | |
| E | 21/07/89 | 1 | 0.0357 | 4 | 0.00892 | 700.0333 | | | 3.269110 | | |
| E | 12/10/89 | 1 | 0.0881 | 12 | 0.00734 | 1369.1130 | | | 1.242810 | | |
| E | 10/01/90 | 1 | 0.0652 | 5 | 0.01304 | 439.2649 | 422.7788 | 23.3148 | 4.634915 | 4.025477 | 0.861875 |
| E | 10/01/90 | 2 | 0.0614 | 5 | 0.01228 | 406.2928 | | | 3.416039 | | |

(continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pisidium spp.* ( pea mussels ) | | | | | | | | | | | | |
| A | 21/07/89 | 1 | 0.0169 | 3 | 0.00563 | 19.6346 | 17.1218 | 2.5009 | 0.241762 | 0.200442 | 0.051427 |
| A | 21/07/89 | 2 | 0.0151 | 3 | 0.00503 | 17.5957 | | | 0.270582 | | |
| A | 21/07/89 | 3 | 0.0314 | 5 | 0.00628 | 14.6596 | | | 0.170666 | | |
| A | 12/10/89 | 1 | 0.0237 | 2 | 0.01185 | 16.5974 | 17.4483 | 1.6069 | 0.118760 | 0.105606 | 0.012097 |
| A | 12/10/89 | 2 | 0.0108 | 2 | 0.00540 | 16.4225 | | | 0.110088 | | |
| A | 12/10/89 | 3 | 0.0110 | 2 | 0.00550 | 16.8459 | | | 0.096607 | | |
| A | 12/10/89 | 4 | 0.0134 | 3 | 0.00447 | 17.0885 | | | 0.089583 | | |
| A | 12/10/89 | 5 | 0.0107 | 4 | 0.00268 | 20.2872 | | | 0.112993 | | |
| A | 10/01/90 | 1 | 0.0026 | 2 | 0.00130 | 32.1786 | | | 3.823544 | | |
| A | 25/04/90 | 1 | 0.0022 | 1 | 0.00220 | 33.8082 | 32.0962 | 1.4955 | 0.241772 | 0.205245 | 0.055432 |
| A | 25/04/90 | 2 | 0.0018 | 2 | 0.00090 | 31.4358 | | | 0.141461 | | |
| A | 25/04/90 | 3 | 0.0045 | 4 | 0.00113 | 31.0445 | | | 0.232502 | | |
| B | 25/04/90 | 1 | 0.0016 | 1 | 0.00160 | 175.4839 | 179.1756 | 14.1042 | 4.703051 | 4.058249 | 0.754336 |
| B | 25/04/90 | 2 | 0.0026 | 2 | 0.00130 | 194.7586 | | | 3.228704 | | |
| B | 25/04/90 | 3 | 0.0021 | 3 | 0.00070 | 167.2844 | | | 4.242991 | | |
| *Gammarus pulex* (freshwater shrimp) | | | | | | | | | | | |
| B | 25/04/90 | 1 | 0.0324 | 1 | 0.03240 | 85.0481 | 88.9665 | 6.0228 | 1.881719 | 1.680490 | 0.188044 |
| B | 25/04/90 | 2 | 0.0207 | 1 | 0.02070 | 85.9500 | | | 1.509230 | | |
| B | 25/04/90 | 3 | 0.0174 | 1 | 0.01740 | 95.9015 | | | 1.650520 | | |
| E | 21/07/89 | 1 | 0.2594 | 15 | 0.01729 | 90.1233 | 87.0112 | 3.1773 | 1.382024 | 1.495938 | 0.284873 |
| E | 21/07/89 | 2 | 0.2299 | 16 | 0.01437 | 91.1401 | | | 1.708443 | | |
| E | 21/07/89 | 3 | 0.2302 | 16 | 0.01439 | 84.0047 | | | 1.209296 | | |
| E | 21/07/89 | 4 | 0.2442 | 25 | 0.00977 | 84.1495 | | | 1.959459 | | |
| E | 21/07/89 | 5 | 0.2332 | 25 | 0.00933 | 84.6556 | | | 1.437796 | | |
| E | 21/07/89 | 6 | 0.2886 | 40 | 0.00721 | 87.9938 | | | 1.278609 | | |
| E | 12/10/89 | 1 | 0.2443 | 20 | 0.01221 | 79.0937 | 80.8396 | 2.4256 | 0.660045 | 0.669599 | 0.026108 |
| E | 12/10/89 | 2 | 0.2393 | 22 | 0.01088 | 81.7781 | | | 0.673836 | | |
| E | 12/10/89 | 3 | 0.2347 | 20 | 0.01173 | 77.3317 | | | 0.687043 | | |
| E | 12/10/89 | 4 | 0.2286 | 22 | 0.01039 | 80.4754 | | | 0.705376 | | |
| E | 12/10/89 | 5 | 0.2290 | 20 | 0.01145 | 82.1537 | | | 0.628806 | | |
| E | 12/10/89 | 6 | 0.2434 | 20 | 0.01217 | 84.2047 | | | 0.662486 | | |
| E | 10/01/90 | 1 | 0.3519 | 22 | 0.01600 | 84.3859 | 81.6689 | 3.2180 | 0.998441 | 1.207947 | 0.211266 |
| E | 10/01/90 | 2 | 0.3557 | 25 | 0.01423 | 79.0340 | | | 1.069184 | | |
| E | 10/01/90 | 3 | 0.3675 | 30 | 0.01225 | 77.9322 | | | 1.113650 | | |
| E | 10/01/90 | 4 | 0.3745 | 35 | 0.01070 | 84.9295 | | | 1.092834 | | |
| E | 10/01/90 | 5 | 0.3807 | 45 | 0.00846 | 76.6161 | | | 1.184194 | | |
| E | 10/01/90 | 6 | 0.3702 | 60 | 0.00617 | 78.7891 | | | 1.027306 | | |
| E | 10/01/90 | 7 | 0.3671 | 24 | 0.01530 | 80.8919 | | | 1.509273 | | |
| E | 10/01/90 | 8 | 0.3493 | 33 | 0.01058 | 82.3253 | | | 1.586184 | | |
| E | 10/01/90 | 9 | 0.3853 | 46 | 0.00838 | 82.5489 | | | 1.287669 | | |
| E | 10/01/90 | 10 | 0.3803 | 56 | 0.00679 | 85.0217 | | | 1.000023 | | |
| E | 10/01/90 | 11 | 0.3089 | 62 | 0.00498 | 85.8834 | | | 1.418659 | | |
| E | 25/04/90 | 1 | 0.4656 | 32 | 0.01455 | 71.7667 | 66.6113 | 5.3567 | 0.968908 | 0.990969 | 0.087826 |
| E | 25/04/90 | 2 | 0.3770 | 25 | 0.01508 | 76.9196 | | | 1.118379 | | |
| E | 25/04/90 | 3 | 0.4062 | 28 | 0.01451 | 67.6865 | | | 1.037984 | | |
| E | 25/04/90 | 4 | 0.4831 | 37 | 0.01306 | 63.0396 | | | 0.933810 | | |
| E | 25/04/90 | 5 | 0.5297 | 38 | 0.01394 | 63.4630 | | | 0.907340 | | |
| E | 25/04/90 | 6 | 0.4692 | 45 | 0.01043 | 59.1204 | | | 1.087196 | | |
| E | 25/04/90 | 7 | 0.4402 | 37 | 0.01190 | 62.0616 | | | 0.957812 | | |
| E | 25/04/90 | 8 | 0.3930 | 40 | 0.00983 | 65.2426 | | | 0.922749 | | |
| E | 25/04/90 | 9 | 0.3833 | 33 | 0.01162 | 71.2745 | | | 1.099997 | | |
| E | 25/04/90 | 10 | 0.4142 | 42 | 0.00986 | 65.5383 | | | 0.875520 | | |
| *Crangonyx pseudogracilis* (freshwater shrimp) | | | | | | | | | | | |
| A | 21/07/89 | 1 | 0.0054 | 5 | 0.00108 | 94.2873 | | | 0.169283 | | |
| A | 25/04/90 | 1 | 0.0031 | 2 | 0.00155 | 27.4068 | 22.9239 | 5.0863 | 0.332573 | 0.300603 | 0.040357 |
| A | 25/04/90 | 2 | 0.0037 | 3 | 0.00123 | 26.9563 | | | 0.314287 | | |
| A | 25/04/90 | 3 | 0.0040 | 3 | 0.00133 | 17.0850 | | | 0.314069 | | |
| A | 25/04/90 | 4 | 0.0032 | 7 | 0.00046 | 20.2477 | | | 0.241482 | | |

(continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 21/07/89 | 1 | 0.0105 | 3 | 0.00350 | 94.2524 | 98.5073 | 5.9602 | 1.636324 | 1.774848 | 0.132909 |
| E | 21/07/89 | 2 | 0.0079 | 4 | 0.00198 | 105.3194 | | | 1.901320 | | |
| E | 21/07/89 | 3 | 0.0073 | 4 | 0.00183 | 95.9502 | | | 1.786899 | | |
| *Asellus aquaticus* (water slaters, hog–lice) | | | | | | | | | | | |
| A | 21/07/89 | 1 | 0.0070 | 1 | 0.00700 | 302.0361 | 241.3766 | 61.5462 | 0.885745 | 0.845251 | 0.173594 |
| A | 21/07/89 | 2 | 0.0062 | 2 | 0.00310 | 243.1133 | | | 0.654990 | | |
| A | 21/07/89 | 3 | 0.0057 | 4 | 0.00143 | 178.9805 | | | 0.995019 | | |
| A | 12/10/89 | 1 | 0.0054 | 1 | 0.00540 | 114.5542 | 103.0377 | 17.7627 | 0.126929 | 0.106720 | 0.011943 |
| A | 12/10/89 | 2 | 0.0049 | 1 | 0.00490 | 107.1987 | | | 0.097483 | | |
| A | 12/10/89 | 3 | 0.0037 | 1 | 0.00370 | 123.2493 | | | 0.106126 | | |
| A | 12/10/89 | 4 | 0.0052 | 2 | 0.00260 | 90.5519 | | | 0.104896 | | |
| A | 12/10/89 | 5 | 0.0040 | 3 | 0.00133 | 79.6345 | | | 0.098167 | | |
| A | 10/01/90 | 1 | 0.0075 | 1 | 0.00750 | 83.8714 | 74.1535 | 14.6624 | 0.583449 | 0.504229 | 0.147986 |
| A | 10/01/90 | 2 | 0.0132 | 1 | 0.01320 | 67.8884 | | | 0.681858 | | |
| A | 10/01/90 | 3 | 0.0103 | 1 | 0.01030 | 52.1587 | | | 0.317938 | | |
| A | 10/01/90 | 4 | 0.0120 | 2 | 0.00600 | 89.3648 | | | 0.548257 | | |
| A | 10/01/90 | 5 | 0.0101 | 3 | 0.00337 | 77.4844 | | | 0.389646 | | |
| A | 28/02/90 | 1 | 0.0095 | 1 | 0.00950 | 190.1204 | 164.5769 | 22.6938 | 0.947423 | 0.693338 | 0.153471 |
| A | 28/02/90 | 2 | 0.0114 | 1 | 0.01140 | 156.8913 | | | 0.654296 | | |
| A | 28/02/90 | 3 | 0.0104 | 1 | 0.01040 | 141.5553 | | | 0.658495 | | |
| A | 28/02/90 | 4 | 0.0149 | 1 | 0.01490 | 147.0944 | | | 0.530162 | | |
| A | 28/02/90 | 5 | 0.0221 | 3 | 0.00737 | 187.2232 | | | 0.676313 | | |
| A | 25/04/90 | 1 | 0.0095 | 1 | 0.00950 | 80.3442 | 70.6342 | 15.3968 | 0.169255 | 0.181188 | 0.024354 |
| A | 25/04/90 | 2 | 0.0066 | 1 | 0.00660 | 65.9941 | | | 0.192188 | | |
| A | 25/04/90 | 3 | 0.0032 | 2 | 0.00160 | 85.1656 | | | 0.209183 | | |
| A | 25/04/90 | 4 | 0.0024 | 6 | 0.00040 | 51.0329 | | | 0.154127 | | |
| B | 10/01/90 | 1 | 0.0102 | 1 | 0.01020 | 672.0280 | 455.2227 | 169.7231 | 3.525062 | 4.722204 | 1.550301 |
| B | 10/01/90 | 2 | 0.0042 | 1 | 0.00420 | 508.7508 | | | 6.421152 | | |
| B | 10/01/90 | 3 | 0.0056 | 1 | 0.00560 | 318.0549 | | | 3.295540 | | |
| B | 10/01/90 | 4 | 0.0041 | 1 | 0.00410 | 322.0573 | | | 5.647060 | | |
| B | 25/04/90 | 1 | 0.0087 | 1 | 0.00870 | 602.1846 | 567.1811 | 146.6485 | 6.954961 | 5.638781 | 1.510778 |
| B | 25/04/90 | 2 | 0.0070 | 1 | 0.00700 | 406.1982 | | | 3.989125 | | |
| B | 25/04/90 | 3 | 0.0086 | 3 | 0.00287 | 693.1606 | | | 5.972257 | | |
| E | 21/07/89 | 1 | 0.0062 | 2 | 0.00310 | 470.0837 | 583.5975 | 100.7128 | 1.072708 | 1.492835 | 0.387652 |
| E | 21/07/89 | 2 | 0.0048 | 2 | 0.00240 | 618.4672 | | | 1.569127 | | |
| E | 21/07/89 | 3 | 0.0053 | 3 | 0.00177 | 662.2415 | | | 1.836669 | | |
| E | 12/10/89 | 1 | 0.0070 | 1 | 0.00700 | 336.6231 | 566.9838 | 97.6744 | 1.756409 | 1.639447 | 0.564148 |
| E | 12/10/89 | 2 | 0.0056 | 1 | 0.00560 | 669.4836 | | | 1.813452 | | |
| E | 12/10/89 | 3 | 0.0037 | 1 | 0.00370 | 707.4329 | | | 0.742897 | | |
| E | 12/10/89 | 4 | 0.0056 | 1 | 0.00560 | 475.1830 | | | 0.560454 | | |
| E | 12/10/89 | 5 | 0.0041 | 1 | 0.00410 | 426.1062 | | | 2.286754 | | |
| E | 12/10/89 | 6 | 0.0067 | 2 | 0.00335 | 546.5763 | | | 2.155727 | | |
| E | 12/10/89 | 7 | 0.0057 | 2 | 0.00285 | 550.8389 | | | 1.576468 | | |
| E | 12/10/89 | 8 | 0.0083 | 2 | 0.00415 | 621.6542 | | | 1.974998 | | |
| E | 12/10/89 | 9 | 0.0085 | 3 | 0.00283 | 599.9865 | | | 1.332329 | | |
| E | 12/10/89 | 10 | 0.0089 | 3 | 0.00297 | 640.2619 | | | 1.666651 | | |
| E | 12/10/89 | 11 | 0.0100 | 3 | 0.00333 | 611.7243 | | | 1.054516 | | |
| E | 12/10/89 | 12 | 0.0086 | 4 | 0.00215 | 579.0926 | | | 1.271509 | | |
| E | 12/10/89 | 13 | 0.0092 | 4 | 0.00230 | 528.3160 | | | 2.036023 | | |
| E | 12/10/89 | 14 | 0.0074 | 4 | 0.00185 | 656.8253 | | | 2.583951 | | |
| E | 12/10/89 | 15 | 0.0079 | 8 | 0.00099 | 554.6520 | | | 1.779563 | | |
| E | 10/01/90 | 1 | 0.0159 | 1 | 0.01590 | 240.2676 | 317.3136 | 64.6906 | 3.808245 | 2.055241 | 0.818834 |
| E | 10/01/90 | 2 | 0.0188 | 1 | 0.01880 | 186.4253 | | | 1.585467 | | |
| E | 10/01/90 | 3 | 0.0086 | 1 | 0.00860 | 262.5552 | | | 2.800465 | | |
| E | 10/01/90 | 4 | 0.0078 | 1 | 0.00780 | 360.7418 | | | 1.351548 | | |
| E | 10/01/90 | 5 | 0.0049 | 1 | 0.00490 | 344.3146 | | | 1.477295 | | |
| E | 10/01/90 | 6 | 0.0118 | 2 | 0.00590 | 364.4879 | | | 2.436928 | | |
| E | 10/01/90 | 7 | 0.0109 | 2 | 0.00545 | 322.9190 | | | 1.519547 | | |
| E | 10/01/90 | 8 | 0.0142 | 3 | 0.00473 | 360.0100 | | | 2.590810 | | |
| E | 10/01/90 | 9 | 0.0105 | 3 | 0.00350 | 355.2499 | | | 1.487342 | | |
| E | 10/01/90 | 10 | 0.0076 | 5 | 0.00152 | 376.1647 | | | 1.494761 | | |

(continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 25/04/90 | 1 | 0.0038 | 1 | 0.00380 | 286.0065 | 273.3199 | 18.7603 | 2.013911 | 2.834802 | 0.919652 |
| E | 25/04/90 | 2 | 0.0096 | 1 | 0.00960 | 267.8895 | | | 2.946354 | | |
| E | 25/04/90 | 3 | 0.0095 | 1 | 0.00950 | 276.8763 | | | 1.935263 | | |
| E | 25/04/90 | 4 | 0.0060 | 1 | 0.00600 | 269.6754 | | | 3.864167 | | |
| E | 25/04/90 | 5 | 0.0084 | 1 | 0.00840 | 297.2829 | | | 3.992262 | | |
| E | 25/04/90 | 6 | 0.0124 | 3 | 0.00413 | 242.1888 | | | 2.256855 | | |
| *Chironomid larvae and pupae* (blood worm) | | | | | | | | | | | |
| A | 21/07/89 | 1 | 0.0144 | 5 | 0.00288 | 28.7031 | 30.4437 | 2.1467 | 0.393862 | 0.464596 | 0.092741 |
| A | 21/07/89 | 2 | 0.0139 | 7 | 0.00199 | 30.4808 | | | 0.534794 | | |
| A | 21/07/89 | 3 | 0.0196 | 10 | 0.00196 | 28.0536 | | | 0.577046 | | |
| A | 21/07/89 | 4 | 0.0140 | 12 | 0.00117 | 33.7516 | | | 0.342185 | | |
| A | 21/07/89 | 5 | 0.0161 | 12 | 0.00134 | 29.6020 | | | 0.417939 | | |
| A | 21/07/89 | 6 | 0.0183 | 14 | 0.00131 | 32.0708 | | | 0.521753 | | |
| A | 12/10/89 | 1 | 0.0487 | 20 | 0.00244 | 18.2924 | 18.9184 | 2.3011 | 0.510362 | 0.662489 | 0.115915 |
| A | 12/10/89 | 2 | 0.0456 | 27 | 0.00169 | 22.0835 | | | 0.707726 | | |
| A | 12/10/89 | 3 | 0.0383 | 32 | 0.00120 | 18.7099 | | | 0.784517 | | |
| A | 12/10/89 | 4 | 0.0432 | 20 | 0.00216 | 16.5877 | | | 0.647353 | | |
| A | 12/10/89 | 5 | 0.0491 | 30 | 0.00164 | 19.3264 | | | 0.732813 | | |
| A | 12/10/89 | 6 | 0.0350 | 25 | 0.00140 | 15.4954 | | | 0.582972 | | |
| A | 10/01/90 | 1 | 0.1706 | 30 | 0.00569 | 15.8599 | 16.1125 | 1.2980 | 0.394231 | 0.440561 | 0.113964 |
| A | 10/01/90 | 2 | 0.1813 | 35 | 0.00518 | 17.5433 | | | 0.433588 | | |
| A | 10/01/90 | 3 | 0.1723 | 45 | 0.00383 | 17.2347 | | | 0.390341 | | |
| A | 10/01/90 | 4 | 0.1647 | 60 | 0.00274 | 16.1077 | | | 0.293033 | | |
| A | 10/01/90 | 5 | 0.1647 | 75 | 0.00220 | 13.8650 | | | 0.625008 | | |
| A | 10/01/90 | 6 | 0.1323 | 130 | 0.00102 | 16.0640 | | | 0.507166 | | |
| A | 25/04/90 | 1 | 0.1551 | | | 12.7423 | 13.5917 | 0.9897 | 0.129485 | 0.151536 | 0.029025 |
| A | 25/04/90 | 2 | 0.1699 | | | 12.3736 | | | 0.146845 | | |
| A | 25/04/90 | 3 | 0.1559 | | | 14.0241 | | | 0.133973 | | |
| A | 25/04/90 | 4 | 0.1593 | | | 13.7754 | | | 0.175308 | | |
| A | 25/04/90 | 5 | 0.1276 | | | 15.1595 | | | 0.125612 | | |
| A | 25/04/90 | 6 | 0.1342 | | | 13.4755 | | | 0.197995 | | |
| B | 21/07/89 | 1 | 0.0118 | 13 | 0.00091 | 53.4458 | 55.3080 | 1.7542 | 1.422242 | 1.774551 | 0.446229 |
| B | 21/07/89 | 2 | 0.0133 | 12 | 0.00111 | 54.1945 | | | 1.361202 | | |
| B | 21/07/89 | 3 | 0.0115 | 15 | 0.00077 | 57.0146 | | | 2.225444 | | |
| B | 21/07/89 | 4 | 0.0074 | 10 | 0.00074 | 56.5773 | | | 2.089316 | | |
| B | 10/01/90 | 1 | 0.0020 | 3 | 0.00067 | 43.7795 | 45.4582 | 1.4841 | 6.922699 | 5.988653 | 0.884082 |
| B | 10/01/90 | 2 | 0.0017 | 3 | 0.00057 | 46.5962 | | | 5.164882 | | |
| B | 10/01/90 | 3 | 0.0037 | 6 | 0.00062 | 45.9988 | | | 5.878378 | | |
| B | 25/04/90 | 1 | 0.0057 | 10 | 0.00057 | 42.7890 | 47.6372 | 4.3270 | 3.997353 | 5.111846 | 0.972054 |
| B | 25/04/90 | 2 | 0.0064 | 11 | 0.00058 | 49.0155 | | | 5.553684 | | |
| B | 25/04/90 | 3 | 0.0102 | 11 | 0.00093 | 51.1072 | | | 5.784500 | | |

(end)

**APPENDIX E.1** Original data of copper and cadmium contents in the tissues of sticklebacks.

Keys:

| | |
|---|---|
| B=Body carcase | K=Kidney and Speen |
| G=Gill | I=Digestion tract |
| L=Liver | F=Food stuff |
| O=Ovary | f=fat and connected tissue |
| T=Testis | P=Parasite |

| Date | Site | Sex | Tissue | wet wt. (g) | dry wt. (g) | COPPER Cu conc. (ug/g dw) | COPPER Total Cu (ug) | CADMIUM Cd conc. (ug/g dw) | CADMIUM Total Cd (ug) |
|---|---|---|---|---|---|---|---|---|---|
| 25/04/90 | A | M1 | B | 2.1059 | 0.4858 | 2.72806 | 1.32529 | 0.04619 | 0.02244 |
| | | | G | 0.1259 | 0.0266 | 3.25986 | 0.08671 | 0.14779 | 0.00393 |
| | | | L | 0.0807 | 0.0169 | 22.63900 | 0.38260 | 0.16772 | 0.00283 |
| | | | K | 0.1154 | 0.0202 | 4.29269 | 0.08671 | 0.16514 | 0.00334 |
| | | | I | 0.1025 | 0.0214 | 5.61990 | 0.12027 | 0.39847 | 0.00853 |
| | | | F | 0.0590 | 0.0095 | 9.68824 | 0.09204 | 0.32220 | 0.00306 |
| | | | T | 0.0128 | 0.0019 | 4.89327 | 0.00930 | 0.13158 | 0.00025 |
| | | | Sum | 2.6022 | 0.5823 | 3.61140 | 2.10292 | 0.07622 | 0.04438 |
| 25/04/90 | A | F1 | B | 1.2514 | 0.2775 | 2.18482 | 0.60629 | 0.08444 | 0.02343 |
| | | | G | 0.0685 | 0.0112 | 3.03605 | 0.03400 | 0.38644 | 0.00433 |
| | | | L | 0.2034 | 0.0418 | 6.31166 | 0.26383 | 0.07730 | 0.00323 |
| | | | K | 0.0257 | 0.0046 | 4.57598 | 0.02105 | 0.05435 | 0.00025 |
| | | | I | 0.0746 | 0.0146 | 3.89637 | 0.05689 | 0.36656 | 0.00535 |
| | | | F | 0.1479 | 0.0265 | 18.35354 | 0.48637 | 2.05378 | 0.05443 |
| | | | O | 0.4505 | 0.1068 | 2.69195 | 0.28750 | 0.44270 | 0.04728 |
| | | | Sum | 2.2220 | 0.4830 | 3.63546 | 1.75593 | 0.28634 | 0.13830 |
| 25/04/90 | A | F2 | B | 1.0726 | 0.1993 | 2.10678 | 0.41988 | 0.09766 | 0.01946 |
| | | | G | 0.0767 | 0.0127 | 2.21472 | 0.02813 | 0.44237 | 0.00562 |
| | | | L | 0.2273 | 0.0435 | 22.99034 | 1.00008 | 0.07885 | 0.00343 |
| | | | K | 0.0252 | 0.0039 | 33.45278 | 0.13047 | 0.06410 | 0.00025 |
| | | | I | 0.0647 | 0.0107 | 5.41610 | 0.05795 | 1.94693 | 0.02083 |
| | | | F | 0.0618 | 0.0086 | 16.70930 | 0.14370 | 2.82074 | 0.02426 |
| | | | O | 0.5194 | 0.1210 | 2.77658 | 0.33597 | 0.36450 | 0.04410 |
| | | | Sum | 2.0477 | 0.3997 | 5.29440 | 2.11617 | 0.29511 | 0.11796 |
| 25/04/90 | A | F3 | B | 0.9775 | 0.1770 | 2.04122 | 0.36130 | 0.15482 | 0.02740 |
| | | | G | 0.0610 | 0.0090 | 2.51690 | 0.02265 | 0.02778 | 0.00025 |
| | | | L | 0.1977 | 0.0373 | 4.78166 | 0.17836 | 0.16644 | 0.00621 |
| | | | K | 0.0213 | 0.0032 | 7.91346 | 0.02532 | 0.07813 | 0.00025 |
| | | | I | 0.0559 | 0.0089 | 3.69891 | 0.03292 | 0.08100 | 0.00072 |
| | | | F | 0.0576 | 0.0906 | 2.16219 | 0.19589 | 0.25461 | 0.02307 |
| | | | O | 0.4605 | 0.0056 | 23.18795 | 0.12985 | 0.82303 | 0.00461 |
| | | | Sum | 1.8315 | 0.3316 | 2.85372 | 0.94629 | 0.18851 | 0.06251 |

(continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 25/04/90 | A | F1 | B | 0.8995 | 0.1924 | 1.71136 | 0.32927 | 0.15274 | 0.02939 |
| | | | G | 0.0500 | 0.0088 | 2.80174 | 0.02466 | 0.13263 | 0.00117 |
| | | | L | 0.1418 | 0.0290 | 3.52528 | 0.10223 | 0.06352 | 0.00184 |
| | | | K | 0.0184 | 0.0034 | 3.12727 | 0.01063 | 0.07353 | 0.00025 |
| | | | I | 0.0541 | 0.0097 | 4.32726 | 0.04197 | 0.40168 | 0.00390 |
| | | | F | 0.0335 | 0.0040 | 13.28972 | 0.05316 | 0.68585 | 0.00274 |
| | | | O | 0.5101 | 0.1005 | 1.57294 | 0.15808 | 0.22163 | 0.02227 |
| | | | Sum | 1.7074 | 0.3478 | 2.07016 | 0.72000 | 0.17700 | 0.06156 |
| 12/10/89 | B | M1 | B | 1.2477 | 0.2677 | 7.32699 | 1.96144 | 1.89444 | 0.50714 |
| | | | G | 0.1108 | 0.0167 | 8.95925 | 0.14962 | 0.45607 | 0.00762 |
| | | | L | 0.0599 | 0.0204 | 397.27931 | 8.10450 | 2.77594 | 0.05663 |
| | | | K | 0.0303 | 0.0043 | 33.34893 | 0.14340 | 2.33115 | 0.01002 |
| | | | I | 0.1378 | 0.0246 | 68.69797 | 1.68997 | 1.97576 | 0.04860 |
| | | | F | 0.1423 | 0.0387 | 832.90666 | 32.23349 | 4.07467 | 0.15769 |
| | | | P | 0.3922 | 0.1319 | 16.67249 | 2.19910 | 0.63239 | 0.08341 |
| | | | Sum | 2.1210 | 0.5043 | 92.17036 | 46.48151 | 1.72738 | 0.87112 |
| | | | P | 0.3922 | 0.1319 | 16.67249 | 2.19910 | 0.63239 | 0.08341 |
| | | | FISH | 1.7288 | 0.3724 | 118.91088 | 44.28241 | 2.11521 | 0.78770 |
| 12/10/89 | B | M2 | B | 1.2237 | 0.2919 | 7.91237 | 2.30962 | 0.26423 | 0.07713 |
| | | | G | 0.1008 | 0.0169 | 9.22122 | 0.15584 | 0.47916 | 0.00810 |
| | | | L | 0.0539 | 0.0121 | 532.73203 | 6.44606 | 4.62704 | 0.05599 |
| | | | K | 0.0425 | 0.0076 | 26.43783 | 0.20093 | 1.01271 | 0.00770 |
| | | | I | 0.0864 | 0.0162 | 63.77116 | 1.03309 | 1.81128 | 0.02934 |
| | | | F | 0.0815 | 0.0160 | 328.38863 | 5.25422 | 15.13044 | 0.24209 |
| | | | P | 0.3735 | 0.1256 | 18.25150 | 2.29239 | 0.32865 | 0.04128 |
| | | | Sum | 1.9623 | 0.4863 | 36.38113 | 17.69214 | 0.94925 | 0.46162 |
| | | | P | 0.3735 | 0.1256 | 18.25150 | 2.29239 | 0.32865 | 0.04128 |
| | | | FISH | 1.5888 | 0.3607 | 42.69408 | 15.39976 | 1.16535 | 0.42034 |
| 12/10/89 | B | F1 | B | 1.4561 | 0.4188 | 4.68347 | 1.96144 | 0.14009 | 0.05867 |
| | | | P | 0.6829 | 0.2156 | 9.33454 | 2.01253 | 0.68159 | 0.14695 |
| | | | G | 0.1152 | 0.0245 | 8.77227 | 0.21492 | 0.34363 | 0.00842 |
| | | | L | 0.0981 | 0.0231 | 133.43944 | 3.08245 | 2.34030 | 0.05406 |
| | | | K | 0.0251 | 0.0053 | 22.65634 | 0.12008 | 0.96763 | 0.00513 |
| | | | O | 0.0825 | 0.0150 | 14.81998 | 0.22230 | 0.33120 | 0.00497 |
| | | | I | 0.1181 | 0.0247 | 65.00605 | 1.60565 | 1.47389 | 0.03641 |
| | | | F | 0.1158 | 0.0340 | 249.18771 | 8.47238 | 3.21312 | 0.10925 |
| | | | Sum | 2.6938 | 0.7610 | 23.24802 | 17.69174 | 0.55696 | 0.42385 |
| | | | P | 0.6829 | 0.2156 | 9.33454 | 2.01253 | 0.68159 | 0.14695 |
| | | | FISH | 2.0109 | 0.5454 | 28.74811 | 15.67922 | 0.50770 | 0.27690 |

(continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 12/10/89 | B | F2 | B | 1.3436 | 0.3296 | 10.83677 | 3.57180 | 0.25092 | 0.08270 |
| | | | P | 0.3088 | 0.0992 | 19.66064 | 1.95034 | 0.30652 | 0.03041 |
| | | | G | 0.0961 | 0.0177 | 20.46501 | 0.36223 | 1.67634 | 0.02967 |
| | | | L | 0.0843 | 0.0179 | 953.20552 | 17.06238 | 3.27124 | 0.05856 |
| | | | K | 0.0299 | 0.0058 | 51.73088 | 0.30004 | 0.42084 | 0.00244 |
| | | | O | 0.2027 | 0.0245 | 20.86104 | 0.51110 | 0.12819 | 0.00314 |
| | | | I | 0.1231 | 0.0229 | 131.67353 | 3.01532 | 1.74394 | 0.03994 |
| | | | F | 0.1715 | 0.0402 | 748.33605 | 30.08311 | 3.75530 | 0.15096 |
| | | | Sum | 2.3600 | 0.5578 | 101.92956 | 56.85631 | 0.71319 | 0.39782 |
| | | | P | 0.3088 | 0.0992 | 19.66064 | 1.95034 | 0.30652 | 0.03041 |
| | | | FISH | 2.0512 | 0.4586 | 119.72520 | 54.90597 | 0.80115 | 0.36741 |
| 10/01/90 | B | M1 | B | 0.9568 | 0.2735 | 8.17839 | 2.23679 | 0.19060 | 0.05213 |
| | | | G | 0.0630 | 0.0180 | 7.52348 | 0.13542 | 0.35096 | 0.00632 |
| | | | L | 0.0689 | 0.0210 | 136.68105 | 2.87030 | 1.34755 | 0.02830 |
| | | | K | 0.0104 | 0.0025 | 21.20114 | 0.05300 | 1.13684 | 0.00284 |
| | | | I | 0.0767 | 0.0139 | 38.38341 | 0.53353 | 2.07299 | 0.02881 |
| | | | F | 0.0828 | 0.0156 | 124.10261 | 1.93600 | 2.29843 | 0.03586 |
| | | | T | 0.0104 | 0.0031 | 42.26406 | 0.13102 | 1.78159 | 0.00552 |
| | | | Sum | 1.2690 | 0.3476 | 22.71595 | 7.89607 | 0.45966 | 0.15978 |
| 10/01/90 | B | M2 | B | 0.8285 | 0.2458 | 9.37648 | 2.30474 | 0.14744 | 0.03624 |
| | | | G | 0.0542 | 0.0130 | 9.88476 | 0.12850 | 0.27209 | 0.00354 |
| | | | L | 0.0490 | 0.0175 | 171.78288 | 3.00620 | 0.99295 | 0.01738 |
| | | | K | 0.0108 | 0.0020 | 27.75974 | 0.05552 | 2.36431 | 0.00473 |
| | | | I | 0.0576 | 0.0107 | 50.21536 | 0.53730 | 1.89121 | 0.02024 |
| | | | F | 0.1304 | 0.0295 | 152.00322 | 4.48410 | 3.49728 | 0.10317 |
| | | | T | 0.0133 | 0.0033 | 42.75307 | 0.14109 | 1.49309 | 0.00493 |
| | | | Sum | 1.1438 | 0.3218 | 33.11822 | 10.65744 | 0.59110 | 0.19022 |
| 10/01/90 | B | F1 | B | 0.7431 | 0.2489 | 5.34673 | 1.33080 | 0.09774 | 0.02433 |
| | | | G | 0.0418 | 0.0098 | 6.11465 | 0.05992 | 0.17856 | 0.00175 |
| | | | L | 0.0695 | 0.0348 | 83.94436 | 2.92126 | 0.28459 | 0.00990 |
| | | | K | 0.0071 | 0.0019 | 24.25373 | 0.04608 | 1.44358 | 0.00274 |
| | | | I | 0.0604 | 0.0149 | 33.78052 | 0.50333 | 0.71734 | 0.01069 |
| | | | f | 0.0074 | 0.0037 | 14.49514 | 0.05363 | 0.44611 | 0.00165 |
| | | | O | 0.0142 | 0.0031 | 21.56269 | 0.06684 | 0.66057 | 0.00205 |
| | | | P | 0.1167 | 0.0306 | 10.58887 | 0.32402 | 0.23241 | 0.00711 |
| | | | Sum | 1.0602 | 0.3477 | 15.25998 | 5.30589 | 0.17320 | 0.06022 |
| | | | p | 0.1167 | 0.0306 | 10.58887 | 0.32402 | 0.23241 | 0.00711 |
| | | | FISH | 0.9435 | 0.3171 | 15.71074 | 4.98188 | 0.16749 | 0.05311 |

(continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 12/10/89 | E | F1 | B | 1.9737 | 0.7268 | 3.05803 | 2.22258 | 0.02000 | 0.01454 |
| | | | P | 0.4110 | 0.1354 | 7.70648 | 1.04346 | 0.01663 | 0.00225 |
| | | | G | 0.1136 | 0.0326 | 5.11418 | 0.16672 | 0.05647 | 0.00184 |
| | | | L | 0.1776 | 0.0752 | 125.06988 | 9.40525 | 0.29228 | 0.02198 |
| | | | K | 0.0397 | 0.0097 | 18.30987 | 0.17761 | 0.12796 | 0.00124 |
| | | | O | 0.0372 | 0.0099 | 16.36948 | 0.16206 | 0.18266 | 0.00181 |
| | | | I | 0.1453 | 0.0372 | 23.10084 | 0.85935 | 0.47259 | 0.01758 |
| | | | F | 0.0380 | 0.0116 | 319.34191 | 3.70437 | 1.42364 | 0.01651 |
| | | | f | 0.0529 | 0.0422 | 2.99284 | 0.12630 | 0.00730 | 0.00031 |
| | | | Sum | 2.9890 | 1.0806 | 16.53497 | 17.86769 | 0.07224 | 0.07806 |
| | | | P | 0.1167 | 0.0306 | 10.58887 | 0.32402 | 0.23241 | 0.00711 |
| | | | FISH | 2.8723 | 1.0500 | 16.70826 | 17.54367 | 0.06757 | 0.07095 |
| 12/10/89 | E | F2 | B | 1.7384 | 0.5402 | 5.88687 | 3.18009 | 0.03185 | 0.01720 |
| | | | G | 0.0958 | 0.0215 | 8.45931 | 0.18188 | 0.10113 | 0.00217 |
| | | | L | 0.1172 | 0.0420 | 194.31963 | 8.16142 | 0.50744 | 0.02131 |
| | | | K | 0.0225 | 0.0046 | 31.84999 | 0.14651 | 0.34228 | 0.00157 |
| | | | O | 0.0403 | 0.0087 | 21.48672 | 0.18693 | 3.11445 | 0.02710 |
| | | | I | 0.1263 | 0.0338 | 51.18439 | 1.73003 | 1.00110 | 0.03384 |
| | | | F | 0.1686 | 0.0743 | 80.54807 | 5.98472 | 0.79195 | 0.05884 |
| | | | f | 0.0137 | 0.0070 | 20.93000 | 0.14651 | 0.33441 | 0.00234 |
| | | | Sum | 2.3228 | 0.7321 | 26.93361 | 19.71810 | 0.22453 | 0.16438 |
| 12/10/89 | E | M1 | B | 1.5647 | 0.5213 | 4.51399 | 2.35315 | 0.01662 | 0.00867 |
| | | | G | 0.1173 | 0.0367 | 3.29655 | 0.12098 | 0.03667 | 0.00135 |
| | | | L | 0.0928 | 0.0362 | 82.28725 | 2.97880 | 0.28790 | 0.01042 |
| | | | K | 0.1339 | 0.0081 | 15.97621 | 0.12941 | 0.28903 | 0.00234 |
| | | | T | 0.0175 | 0.0042 | 24.51807 | 0.10298 | 0.18827 | 0.00079 |
| | | | I | 0.1111 | 0.0344 | 21.29362 | 0.73250 | 0.30723 | 0.01057 |
| | | | F | 0.0590 | 0.0038 | 190.03557 | 0.72214 | 2.67926 | 0.01018 |
| | | | f | 0.0120 | 0.0083 | 6.41237 | 0.05322 | 0.45135 | 0.00375 |
| | | | Sum | 2.1083 | 0.6530 | 11.01557 | 7.19317 | 0.07360 | 0.04806 |
| 10/01/90 | E | M1 | B | 1.7583 | 0.5993 | 9.19352 | 5.50967 | 0.05053 | 0.03028 |
| | | | G | 0.1087 | 0.0305 | 6.81233 | 0.20778 | 0.17782 | 0.00542 |
| | | | L | 0.0772 | 0.0270 | 203.19799 | 5.48635 | 0.49648 | 0.01340 |
| | | | K | 0.0267 | 0.0072 | 16.44936 | 0.11844 | 0.78087 | 0.00562 |
| | | | I | 0.0993 | 0.0224 | 40.41797 | 0.90536 | 0.82088 | 0.01839 |
| | | | F | 0.1133 | 0.0257 | 81.94061 | 2.10587 | 4.80204 | 0.12341 |
| | | | T | 0.0133 | 0.0035 | 28.80541 | 0.10082 | 0.75529 | 0.00264 |
| | | | Sum | 2.1968 | 0.7156 | 20.17089 | 14.43429 | 0.27834 | 0.19918 |
| 10/01/90 | E | F2 | B | 0.9589 | 0.2351 | 14.33124 | 3.36928 | 0.11192 | 0.02631 |
| | | | G | 0.0908 | 0.0155 | 22.49727 | 0.34871 | 0.28585 | 0.00443 |
| | | | L | 0.0546 | 0.0140 | 258.41021 | 3.61774 | 1.08294 | 0.01516 |
| | | | K | 0.0157 | 0.0026 | 43.13223 | 0.11214 | 1.24587 | 0.00324 |
| | | | I | 0.0980 | 0.0158 | 96.47016 | 1.52423 | 0.87114 | 0.01376 |
| | | | F | 0.1275 | 0.0206 | 270.45059 | 5.57128 | 2.81721 | 0.05803 |
| | | | O | 0.0204 | 0.0035 | 72.48703 | 0.25370 | 3.33685 | 0.01168 |
| | | | Sum | 1.3659 | 0.3071 | 48.18328 | 14.79709 | 0.43185 | 0.13262 |

(end)

**APPENDIX E.2** Copper and cadmium concentrations in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish.

COPPER CONCENTRATION (ug/g dry weight)

| (LNP) | 25/04/90 | 25/04/90 | 25/04/90 | 25/04/90 | 25/04/90 | | |
|---|---|---|---|---|---|---|---|
| Tissue | A–M1 | A–F1 | A–F2 | A–F3 | A–F4 | MEAN | S.D. |
| BODY | 2.7281 | 2.1848 | 2.1068 | 2.0412 | 1.7114 | 2.1544 | 0.3679 |
| GILL | 3.2599 | 3.0360 | 2.2147 | 2.5169 | 2.8017 | 2.7659 | 0.4135 |
| LIVER | 22.6390 | 6.3117 | 22.9903 | 4.7817 | 3.5253 | 12.0496 | 9.8773 |
| KIDNEY | 4.2927 | 4.5760 | 33.4528 | 7.9135 | 3.1273 | 10.6724 | 12.8584 |
| INTESTINE | 5.6199 | 3.8964 | 5.4161 | 3.6989 | 4.3273 | 4.5917 | 0.8785 |
| FOOD STUFF | 9.6882 | 18.3535 | 16.7093 | 2.1622 | 13.2897 | 12.0406 | 6.4484 |
| GONAD | 4.8933 | 2.6920 | 2.7766 | 23.1880 | 1.5729 | 7.0245 | 9.1150 |

| (HNP) | 10/01/90 | 10/01/90 | 12/10/89 | 12/10/89 | 10/1/90 | 10/1/90 | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | B–M1 | B–M2 | E–F2 | E–M1 | E–M1 | E–F1 | MEAN | S.D. |
| BODY | 8.1784 | 9.3765 | 5.8869 | 4.5140 | 9.1935 | 14.3312 | 8.5801 | 3.4069 |
| GILL | 7.5235 | 9.8848 | 8.4593 | 3.2966 | 6.8123 | 22.4973 | 9.7456 | 6.6244 |
| LIVER | 136.6810 | 171.7829 | 194.3196 | 82.2873 | 203.1980 | 258.4102 | 174.4465 | 60.3293 |
| KIDNEY | 21.2011 | 27.7597 | 31.8500 | 15.9762 | 16.4494 | 43.1322 | 26.0614 | 10.4454 |
| INTESTINE | 38.3834 | 50.2154 | 51.1844 | 21.2936 | 40.4180 | 96.4702 | 49.6608 | 25.3419 |
| FOOD STUFF | 124.1026 | 152.0032 | 80.5481 | 190.0356 | 81.9406 | 270.4506 | 149.8468 | 72.4450 |
| GONAD | 42.2641 | 42.7531 | 21.4867 | 24.5181 | 28.8054 | 72.4870 | 38.7191 | 18.7937 |
| FAT | | | 20.9300 | 6.4124 | | | 13.6712 | 10.2655 |

| (HP) | 12/10/89 | 12/10/89 | 12/10/89 | 12/10/89 | 10/01/90 | 12/10/89 | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | B–M1P | B–M2P | B–F1P | B–F2P | B–F1P | E–F1P | MEAN | S.D. |
| BODY | 7.3270 | 7.9124 | 4.6835 | 10.8368 | 5.3467 | 3.0580 | 6.5274 | 2.7553 |
| GILL | 8.9593 | 9.2212 | 8.7723 | 20.4650 | 6.1147 | 5.1142 | 9.7744 | 5.5023 |
| LIVER | 397.2793 | 532.7320 | 133.4394 | 953.2055 | 83.9444 | 125.0699 | 370.9451 | 336.1930 |
| KIDNEY | 33.3489 | 26.4378 | 22.6563 | 51.7309 | 24.2537 | 18.3099 | 29.4563 | 11.9831 |
| INTESTINE | 68.6980 | 63.7712 | 65.0061 | 131.6735 | 33.7805 | 23.1008 | 64.3383 | 37.9160 |
| FOOD STUFF | 832.9067 | 328.3886 | 249.1877 | 748.3361 | | 319.3419 | 495.6322 | 272.6705 |
| GONAD | | | 14.8200 | 20.8610 | 21.5627 | 16.3695 | 18.4033 | 3.3166 |
| FAT | | | | | 14.4951 | 2.9928 | 8.7440 | 8.1334 |
| PARASITES | 16.6725 | 18.2515 | 9.3345 | 19.6606 | 10.5889 | 7.7065 | 13.7024 | 5.0939 |

(continued)

CADMIUM CONCENTRATION (ug/g dry weight)

| (LNP) | 25/04/90 | 25/04/90 | 25/04/90 | 25/04/90 | 25/04/90 | | |
|---|---|---|---|---|---|---|---|
| Tissue | A–M1 | A–F1 | A–F2 | A–F3 | A–F4 | MEAN | S.D. |
| BODY | 0.04619 | 0.08444 | 0.09766 | 0.15482 | 0.15274 | 0.1072 | 0.0466 |
| GILL | 0.14779 | 0.38644 | 0.44237 | 0.02778 | 0.13263 | 0.2274 | 0.1780 |
| LIVER | 0.16772 | 0.07730 | 0.07885 | 0.16644 | 0.06352 | 0.1108 | 0.0518 |
| KIDNEY | 0.16514 | 0.05435 | 0.06410 | 0.07813 | 0.07353 | 0.0870 | 0.0446 |
| INTESTINE | 0.39847 | 0.36656 | 1.94693 | 0.08100 | 0.40168 | 0.6389 | 0.7434 |
| FOOD STUFF | 0.32220 | 2.05378 | 2.82074 | 0.25461 | 0.68585 | 1.2274 | 1.1490 |
| GONAD | 0.13158 | 0.44270 | 0.36450 | 0.82303 | 0.22163 | 0.3967 | 0.2673 |

| (HNP) | 10/01/90 | 10/01/90 | 12/10/89 | 12/10/89 | 10/1/90 | 10/1/90 | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | B–M1 | B–M2 | E–F2 | E–M1 | E–M1 | E–F1 | MEAN | S.D. |
| BODY | 0.19060 | 0.14744 | 0.03185 | 0.01662 | 0.05053 | 0.11192 | 0.0915 | 0.0696 |
| GILL | 0.35096 | 0.27209 | 0.10113 | 0.03667 | 0.17782 | 0.28585 | 0.2041 | 0.1202 |
| LIVER | 1.34755 | 0.99295 | 0.50744 | 0.28790 | 0.49648 | 1.08294 | 0.7859 | 0.4137 |
| KIDNEY | 1.13684 | 2.36431 | 0.34228 | 0.28903 | 0.78087 | 1.24587 | 1.0265 | 0.7645 |
| INTESTINE | 2.07299 | 1.89121 | 1.00110 | 0.30723 | 0.82088 | 0.87114 | 1.1608 | 0.6810 |
| FOOD STUFF | 2.29843 | 3.49728 | 0.79195 | 2.67926 | 4.80204 | 2.81721 | 2.8144 | 1.3260 |
| GONAD | 1.78159 | 1.49309 | 3.11445 | 0.18827 | 0.75529 | 3.33685 | 1.7783 | 1.2546 |
| FAT | | | 0.33441 | 0.45135 | | | 0.3929 | 0.0827 |

| (HP) | 12/10/89 | 12/10/89 | 12/10/89 | 12/10/89 | 10/01/90 | 12/10/89 | | |
|---|---|---|---|---|---|---|---|---|
| Tissue | B–M1P | B–M2P | B–F1P | B–F2P | B–F1P | E–F1P | MEAN | S.D. |
| BODY | 1.89444 | 0.26423 | 0.14009 | 0.07747 | 0.09774 | 0.02000 | 0.4157 | 0.7291 |
| GILL | 0.45607 | 0.47916 | 0.34363 | 1.67634 | 0.17856 | 0.05647 | 0.5317 | 0.5839 |
| LIVER | 2.77594 | 4.62704 | 2.34030 | 3.27124 | 0.28459 | 0.29228 | 2.2652 | 1.7130 |
| KIDNEY | 2.33115 | 1.01271 | 0.96763 | 0.42084 | 1.44358 | 0.12796 | 1.0506 | 0.7811 |
| INTESTINE | 1.97576 | 1.81128 | 1.47389 | 1.74394 | 0.71734 | 0.47259 | 1.3658 | 0.6235 |
| FOOD STUFF | 4.07467 | 15.13044 | 3.21312 | 3.75530 | | 1.42364 | 5.5194 | 5.4696 |
| GONAD | | | 0.33120 | 0.12819 | 0.66057 | 0.18266 | 0.3257 | 0.2392 |
| FAT | | | | | 0.44611 | 0.00730 | 0.2267 | 0.3103 |
| PARASITES | 0.63239 | 0.32865 | 0.68159 | 0.30652 | 0.23241 | 0.01663 | 0.3664 | 0.2512 |

(end)

**APPENDIX E.3** Percentage of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish.

Included Food Stuff

| | PERCENTAGE OF COPPER | | | | | | | | PERCENTAGE OF CADMIUM | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (LNP) Tissue | 25/04 A-M1 | 25/04 A-F1 | 25/04 A-F2 | 25/04 A-F3 | 25/04 A-F4 | MEAN | S.D. | | 25/04 A-M1 | 25/04 A-F1 | 25/04 A-F2 | 25/04 A-F3 | 25/04 A-F4 | MEAN | S.D. | |
| Body | 63.02 | 34.53 | 19.84 | 38.18 | 45.73 | 40.26 | 15.83 | | 50.57 | 16.94 | 16.50 | 43.84 | 47.74 | 35.12 | 16.96 | |
| Gill | 4.12 | 1.94 | 1.33 | 2.39 | 3.42 | 2.64 | 1.13 | | 8.86 | 3.13 | 4.76 | 0.40 | 1.90 | 3.81 | 3.25 | |
| Liver | 18.19 | 15.02 | 47.26 | 18.85 | 14.20 | 22.70 | 13.87 | | 6.39 | 2.34 | 2.91 | 9.93 | 2.99 | 4.91 | 3.23 | |
| Kidney | 4.12 | 1.20 | 6.17 | 2.68 | 1.48 | 3.13 | 2.05 | | 7.52 | 0.18 | 0.21 | 0.40 | 0.41 | 1.74 | 3.23 | |
| Intestine | 5.72 | 3.24 | 2.74 | 3.48 | 5.83 | 4.20 | 1.46 | | 19.21 | 3.87 | 17.66 | 1.15 | 6.33 | 9.65 | 8.25 | |
| Food | 4.38 | 27.70 | 6.79 | 20.70 | 7.38 | 13.39 | 10.24 | | 6.90 | 39.35 | 20.57 | 36.90 | 4.46 | 21.63 | 16.28 | |
| Gonad | 0.44 | 16.37 | 15.88 | 13.72 | 21.96 | 13.67 | 8.00 | | 0.56 | 34.19 | 37.39 | 7.37 | 36.18 | 23.14 | 17.70 | |
| (HNP) Tissue | 10/01 B-M1 | 10/01 B-M2 | 12/10 E-F2 | 12/10 E-M1 | 10/01 E-M1 | 10/01 E-F1 | MEAN | S.D. | 10/01 B-M1 | 10/01 B-M2 | 12/10 E-F2 | 12/10 E-M1 | 10/01 E-M1 | 10/01 E-F1 | MEAN | S.D. |
| Body | 28.33 | 21.63 | 16.13 | 32.71 | 38.17 | 22.77 | 26.62 | 8.04 | 32.63 | 19.05 | 10.47 | 18.03 | 15.20 | 19.84 | 19.20 | 7.41 |
| Gill | 1.72 | 1.21 | 0.92 | 1.68 | 1.44 | 2.36 | 1.55 | 0.49 | 3.95 | 1.86 | 1.32 | 2.80 | 2.72 | 3.34 | 2.67 | 0.96 |
| Liver | 36.35 | 28.21 | 41.39 | 41.41 | 38.01 | 24.45 | 34.97 | 7.08 | 17.71 | 9.14 | 12.97 | 21.68 | 6.73 | 11.43 | 13.28 | 5.55 |
| Kidney | 0.67 | 0.52 | 0.74 | 1.80 | 0.82 | 0.76 | 0.89 | 0.46 | 1.78 | 2.49 | 0.96 | 4.87 | 2.82 | 2.44 | 2.56 | 1.31 |
| Intestine | 6.76 | 5.04 | 8.77 | 10.18 | 6.27 | 10.30 | 7.89 | 2.18 | 18.03 | 10.64 | 20.58 | 21.99 | 9.23 | 10.38 | 15.14 | 5.71 |
| Food | 24.52 | 42.07 | 30.35 | 10.04 | 14.59 | 37.65 | 26.54 | 12.64 | 22.44 | 54.24 | 35.80 | 21.18 | 61.96 | 43.76 | 39.90 | 16.61 |
| Gonad | 1.66 | 1.32 | 0.95 | 1.43 | 0.70 | 1.71 | 1.30 | 0.40 | 3.46 | 2.59 | 16.48 | 1.65 | 1.33 | 8.81 | 5.72 | 5.94 |
| Fat | | | 0.74 | 0.74 | | | 0.74 | 0.00 | | | 1.42 | 7.79 | | | 4.61 | 4.50 |
| (HP) Tissue | 12/10 B-M1P | 12/10 B-M2P | 12/10 B-F1P | 12/10 B-F2P | 10/01 B-F1P | 12/10 E-F1P | MEAN | S.E. | 12/10 B-M1P | 12/10 B-M2P | 12/10 B-F1P | 12/10 B-F2P | 10/01 B-F1P | 12/10 E-F1P | MEAN | S.D. |
| Body | 4.22 | 13.05 | 11.09 | 6.28 | 25.08 | 12.44 | 12.03 | 7.30 | 58.22 | 16.71 | 13.84 | 20.79 | 40.40 | 18.62 | 28.10 | 17.52 |
| Gill | 0.32 | 0.88 | 1.21 | 0.64 | 1.13 | 0.93 | 0.85 | 0.33 | 0.87 | 1.75 | 1.99 | 7.46 | 2.91 | 2.36 | 2.89 | 2.34 |
| Liver | 17.44 | 36.43 | 17.42 | 30.01 | 55.06 | 52.64 | 34.83 | 16.48 | 6.50 | 12.13 | 12.75 | 14.72 | 16.45 | 28.16 | 15.12 | 7.22 |
| Kidney | 0.31 | 1.14 | 0.68 | 0.53 | 0.87 | 0.99 | 0.75 | 0.31 | 1.15 | 1.67 | 1.21 | 0.61 | 4.55 | 1.59 | 1.80 | 1.40 |
| Intestine | 3.64 | 5.84 | 9.08 | 5.30 | 9.49 | 4.81 | 6.36 | 2.38 | 5.58 | 6.36 | 8.59 | 10.04 | 17.75 | 22.52 | 11.81 | 6.81 |
| Food | 69.35 | 29.70 | 47.89 | 52.91 | | 20.73 | 44.12 | 19.25 | 18.10 | 52.44 | 25.77 | 37.95 | | 21.16 | 31.08 | 14.13 |
| Gonad | | | 1.26 | 0.90 | 1.26 | 0.91 | 1.08 | 0.21 | | | 1.17 | 0.79 | 3.40 | 2.32 | 1.92 | 1.18 |
| Fat | | | | | 1.01 | 0.71 | 0.86 | 0.21 | | | | | 2.74 | 0.39 | 1.57 | 1.66 |
| Parasite | 4.73 | 12.96 | 11.38 | 3.43 | 6.11 | 5.84 | 7.41 | 3.84 | 9.58 | 8.94 | 34.67 | 7.64 | 11.81 | 2.89 | 12.59 | 11.22 |

(continued)

## Not Included Food Stuff

| (LNP) | PERCENTAGE OF COPPER | | | | | | | PERCENTAGE OF CADMIUM | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | 25/04 A-M1 | 25/04 A-F1 | 25/04 A-F2 | 25/04 A-F3 | 25/04 A-F4 | MEAN | S.D. | 25/04 A-M1 | 25/04 A-F1 | 25/04 A-F2 | 25/04 A-F3 | 25/04 A-F4 | MEAN | S.D. |
| Body | 65.91 | 47.76 | 21.29 | 48.15 | 49.38 | 46.49 | 16.01 | 54.31 | 27.94 | 20.77 | 69.48 | 49.96 | 44.49 | 19.92 |
| Gill | 4.31 | 2.68 | 1.43 | 3.02 | 3.70 | 3.03 | 1.09 | 9.51 | 5.16 | 6.00 | 0.63 | 2.98 | 4.86 | 3.33 |
| Liver | 19.03 | 20.78 | 50.70 | 23.77 | 15.33 | 25.92 | 14.18 | 6.86 | 3.85 | 3.66 | 15.74 | 3.13 | 6.65 | 5.29 |
| Kidney | 4.31 | 1.66 | 6.61 | 3.37 | 1.59 | 3.51 | 2.09 | 8.07 | 0.30 | 0.27 | 0.63 | 0.43 | 1.94 | 3.43 |
| Intestine | 5.98 | 4.48 | 2.94 | 4.39 | 6.29 | 4.82 | 1.36 | 20.64 | 6.38 | 22.23 | 1.83 | 6.62 | 11.54 | 9.25 |
| Gonad | 0.46 | 22.65 | 17.03 | 17.30 | 23.71 | 16.23 | 9.32 | 0.61 | 56.37 | 47.07 | 11.69 | 37.87 | 30.72 | 23.69 |

| (HNP) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | 10/01 B-M1 | 10/01 B-M2 | 12/10 E-F2 | 12/10 E-M1 | 10/01 E-M1 | 10/01 E-F1 | MEAN | S.D. | 10/01 B-M1 | 10/01 B-M2 | 12/10 E-F2 | 12/10 E-M1 | 10/01 E-M1 | 10/01 E-F1 | MEAN | S.D. |
| Body | 37.53 | 37.33 | 23.16 | 36.36 | 44.69 | 36.52 | 35.93 | 7.00 | 42.06 | 41.63 | 16.30 | 22.88 | 39.97 | 35.28 | 33.02 | 10.88 |
| Gill | 2.27 | 2.08 | 1.32 | 1.87 | 1.69 | 3.78 | 2.17 | 0.85 | 5.10 | 4.06 | 2.06 | 3.55 | 7.16 | 5.94 | 4.65 | 1.81 |
| Liver | 48.16 | 48.70 | 59.43 | 46.03 | 44.50 | 39.21 | 47.67 | 6.69 | 22.84 | 19.96 | 20.19 | 27.51 | 17.69 | 20.33 | 21.42 | 3.40 |
| Kiney | 0.89 | 0.90 | 1.07 | 2.00 | 0.96 | 1.22 | 1.17 | 0.42 | 2.29 | 5.43 | 1.49 | 6.18 | 7.42 | 4.34 | 4.53 | 2.29 |
| Intestine | 8.95 | 8.70 | 12.60 | 11.32 | 7.34 | 16.52 | 10.91 | 3.34 | 23.25 | 23.25 | 32.06 | 27.90 | 24.27 | 18.45 | 24.86 | 4.64 |
| Gonad | 2.20 | 2.29 | 1.36 | 1.59 | 0.82 | 2.75 | 1.83 | 0.71 | 4.46 | 5.66 | 25.67 | 2.09 | 3.49 | 15.66 | 9.50 | 9.28 |
| fat | | | 1.07 | 0.82 | | | 0.94 | 0.17 | | | 2.22 | 9.89 | | | 6.05 | 5.42 |

| (HP) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | 12/10 B-M1P | 12/10 B-M2P | 12/10 B-F1P | 12/10 B-F2P | 10/01 B-F1P | 12/10 E-F1P | MEAN | S.D. | 12/10 B-M1P | 12/10 B-M2P | 12/10 B-F1P | 12/10 B-F2P | 10/01 B-F1P | 12/10 E-F1P | MEAN | S.D. |
| Body | 13.77 | 18.57 | 21.28 | 13.34 | 25.08 | 15.69 | 17.95 | 4.61 | 71.09 | 35.13 | 18.65 | 33.50 | 40.40 | 23.62 | 37.06 | 18.47 |
| Gill | 1.05 | 1.25 | 2.33 | 1.35 | 1.13 | 1.18 | 1.38 | 0.48 | 1.07 | 3.69 | 2.68 | 12.02 | 2.91 | 2.99 | 4.23 | 3.92 |
| Liver | 56.88 | 51.83 | 33.43 | 63.73 | 55.06 | 66.41 | 54.56 | 11.69 | 7.94 | 25.50 | 17.18 | 23.72 | 16.45 | 35.71 | 21.08 | 9.49 |
| Kidney | 1.01 | 1.62 | 1.30 | 1.12 | 0.87 | 1.25 | 1.19 | 0.26 | 1.41 | 3.51 | 1.63 | 0.99 | 4.55 | 2.02 | 2.35 | 1.38 |
| Intestine | 11.86 | 8.31 | 17.42 | 11.26 | 9.49 | 6.07 | 10.73 | 3.88 | 6.81 | 13.37 | 11.57 | 16.18 | 17.75 | 28.56 | 15.71 | 7.37 |
| Gonad | | | 2.41 | 1.91 | 1.26 | 1.14 | 1.68 | 0.59 | | | 1.58 | 1.27 | 3.40 | 2.94 | 2.30 | 1.03 |
| Fat | | | | | 1.01 | 0.89 | 0.95 | 0.08 | | | | | 2.74 | 0.50 | 1.62 | 1.58 |
| Parasite | 15.43 | 18.43 | 21.83 | 7.28 | 6.11 | 7.37 | 12.74 | 6.71 | 11.69 | 18.80 | 46.71 | 12.32 | 11.81 | 3.66 | 17.50 | 15.10 |

(end)

**APPENDIX E.4** One-way ANOVA of Cu and Cd distributions in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. * = P<0.05, ** = P<0.01, *** = P<0.001, **** =P<0.0001.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Copper Concentration | | | | | | |
| LNP | Sum of Sq. Between Gr. | 6 | 554.16 | 92.36 | 1.664 | n.s. |
| | Sum of Sq. Within Gr. | 28 | 1554.49 | 55.52 | | |
| | Sum of Sq. of Total | 34 | 2108.65 | | | |
| HNP | Sum of Sq. Between Gr. | 6 | 167132.4 | 27855.4 | 19.406 | **** |
| | Sum of Sq. Within Gr. | 35 | 50239.4 | 1435.4 | | |
| | Sum of Sq. of Total | 41 | 217371.8 | | | |
| HP | Sum of Sq. Between Gr. | 7 | 1410040.1 | 201434.3 | 8.559 | **** |
| | Sum of Sq. Within Gr. | 37 | 870793.5 | 23535.0 | | |
| | Sum of Sq. of Total | 44 | 2280833.7 | | | |
| Cadmium Concentration | | | | | | |
| LNP | Sum of Sq. Between Gr. | 6 | 5.195 | 0.866 | 3.057 | * |
| | Sum of Sq. Within Gr. | 28 | 7.931 | 0.283 | | |
| | Sum of Sq. of Total | 34 | 13.125 | | | |
| HNP | Sum of Sq. Between Gr. | 6 | 31.938 | 5.323 | 8.151 | **** |
| | Sum of Sq. Within Gr. | 35 | 22.856 | 0.653 | | |
| | Sum of Sq. of Total | 41 | 54.794 | | | |
| HP | Sum of Sq. Between Gr. | 7 | 111.014 | 15.859 | 4.072 | ** |
| | Sum of Sq. Within Gr. | 37 | 144.088 | 3.894 | | |
| | Sum of Sq. of Total | 44 | 255.102 | | | |

APPENDIX E.5 One-way ANOVA of Cu and Cd concentrations in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish. * = P<0.05, ** = P<0.01, *** = P<0.001, **** =P<0.0001.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Copper Concentration | | | | | | |
| Body | Sum of Sq. Between Gr. | 2 | 115.55 | 67.78 | 8.377 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 96.56 | 6.90 | | |
| | Sum of Sq. of Total | 16 | 212.11 | | | |
| Gill | Sum of Sq. Between Gr. | 2 | 172.61 | 86.30 | 3.250 | **** |
| | Sum of Sq. Within Gr. | 14 | 371.71 | 26.55 | | |
| | Sum of Sq. of Total | 16 | 544.32 | | | |
| Liver | Sum of Sq. Between Gr. | 2 | 355609.8 | 177804.9 | 4.264 | **** |
| | Sum of Sq. Within Gr. | 14 | 583723.5 | 41694.5 | | |
| | Sum of Sq. of Total | 16 | 93933.3 | | | |
| Kidney | Sum of Sq. Between Gr. | 2 | 1065.06 | 532.53 | 3.874 | * |
| | Sum of Sq. Within Gr. | 14 | 1924.48 | 137.46 | | |
| | Sum of Sq. of Total | 16 | 2989.54 | | | |
| Intestine | Sum of Sq. Between Gr. | 2 | 10339.19 | 5169.59 | 6.958 | **** |
| | Sum of Sq. Within Gr. | 14 | 10402.12 | 743.01 | | |
| | Sum of Sq. of Total | 16 | 20741.31 | | | |
| Cadmium Concentration | | | | | | |
| Body | Sum of Sq. Between Gr. | 2 | 0.4653 | 0.2327 | 1.254 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 2.5982 | 0.1856 | | |
| | Sum of Sq. of Total | 16 | 3.0635 | | | |
| Gill | Sum of Sq. Between Gr. | 2 | 0.3917 | 0.1958 | 1.440 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 1.9033 | 0.1360 | | |
| | Sum of Sq. of Total | 16 | 2.2950 | | | |
| Liver | Sum of Sq. Between Gr. | 2 | 13.6299 | 6.8149 | 6.140 | * |
| | Sum of Sq. Within Gr. | 14 | 15.5383 | 1.1099 | | |
| | Sum of Sq. of Total | 16 | 29.1682 | | | |
| Kidney | Sum of Sq. Between Gr. | 2 | 3.1975 | 1.5987 | 3.742 | * |
| | Sum of Sq. Within Gr. | 14 | 5.9809 | 0.4272 | | |
| | Sum of Sq. of Total | 16 | 9.1783 | | | |
| Intestine | Sum of Sq. Between Gr. | 2 | 1.5019 | 0.7509 | 1.624 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 6.4731 | 0.4624 | | |
| | Sum of Sq. of Total | 16 | 7.9750 | | | |

**APPENDIX E.6** One-way ANOVA of percentage of the total amount of Cu and Cd in the tissues of three groups of sticklebacks; LNP: low-metal non-parasitised fish, HNP: high-metal non-parasitised fish, and HP: high-metal parasitised fish.
* = P<0.05, ** = P<0.01, *** = P<0.001, **** =P<0.0001.

| Source | Source of Variance | d.f. | Sum of Sq. | Mean Sq. | F value | Sig. level |
|---|---|---|---|---|---|---|
| Percentage of the Total Amount of Copper | | | | | | |
| Body | Sum of Sq. Between Gr. | 2 | 2319.05 | 1159.52 | 11.798 | ** |
| | Sum of Sq. Within Gr. | 14 | 1376.00 | 98.29 | | |
| | Sum of Sq. of Total | 16 | 3695.04 | | | |
| Gill | Sum of Sq. Between Gr. | 2 | 7.40 | 3.70 | 5.409 | * |
| | Sum of Sq. Within Gr. | 14 | 9.58 | 0.68 | | |
| | Sum of Sq. of Total | 16 | 16.98 | | | |
| Liver | Sum of Sq. Between Gr. | 2 | 2381.98 | 1190.99 | 9.736 | ** |
| | Sum of Sq. Within Gr. | 14 | 1712.67 | 122.33 | | |
| | Sum of Sq. of Total | 16 | 4094.65 | | | |
| Kidney | Sum of Sq. Between Gr. | 2 | 19.11 | 9.56 | 7.175 | ** |
| | Sum of Sq. Within Gr. | 14 | 18.65 | 1.33 | | |
| | Sum of Sq. of Total | 16 | 37.76 | | | |
| Intestine | Sum of Sq. Between Gr. | 2 | 127.22 | 63.61 | 6.432 | * |
| | Sum of Sq. Within Gr. | 14 | 138.67 | 9.91 | | |
| | Sum of Sq. of Total | 16 | 265.89 | | | |
| Percentage of the Total Amount of Cadmium | | | | | | |
| Body | Sum of Sq. Between Gr. | 2 | 364.11 | 182.06 | 0.656 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 3883.60 | 277.40 | | |
| | Sum of Sq. of Total | 16 | 4247.72 | | | |
| Gill | Sum of Sq. Between Gr. | 2 | 0.71 | 0.35 | 0.035 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 141.96 | 10.14 | | |
| | Sum of Sq. of Total | 16 | 142.67 | | | |
| Liver | Sum of Sq. Between Gr. | 2 | 753.08 | 376.54 | 8.505 | ** |
| | Sum of Sq. Within Gr. | 14 | 619.84 | 44.27 | | |
| | Sum of Sq. of Total | 16 | 1372.91 | | | |
| Kidney | Sum of Sq. Between Gr. | 2 | 22.10 | 11.05 | 1.865 | n.s. |
| | Sum of Sq. Within Gr. | 14 | 82.96 | 5.93 | | |
| | Sum of Sq. of Total | 16 | 105.06 | | | |
| Intestine | Sum of Sq. Between Gr. | 2 | 521.41 | 260.70 | 5.059 | * |
| | Sum of Sq. Within Gr. | 14 | 721.43 | 51.53 | | |
| | Sum of Sq. of Total | 16 | 1242.84 | | | |