The pollution of the earth's freshwater habitats is a topic of major concern. This timely synthesis considers the effects of pollutants on aquatic animals via a series of research and review articles which present experimental evidence of sublethal and lethal effects of a range of toxicants at the physiological, cellular and subcellular levels, and which explore new techniques for detection of pollution damage. Topics covered include routes of uptake of toxicants; the effects of acute and chronic exposure to toxic metal ions, particularly zinc, copper and aluminium, with emphasis on the mechanisms of toxicity and responses to chronic exposure to sublethal levels; the impact on fish biology of two chemicals of current concern, nitrites and polyaromatic hydrocarbons which may act as oestrogenic substances or potent mutagens; and *in vitro* studies of the mechanisms of toxicity at the cellular and subcellular level, including damage of DNA, using cultured fish cells.

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Edited by

E.W. Taylor

Department of Biological Sciences
University of Birmingham

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Contents

Contributors

BEAUMONT, M.W.
School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
BRAUNER, C.J.
Department of Zoology, University of British Columbia, V6T 1Z4, Canada.
BROWN, J.A.
Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Exeter EX4 4PS, UK.
BUTLER, P.J.
School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
CHIPMAN, J.K.
School of Biochemistry, University of Birmingham, Birmingham, B15 2TT, UK.
GEORGE, S.G.
School of Biochemistry, University of Birmingham, Birmingham, B15 2TT, UK.
HANDY, R.D.
Department of Biological Sciences, The University, Dundee DD1 4HN, UK.
HOGSTRAND, C.
McMaster University, Department of Biology, Hamilton, On L8S 4L1, Canada.
JENSEN, F.B.
Institute of Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark.
JOBLING, S.
Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.
LEAVER, M.J.
NERC Unit of Aquatic Biochemistry, Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, UK.

LIVINGSTONE, D.R.
NERC Plymouth Marine Laboratory, Citadell Hill, Plymouth PL1 2PB, UK.
MAIR, J.
School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
MUJALLID, M.S.I.
School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
NEUMAN, J.F.
Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana 59717, USA.
NUNN, J.W.
School of Biochemistry, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
OLSSON, P.-E.
Department of Cellular and Developmental Biology, Umea University, S-901 87 Umea, Sweden.
RANDALL, D.J.
Department of Zoology, University of British Columbia, V6T 1Z4, Canada.
SUMPTER, J.P.
Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.
TAYLOR, E.W.
School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.
THURSTON, R.V.
Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana 59717, USA.
TYLER, C.R.
Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.
WARING, C.P.
MAFF Fisheries Laboratory, Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK.
WILSON, R.W.
School of Biological Sciences, University of Manchester, G 38 Stopford Building, Oxford Road, Manchester M13 9PT, UK.
WOOD, C.M.
McMaster University, Department of Biology, Hamilton, On L8S 4L1, Canada.

Preface

This book originated from a symposium on Aquatic Toxicology at the Easter meeting of the Society for Experimental Biology at the University of Kent in Canterbury in 1993. It is in some respects a sequel to the volume entitled *Acid Toxicity and Aquatic Animals* (Seminar Series volume 34), as it is soft, acid waters into which toxic metal ions are leached from rocks and industrial waste. In this previous volume the role of aluminium in determining acid toxicity was discussed, and this account is extended into the current volume and amplified by consideration of the mechanisms of toxicity of other metal species and of the aromatic hydrocarbons, at low to sublethal levels. New approaches to the problem of pollution monitoring are considered, including the construction of large-scale models for the exchange of xenobiotics between fish and their environment, and the alternative use of sensitive cellular and molecular markers of pollution. A contemporary account of this general area is also provided by *Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives* edited by Donald C. Malins and Gary K. Ostrander, published by Lewis Publishers, 1994. Fortunately, there is little direct overlap between the two accounts, and the overall approach is differerent so that the volumes complement one another and both provide valuable background for students and practitioners of aquatic toxicology.

Pollution of the aquatic environment is a problem with its origins in the urbanization of the human population which, in Europe, accompanied the industrial revolution about 250 years ago. I am writing this preface in Birmingham, at the centre of the industrial West Midlands, a city with no major river, a population of over one million, an economy based on heavy industry and associated high traffic flows. The problems of aerial and aquatic pollution are intense, with most local waterways devoid of fish. Public awareness of the problem is more recent, and the history of pollution control and monitoring has been one of slowly evolving standards and techniques. Although some progress is being made in northern post-industrial countries (for example, the return of salmon has been reported in the Thames) the

problem is worldwide and continuing to escalate in the Far East, Asia and Africa to the point where human health has suffered, as in the incidence of Minimato disease in Japan, caused by the ingestion of shellfish contaminated with methyl mercury.

Large numbers of new chemicals are manufactured, then released into the environment each year, and assessment of the potential impact of these xenobiotic (i.e. 'foreign to life') contaminants on aquatic organisms is being afforded a progressively higher priority by many nations and international agencies. Monitoring gross environmental pollution has always been the province of chemists. Biological means of assessment have included observation and quantification of faunal changes in polluted water, with the development of marker species or communities as indicators of of levels of pollution, as well as routine testing of the toxicity of effluents on aquatic animals, typically fish, using the LC_{50} test of lethality. However, it is now recognised that the effects of chronic exposure to sublethal levels of toxicants, with the risk of their accumulation over time or via a food chain, can have important limiting effects upon the survival of individual animals and on the survival or species diversity of communities of aquatic organisms. Accordingly, the assessment of pollution has more recently broadened to include the study of chronic exposure to sublethal levels of toxicants and their effects on vulnerable stages in the life-cycle of species.

For a full understanding of the nature of the problem of aquatic pollution, it is now deemed necessary to study the mechanisms of action of pollutants on living organisms at physiological, cellular and molecular levels. This new approach is the subject of this volume which combines the expertise of fish physiologists interested in the basic mechanisms of respiratory gas exchange, ion regulation and endocrine control with that of cell and molecular biologists similarly interested in the fundamental responses of aquatic organisms to toxic pollutants. It assesses the effects of two major classes of aquatic polluntants, toxic metal ions and in particular aluminium, copper and zinc and the polyaromatic hydrocarbons (PAHs) derived from pesticides and products of the petrochemical industry. Both classes are lethally toxic at high concentrations, but the emphasis is on the study of sublethal effects of chronic exposure to these chemicals and their bioaccumulation in a range of aquatic species, and on the development of new methods of early detection of these more subtle effects.

Differences in the rates of accumulation of organic xenobiotics by fish, based on species differences, activity levels and the effects of environmental variables such as temperature are reported by Randall and Thurston who, together with their coworkers, have developed a

model for accumulation of these compounds based on their partition coefficients between water and lipid (representing the membrane components of tissue) which, together with a database for rates of oxygen uptake, will enable them to predict the likely outcome of a measured pollutant load on a fish population. Accumulation of a range of PAHs and related compounds at lower levels have been found to have oestrogenic effects on fish. Their bioaccumulation up the food chain may result in sterilization of males with consequent loss of fecundity. This cause of public anxiety is considered by Sumpter and colleagues. The same range of chemicals can act as genetic toxicants to aquatic organisms, particularly in their active forms following biotransformation to procarcinogens or free radicals. These effects include DNA damage with consequent mutagenic and reproductive effects and are described by Nunn, Livingstone, and Chipman who consider the utility of detection of DNA damage as a sensitive marker of environmental contamination by pesticides and other organic residues.

The use of *in vitro* techniques using primary cultures of fish cells, such as hepatocytes, to detect pollution damage by organic hydrocarbons is explored by George, and this cellular approach is extended to the molecular level by Leaver, who considers the measurement of the levels of cytochrome P450 messenger RNA, protein or enzymic activity in fish as a means of monitoring low levels of organic pollution. This enzyme is involved in biotransformation, by insertion of oxygen, of aromatic hydrocarbons, often rendering them available for excretion, and consequently countering their progressive accumulation in the lipid components of tissues; though the transformed products may be potent mutagens.

This consideration of major topics of current public concern in aquatic toxicology is continued in a chapter on nitrate accumulation by Jensen. Nitrite arises from the incomplete bacterial decomposition of organic waste or from agricultural run-off and is toxic to aquatic animals. It is actively accumulated, apparently over chloride channels, affects oxygen transport and ionoregulation and many be carcinogenic, following its conversion to *N*-nitroso compounds.

Toxic metal ions at high concentrations cause gross, non-specific gill damage to fish, resulting in loss of ionoregulatory ability and ultimately a breakdown in respiratory gas exchange which proves fatal. These changes resulting from exposure of trout to acutely toxic levels of copper in freshwater are described in detail by Taylor and colleagues, while the effects of zinc pollution are described by Hogstrand and Wood, who also consider the role of zinc in metabolism, routes and mechanisms for its uptake over the gills and following ingestion over

the gut wall and regulation of its internal concentration. The role of ingestion in the accumulation of a wide range of metal ions is covered in detail by Handy. Taylor and colleagues progress to a consideration of the effects of exposure to sublethal levels of copper upon the swimming performance of trout. These effects are temperature dependent and apparently associated with problems of oxygen supply to aerobic tissues and accumulation of ammonia, though the mechanisms underlying this accumulation and its effects on aerobic exercise are as yet not understood.

The toxic effects of sublethal levels of zinc and copper seem to reside in their competition for calcium channels in the apical membrane of gill epithelial cells and inhibition of calcium–ATPase on the basolateral membranes. Calcium is important in the regulation of cell permeability and adhesion. The toxic effects of aluminium, including the hormonal control of observed physiological responses to aluminium exposure in trout, are described by Brown and Waring. Aluminium, which is solubilized in soft, acid water, may precipitate on to the surface of the gills due to alkalinization of the microenvironment between the lamellae. Aspects of the complex nature of this microenvironment, which are influenced by active ionoregulatory activity, including an electrogenic proton pump, are considered by several authors, as there are common features of the toxic effects of a range of metal ions on the functioning of the gills in ionoregulation, acid–base regulation and ultimately in respiratory gas exchange.

Wilson considers the effects of chronic exposure of trout to low levels of aluminium, a common feature of acidified upland rivers. They can undergo a process of acclimation which increases their resistance to any subsequent exposure, this is accompanied by cell proliferation on the gills, including the production of mucocytes and subsequent increased mucous production, which will bind metal ions removing them from solution. However successful these processes of acclimation, chronic exposure has costs for the fish which may manifest themselves in reduced appetite, swimming performance, growth rate and fecundity. Another protective response to exposure to toxic metal ions is the production of metallothioneins, proteins which bind toxic metal ions intracellularly. Their occurrence, structure and the endocrinology and molecular biology of their induction in fish are considered by Olsson. He describes techniques for their measurement in the tissues of animals exposed to toxic metals and discusses their use as early indicators of environmental pollution.

Each chapter is written by experts in the field and they have provided an up-to-date list of references to support their texts. The index will

enable many of the major themes in the book to be traced within and between chapters. I am grateful to all authors for their hard work on their manuscripts and to Maria Murphy of Cambridge University Press and Linda Kachur of the University of British Columbia for their cheerful patience during the final stages of the preparation of this book.