ASSESSMENT OF FACTORS AFFECTING METAL BURDEN IN THE STONE LOACH (*NOEMACHEILUS BARBATULUS* L.)

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Proefschrift
ter verkrijging van de graad van
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STATEMENTS

1. Body size of the stone loach is an important factor to explain fluctuations in metal levels in the fish.
   this thesis

2. Rates of uptake and loss of cadmium by stone loach increase with temperature but to different extents: the first is more affected than the latter.
   this thesis

3. Metabolic rate, which is affected by feeding, is likely to govern rates of uptake and loss of cadmium to an appreciable extent; this is less important for lead.
   this thesis

4. Comparison of bio-magnification and bio-concentration factors do not elucidate the relative importance of food and water to the metal burden of aquatic organisms.
   this thesis; Kay, S.H. (1985) Cadmium in aquatic food webs. Residue Reviews 96, 13-43.

5. The importance of sediment as a source of metal for fish should be assessed by both chemical and biological assay. Metal physically present may not be biologically available.
   this thesis

6. There will be a continuing lack of consensus over the relative importance of water, food and sediment as sources of cadmium and lead in fish, even within one species, under field circumstances, because this is affected by factors including weight of fish, species of fish, and environmental factors, such as cadmium concentration and temperature.
   this thesis

7. Ignoring the possibility that sediments can serve both as a sink and as a source for heavy metals will lead to misinterpretation of data.
   Salomons, W., Rooij, N.M. de, Kerdijk, H. & Bril, J. (1987) Sediments as a source for contaminants? Hydrobiologia 149, 13-30.

8. Mathematical models are a useful tool in explaining and predicting metal levels, despite the simplification of the complexity of processes in the environment.
   this thesis

9. A world standard for word-processing packages is urgently needed.

10. To turn an insult into a compliment: go Dutch.

Peter E.T. Douben

Assessment of factors affecting metal burden in the stone loach (*Noemacheilus barbatulus* L.).

26 May 1989

CONTENTS

To my mother

PREFACE

I wish to thank all those who have contributed to this thesis in one way or other.

First, the person who made me familiar with the principles of toxicology, and encouraged me to pursue the path of research (which has resulted in my presence here today), the promoter Prof. Dr. J.H. Koeman. Secondly, Dr. F. Moriarty, who has supervised me during my stay at Monks Wood.

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I am sure there are other people who have encouraged me; last but not least is my mother: she enabled me to set the first steps in the direction of science. To her I say: **Mamma bedankt**.

Cambridge, May 1989.

P.E.T. Douben

PART I GENERAL

Chapter 1 : Introduction

INTRODUCTION

1. General

Heavy metals are present naturally in the environment in mineral ores. Exploitation of these ores has resulted in the wider distribution of heavy metals in the environment. Some metals, such as lead, have been used by man for millenia whilst others, such as cadmium, have only been exploited this century. Contamination of organisms by heavy metals derives partly from metals remaining in mining waste, partly from refining of ores and partly from the end use of metals. Ores are not pure and metals other than the target metal are frequently discarded to the environment during refining. Heavy metals can end up in different environmental compartments such as air, water and sediment.

Since the mid fifties, the problem of environmental pollution was approached in a much more organised way (see review by Koeman, 1989). Ever since, legislation to protect the environment has proliferated and research into processes in nature and disruption of these processes by pollutants has been radically increased. Also during this era PCB's were discovered (Jensen, 1966). At present, the general public is aware of the consequences of the use of chemicals and the problems of pollution.

Usually explanations for amounts of pollutants found in field specimens are extrapolated from laboratory exposure of organisms. Most laboratory studies measure exposure, whilst most field studies measure dose. It is, however, necessary to link laboratory and field studies to form a complete picture of what influences metal burden of organisms, and to facilitate extrapolation of laboratory data to the field. Models can help to bridge the gap between these two approaches.

The nature of the link between exposure to a contaminant (the concentration of a contaminant in the environment to which an organism is exposed) and the dose (the amount of contaminant taken into the body of an organism) is a central problem in studies of the effects of pollutants in the environment. Although exposure determines the dose, followed by a response/effect of the organism, the exposure-dose relationship is not straight-forward (Doull *et al.*, 1980). For example, exposure is usually kept constant during laboratory studies, while under field conditions it fluctuates. These fluctuations have consequences for the dose. Change in environmental conditions (such as pH, alkalinity) may affect speciation of heavy metals and consequently the readiness with which these metals can be taken up by aquatic organisms. Sorption and desorption processes onto sediment may influence the distribution of pollutants between the different environmental compartments. Increasing temperature results in increasing growth rate and, therefore, possibly the relative contribution of water and food to the body burden of fish (see also chapter 7). Body size is often overlooked as a factor affecting metal burden. Relatively less food is consumed by bigger individuals than by smaller ones of the same species (Niimi, 1983), hence exposure through the diet can be affected by body size. The topic of the exposure-dose relationship is of central importance in the development of ecotoxicology: deeper knowledge of this relationship is important both for understanding and predicting effects of pollutants on wildlife.

The studies in this thesis deal with aquatic systems and the exposure of one species of fish, the stone loach (*Noemacheilus barbatulus* L.), to lead and cadmium.

2. Sources of, use of and pollution by lead and cadmium

Lead is ubiquitous in the natural environment; the most important lead minerals contain the metal as sulphide (galena), carbonate (cerrusite) or sulphate (anglesite). Concentrations of lead differ widely between regions and locations: in freshwater, background levels of lead are in the order of 1 - 4 $\mu g\ l^{-1}$, whilst, for water samples from naturally contaminated areas, levels of 200 $\mu g\ l^{-1}$ in unfiltered water samples and of 150 $\mu g\ l^{-1}$ in filtered samples (0.45 µm pore size) have been reported (Aston and Thornton, 1977; Thorne *et al.*, 1979). Lead concentrations varied between 25 and 140 mg kg^{-1} in sediment from unmineralized areas, and levels of 8500 mg kg^{-1} have been published for sediments from areas with metalliferous mineralisations (Aston and Thornton, 1977).

Cadmium is a relatively rare metal which occurs in nature as a minor component of other, ubiquitous, non-ferrous metal ores such as zinc, lead and copper; substitution of zinc by cadmium in metal ores (sphalerite) is the most common. Background cadmium concentrations in freshwater range up to 1 $\mu g\ l^{-1}$ (Thorne *et al.*, 1979) (see also chapter 6). In areas regarded as non-polluted, the cadmium concentration in soil is up to 4 mg kg^{-1}. Occasionally much higher values are found (up to 30 mg kg^{-1} soil) originating from natural or anthropogenic sources (Aston and Thornton, 1977, Webb *et al.*, 1978) (see also chapter 7).

Lead is used in:

- storage batteries;
- metal products such as ammunition and solder, the manufacture of sound proofing material (both as sheets and composition paneling);
- in petrol as an anti-knocking agent (as tetra-ethyl lead);
- in paints as pigments (as red lead and lead chromates);
- other uses include: various alloys, radiation shielding against gamma rays.

The lead in petrol additive and in batteries formed 75% of the total lead consumption in 1975 in the USA (Nriagu, 1979). In 1979, a total of 123 000 tons were emitted in Europe, of which about 60% was accounted-for by petrol combustion alone (Pacyna, 1986).

Cadmium is used in:

- protective plating on steel (anti-corrosive). The first patent for electroplating of cadmium was issued in England in the 1840s;
- nickel-cadmium batteries, (most for consumer use but also in emergency lighting supplies);
- pigments, particularly cadmium sulfides to give yellow-to-orange colours and cadmium sulphoselenides to give pink-to-red and maroon. Applications of this type are found in traffic paints and, until recently in crates for beer;
- stabilizers for polymers in plastic, for example in the production of PVC;
- various alloys.

Cadmium used in the electroplating formed 34% of the US consumption in 1979, followed by its use in batteries (22%), pigments (13%), in plastics (11%) and for other purposes 3%. About 80% of all the pigment sales in Europe goes to the plastic industry; the corresponding figures for Japan and the US are 60-80% and 75% respectively (Nriagu, 1980b). Total US consumption of cadmium was 4818 tons in 1978 (Moore and Ramamoorthy, 1984). All time production of cadmium up to 1980 was estimated to be 500 x 10^3 tons (Nriagu, 1980a).

Emission of lead due to human activity has increased over the last few centuries to such an extent that anthropogenic emission has now exceeded natural release of lead (Nriagu, 1979; Thornton and Abrams, 1984). Currently, major sources of lead pollution are lead ore mining (usually lead sulphides) and associated activities, and organo-lead fuel additives. Transport of anthropogenic lead is mainly via air. This has resulted in lead contamination at both the North and South Poles (see report by Jaworski *et al.*, 1987). For further details see section 3.

Release of cadmium through natural processes, such as weathering, rarely results in toxic levels of the metal in the environment (Hutton *et al.*, 1987). Environmental contamination due to human activities, such as mining, is not confined to active mines; many disused zinc-lead mines act as a persistent source of cadmium for centuries. About 5% of the cadmium consumed is recovered (15-20% in the USA), which is strikingly low in comparison with the figure of about 40% for lead (Hutton *et al.*, 1987).

There has been a recent increase in cadmium consumption; over half the cadmium ever produced has been refined in the last 20 years (Nriagu, 1979). In contrast to lead, human exposure to cadmium is still very limited. There has not yet been any increase in cadmium content of recent ice cores from the Antarctic or Greenland (Zoller, 1984). Further details of the cycling of cadmium are given in section 3.

Awareness of the toxicity of lead has resulted in measures to reduce the human contribution to environmental load. In particular, progress is being made towards eliminating lead additives from petrol. Also the lead in pipes and tanks, which formerly carried much of the supply of drinking water, is being replaced by copper or plastic (Duffus, 1980). These measures are primarily designed to reduce human exposure to lead but also tend to reduce lead input to the general environment.

3. Global cycling of lead and cadmium

The global cycle of lead and cadmium includes the following compartments and transfers:

1. presence on earth (rocks, water, etc., but also biota);
2. emission into the atmosphere;
3. dispersal in the atmosphere;
4. deposition of airborne particles;
5. redistribution between compartments.

The presence of lead and cadmium in different rock formations has been described in section 2. Emission of both metals due to human acitivity, exceeds the amount emitted through natural activity (table 1). Most of the lead released into the atmosphere is caused by combustion of petrol.

Cadmium is released from natural sources through activities like ocean sprays and volcanic action. These are responsible for about 10% of the

Table 1 Estimated global emission of lead and cadmium (10^3 tons per year) in 1975 from different sources[a].

Source	Metal Lead	Cadmium
Anthropogenic activity		
Metal production and mining	135.5	5.3
Waste incineration	8.9	1.4
Fertilizer manufacture	0.05	0.2
Oil combustion (including petrol)	273[b]	< 0.01
Others	21.5	0.4
Total	43.95	7.3
Natural activity		
Windblown dust	10	0.1
Vulcanic action	6.4	0.5
Vegetation (e.g. exudates, slouch)	1.6	0.2
Others	0.6	0.01
Total	18.6	0.8

[a] data compiled for cadmium from Nriagu (1980a) and for lead from Nriagu (1978a) and Pacyna (1986);
[b] about 267 x 10^3 tons were attributed to anti-knock additives.

atmospheric load (Dederen, 1987; Hutton *et al.*, 1987). Weathering of crystal materials plays a major role in the release of cadmium into the global cycle (Förstner and Wittman, 1983). Non-ferrous metal mines, particularly those which exploit lead and zinc ores, can be a significant source of cadmium contamination. The extent of the pollution varies widely between different ore bodies. Human activity also leads to elevated cadmium levels in the environment, for example through industrial manufacturing of cadmium-containing products, refineries and smelters. It is noteworthy that metal production and mining differ enormously between countries because of the importance of production of steel and base metals. Metal production, then, constitutes a major source of emission of cadmium. Thermal smelters for refining zinc emit cadmium. In the last two decades electrolytic refining has taken over as the major process of zinc production. Cadmium discharges to the environment arise both during the manufacture of cadmium-containing materials (Dederen, 1987) and when these products are discarded. Also, phosphate fertilizers frequenly contain higher concentrations of cadmium than the soil onto which they are applied. The cadmium concentrations of rock phosphate depends on its geographical origin (5-100 mg kg^{-1}); most of it remains in the fertilizer prepared from it (Williams and David, 1976). These fertilizers can be a substantial source of environmental contamination (Andersson, 1976).

Residence time of metals in the atmosphere is in the order of days to weeks (Salomons, 1986). Within this time they can travel large distances. The spread is affected by the type of source (e.g. smelters), location and height of the emitter, as well as weather conditions. Particles with a diameter in the range from 1 to 10 μm stay between 0.1 and 4 days in the atmosphere, smaller ones remain airborne longer in polluted areas (Nriagu, 1980c). This explains why concentrations of both lead and cadmium can be considerably higher in urban air than in rural air (Bewers *et al.*, 1987). In cities, cadmium concentrations between 5 and 50 ng m^{-3} have been measured; about a factor 10-100 above background levels (Nriagu, 1980c). For lead, concentrations of about 0.23 ng m^{-3} have been measured above the Cape of Desire (Novaya Zemla, USSR); in general the lead concentration is between 0.1 and 10 ng m^{-3} whilst levels ranging from 0.5 and 10 μg m^{-3} have been measured in urban air (Nriagu, 1978b).

Two types of deposition of metal from the atmosphere can be distinguished:

1. dry deposition, which occurs by impaction of particles or by absorption of gasses and vapours onto water and vegetation surfaces (Bewers *et al.*, 1987);
2. wet deposition, which consists of rainout (incorporation of trace substances into cloud droplets within the clouds) and washout (scavenging of airborne particles by falling precipitation below the clouds).

The relative importance of each type depends, among other factors, on the distance from the source, the type of pollution, and wheather conditions. Larger particles (diameter 1-10 μm) are being removed more efficiently by both rainout and washout than smaller particles (diameter <0.1 μm). The latter are mainly transported by air turbulence. In general, large proportions of cadmium, (particularly that from industrial sources) are removed rapidly by dry precipitation or washout. This explains the lack of cadmium, released by human activities, in more remote areas (Bewers *et al.*, 1987). It has been suggested that removal of lead in heavily polluted areas is mostly achieved by washout, whereas in remote areas, rainout is more effective (Nriagu, 1978b). About 70% of the atmospheric load of both cadmium and lead are deposited onto land, the remaining 30% ends up in the sea. The sea has an additional input from stream runoff. Cadmium in precipitation is in the soluble form and, therefore, readily available to biota. Concentrations in rainfall are between 0.1 and 50 μg l^{-1} (Nriagu, 1980c); highest levels have been measured near emission sources, for example an average of 3 μg l^{-1} near a smelter at Sudbury compared with 0.8 μg l^{-1} for the remainder of Ontario (Canada). In rural areas levels below 1 ng l^{-1} have been measured (Nriagu, 1980c). Lead concentrations in US rainwater averaged 34 μg l^{-1} (Nriagu, 1978b).

After deposition in, for example, rivers and lakes, the metals are mixed with industrial and municipal wastes, which are often already contaminated with lead and cadmium (Rickard and Nriagu, 1978). Speciation of both metals can alter because of the influence of physico-chemical processes. This has consequences for the exposure of aquatic organisms to these metals.

4. Exposure to heavy metals

There has been long debate about the relative importance of different environmental sources of metals contributing to uptake by organisms. For example, fish live in water, consume food organisms and, sometimes, ingest sediment particles. The water, organisms and sediment could all contain metals. Thus, fish may, in principle, acquire heavy metals from water, food or sediment, or from a combination of all three. There is still discussion over the relative importance of these sources for the metal burden of fish and this may differ between species. For example, McCracken (1987) concluded in his review that uptake of cadmium was mostly from water and that food contributed little to the fish's body burden; sediment was not mentioned as a possible source. However, Kay (1985) suggested that uptake via the diet is an important pathway; again sediment is ignored, although there is some evidence that metal associated with sediment can be taken up by aquatic organisms. Guppies (*Poecilia reticulata*) were exposed to sediments containing various forms of mercury (Gillespie, 1972). Whole body burden of total mercury rose rapidly. *Asellus communis* appeared to take up lead and zinc from clay loam over 20 days' exposure. When the pH rose to 7.5 no significant increase was observed (Lewis and McIntosh, 1986). It has to be mentioned that there is no satisfactory method of distinguishing between uptake from interstitial water (water between sediment particles) and from sediment particles directly. Some authors have attempted to assess the relative importance of the different routes of exposure of fish (table 2).

It is now recognised that not all the metal present is equally available for uptake (Khalid, 1980; see also below). The term "bio-availability" is used to define the readiness with which metal in different forms or from different sources can be taken up by, for example, fish. Bio-availability is governed by chemical speciation of the metal, which is in turn determined by the physical properties of the metal. Changes in chemical speciation can be induced by changes in the physical environment; for example characteristics of water may change, which influence availability of the metal and thus the effective exposure.

Lead in rivers comes from runoff (largely anthropogenic), erosion (mostly natural) and precipitation (Jaworski *et al.*, 1987). It can be present as Pb(2+), as inorganic (e.g. Cl^{-1}) and organic lead complexes or bound to suspended solids. In water with pH between 6 and 8, lead will be entirely complexed, especially as $Pb(CO_3)_2^{2-}$. A large proportion of lead in discharges is organically bound whilst, during storm runoff, transport of lead is mostly as suspended solid (in contrast to cadmium, which is mostly dissolved) (Rickard and Nriagu, 1978). Laboratory studies have revealed that lead is rapidly adsorbed onto sediment particles (Gardiner, 1974b). Sorption is correlated with pH, salinity, organic content and grain size of the sediment particles. The larger the surface area, the greater the binding between metal and particle, which reduces availability to organisms (Duffus, 1980). It forms stable complexes, thus relatively little lead (≤ 3 $\mu g\ l^{-1}$) is in solution (i.e. fractions smaller than 0.45 µm) in uncontaminated water (Förstner and Wittman, 1983). Transport of lead in freshwater is determined by the

Table 2 Comparison of relative importance of water, food and sediment as sources of cadmium and lead for different fish species.

Metal	Fish species	Type of observation	Relative importance of uptake from water	food	sediment	Reference
Cd	*Etheostoma flabellare*	Field	+	+	+	Ney and Van Hassel, 1983
	Gambusia affinis	Laboratory	++	+	n.a.	Williams and Giesy, 1978
	Leucaspius delineatus	Laboratory	+	++	n.a.	Ferard *et al.*, 1983
	Pleuronectes platessa	Laboratory	+	+	n.a.	Pentreath, 1977
	Poecilia reticulata	Laboratory	++	+	n.a.	Hatakeyama and Yasuno, 1982
	Raja clavata	Laboratory	+	+	n.a.	Pentreath, 1977
	Rhinicthys atratulus	Field	+	+	+	Ney and Van Hassel, 1983
	Salmo gairdneri	Field	+	+	n.a.	Dallinger and Kautzky, 1985
Pb	*Etheostoma flabellare*	Field	+	+	++	Ney and Van Hassel, 1983
	Poecilia reticulata	Laboratory	+	++	n.a.	Vighi, 1981
	Rhinicthys atratulus	Field	+	+	++	Ney and Van Hassel, 1983
	Salmo gairdneri	Laboratory	++	0	n.a.	Hodson *et al.*, 1978a
		Field	+	+	n.a.	Dallinger and Kautzky, 1985

n.a.: not addressed;
0 : indicates no contribution from this source;
+ : indicates substantial contribution from this source;
++ : indicates most contribution from this source.

movement of particles to which it is bound (Thorne *et al.*, 1979). (For comparison with this study see chapter 6).

Most cadmium in fresh water exists as Cd(+2) ions (Gardiner, 1974a). It begins to hydrolyse when pH rises to about 9, forming $Cd(OH)^+$ species. Binding to inorganic ligands, such as chloride, to form $CdCl^+$ and $CdCl_2$, depends on the concentration of chloride. Organic content of water generally decreases uptake and toxic effect by binding cadmium and reducing availability to organisms. Increasing salinity or water hardness both reduce the proportion of free ionic cadmium and decrease toxic effect of cadmium (Taylor, 1983; Calamari *et al.*, 1980), while rates of uptake are less affected (Wiener and Giesy, 1979). It is thought that mainly ionic cadmium (free cadmium ion) is taken up by aquatic organisms (Pärt *et al.*, 1985). It is not clear from the published literature whether this is due to preferential uptake of cadmium or because most of the cadmium is in that form. Not all cadmium in food items (Siewicki *et al.*, 1983) and associated with sediment particles (Khalid, 1980) may be available to organisms. The readily available fraction of cadmium in sediments is the cadmium adsorbed on exchange complexes, whilst cadmium in the crystalline lattice of clay minerals is very much unavailable (Khalid, 1980).

Chemical extraction (e.g. sequential extraction) of metals provides information on the binding between heavy metals and sediment particles (Tessier *et al.*, 1979). Adsorption and/or precipitation with oxides, hydroxides and hydrous oxides of iron and manganese are important processes that affect availability to aquatic organisms (Bewers *et al.*,1987; Khalid, 1987). However, there are few studies comparing results from chemical extraction with biological uptake (Calmano and Förstner, 1983).

5. Function, fate and effects of heavy metals

Some heavy metals are required by organisms (in small amounts) as cofactors to enzymes, but at high exposure they become toxic, e.g. zinc, molybdenum. Others have no known essential function and are toxic to organisms even at low concentration. Metals can interact with each other, for example by competitive binding, and cause harmful effects. Competitive binding can occur with detoxifying metal-binding proteins such as metallothioneins. Since metals are elements they cannot, of course, be broken down; once absorbed into an organism, they can only be bound (to reduce their toxic effect) or excreted. Similarly metals are highly persistent in the environment (Friberg *et al.*, 1979).

Lead is taken up by many organisms. It can affect the central nervous system and peripheral nerves (affecting Na^+-K^+-ATPase activity), kidney or the haematopoietic system (Doull *et al.*, 1980), according to the type of exposure. Lead has great affinity for bone and cartilage (Doull *et al.*, 1980; Moore and Ramamoorthy, 1984). High levels have been found in gills, liver and kidney (and also in blood) of fish (Hodson *et al.*, 1978b). Although the final concentrations of lead in the body can be high, apparently no biomagnification (concentration increasing via a food chain) occurs (WHO, 1989). Steady-state concentrations of inorganic lead are reached after exposures in the order of weeks. Tetra-alkyl lead, however, is rapidly taken up and eliminated.

Cadmium is readily accumulated by many organisms resulting in high bioconcentration factors (ratio of concentration of the metal in the organism to the concentration of metal in the medium) (Moore and Ramamoorthy, 1984).

The metal is bound in liver and kidney to specific cadmium-binding proteins of low molecular weight: metallothioneins. Induction of metallothionein production occurs in the order of a few days (Sheehan, 1984; Siewicki *et al.*, 1983). Cadmium seems to displace zinc in many enzymatic reactions which leads to disruption or cessation of activity (Moore and Ramamoorthy, 1984). Uptake and loss of zinc is regulated by the organism, whilst the evidence suggests that cadmium is not. Loss of cadmium from organisms is principally through the kidney, although considerable amounts can be eliminated through the gills (Coombs, 1979).

There exist a variety of sublethal toxic effects of lead in fish, including the 'black tail effect' (darkening of the caudal region), scoliosis (spinal curvature) and effect on enzymes. Fingerling trout showed the 'black tail effect' after 6 months' exposure to 31.6 $\mu g\ l^{-1}$ dissolved lead in hard water (353 mg l^{-1} as $CaCO_3$) and after exposure to 14.6 $\mu g\ l^{-1}$ in soft water (28 mg l^{-1}). When the fish had been hatched from pre-exposed eggs in soft water, the effect was noted at 7.6 $\mu g\ l^{-1}$ (Davies *et al.*, 1976). Spinal deformities were observed about one month after blackening of the tail. Older rainbow trout (*Salmo gairdneri*) developed black tails after 32 weeks exposure to 120 $\mu g\ l^{-1}$ lead (hardness 135 mg l^{-1} as $CaCO_3$) (Hodson *et al.*, 1978a). Spinal deformities in second and third generation of brook trout (*Salvelinus fontinalis*) were observed after exposure to 119 $\mu g\ l^{-1}$ total lead (84 $\mu g\ l^{-1}$ dissolved lead), whilst hardness of the water was 44 mg $CaCO_3$ per litre (Holcombe *et al.*, 1976). Activity of amino levulinic acid dehydratase (delta-ALAD) in red blood cells of rainbow trout was reduced at concentrations of 13 $\mu g\ l^{-1}$ lead over 4 weeks (Hodson *et al.*, 1976 and 1978a) and of 10 $\mu g\ l^{-1}$ for 30 days (Johansson-Sjöbeck & Larsson, 1979). Avoidance reactions were observed in trout exposed to 26 $\mu g\ l^{-1}$ (Giattina and Garten, 1983).

Exposure to cadmium concentrations of 7.5 μg l^{-1} over 70 days resulted in spinal deformities in minnows (*Phoxinus phoxinus*) (Bengtsson *et al.*, 1975). Fathead minnows (*Pimephales promelas*) appeared to be more prone to predation after sub-lethal exposure to 25 μg l^{-1} for 21 days (Sullivan *et al.*, 1978), which is below the maximum acceptable toxicant concentration (between 37 and 57 μg cadmium l^{-1}) for this species with respect to survival and reproduction effects (Pickering and Gast, 1972).

6. The present study

6.1 Aspects of the biology of the stone loach (*Noemacheilus barbatulus* L.)

This thesis comprises work on the exposure-dose relationship of heavy metals, particularly cadmium and lead, in the fish *Noemacheilus barbatulus* L. (stone loach). It has been centred around factors which might influence the cadmium and lead burden in the stone loach, a common fish species in British rivers that is suitable for laboratory experiments.

Stone loach is a 'elongated' fish, usually brown (figure 1), but can adapt its colour varying from yellow/grey to green/brown to match the background. It is abundant in many parts of Europe, as well as in some streams in Siberia, but not in Greece and Mid and Southern Italy. The loach lives near the bottom of rivers, with sandy beds covered with stones, under which it sometimes hides. Hyslop (1982) reported that stone loach bury themselves in the river bed, particularly during the winter period (see also chapter 5). The activity peak of loach occurs for a few hours immediately following dusk (Welton *et al.*, 1983). The stone loach is a non-visually foraging predator (Street and Hart, 1985), locates food with its barbels and feeds on a variety

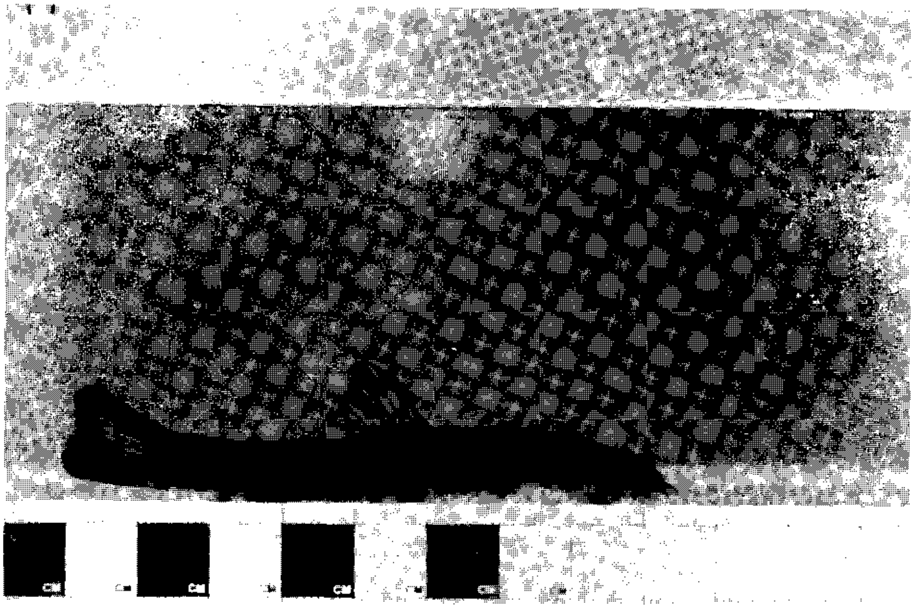

Fig. 1 Stone loach (*Noemacheilus barbatulus* L.)

of invertebrates, the species differing with season (Hyslop, 1982; Welton *et al.*, 1983). (See also chapter 7).

6.2 Particulars of the present study

The work described here comprised both laboratory experiments and field studies; most of the laboratory work only studied cadmium, while field work included both lead and cadmium. The work was based on the premise of

DeFreitas and Hart (1975) that metabolic rate is an important factor affecting metal burden. Metabolism of fish (including growth) depends on body weight and temperature of the water and affects rates of uptake and loss of metal. This appeared to be a valid approach to the body burden of methyl mercury in yellow perch (*Perca flavescens*) in the Ottawa River (Norstrom *et al.*, 1976) and for the herring (*Clupea harengus harengus*) (Braune, 1987).

Although fish of similar size are commonly used in experiments to reduce variation, this study deliberately included loach covering a wide range of weight. Metabolic rate is, to a very large extent, affected by body size, and so is metal burden. Applying statistical analysis (analysis of covariance) separated out information on the effect of body size on, for example, metal burden. Field studies also included fish of different body weight.

Laboratory experiments were used to estimate rates of uptake and loss of cadmium during and after exposure to cadmium in water (chapter 2), food (chapter 3) and sediment (chapter 4). Metabolic rate was estimated by measuring oxygen consumption and feeding rate at different temperatures and under different light regimes (chapter 3). During experiments, loach were kept individually in aquaria to avoid interaction between fish (Street and Hart, 1985). They were exposed in a continuous-flow system based on one described by Benoit *et al.* (1982).

Stone loach are present in a similar habitat to that of the bullhead (Hynes, 1972). Since there is some published work on bullhead (Moriarty *et al.*, 1984), sites for the present study were selected in the same area, so that the species could be compared where appropriate. Also, Nichol *et al.* (1970) reported high levels of lead and zinc in stream sediments from the valley of the River Ecclesbourne, which probably originate from mineral veins at the head of the valley. The mining activity started at least with the Romans and ceased around the 17th century.

A one year sampling programme was carried out to obtain information on growth rate and fluctuations in metal burden in stone loach under field conditions (chapter 5). Field studies were restricted to three sites covering a range of concentrations of cadmium and lead in the environment, particularly in sediment (Webb *et al.*, 1978): the River Ecclesbourne (national grid reference SK 288 505), the Brailsford Brook South (national grid reference SK 284 452) and a site in the Suttonbrook (national grid reference SK 222 342). This provided information on the relationship between metal concentration in the environment and the metal burden of stone loach. Information on changes of metal concentration in water (chapter 6), food and sediment (chapter 7) is important for assessing the magnitude of exposure for aquatic organisms.

For fish from both laboratory experiments and field studies, total body burden was calculated by adding the mass of metal in gills and the rest of the body together, since gills were analysed separately. Total body burden was used to compare different fish because, at least for bullhead, most of the variation in metal content of specific organs of bullhead was related to differences in total body burden (Moriarty *et al.*, 1984). Most of the cadmium in fish is usually found in the kidney and liver, followed by the gills, according to the route of exposure (Harrison and Klaverkamp, 1989). To study the transfer of cadmium through the food chain and to assess the relative importance of different sources of cadmium, whole-body burdens should be considered since the predator will not be selective and feed only on certain tissues (Kay, 1985). Additionally, comparisons between fish taken from different sites or at different times were best based on total mass of metal in each fish because differences in body burden can be masked by differences in weight.

6.3 Chemical analyses

Details of methods of metal analyses have been given in the individual papers (e.g. chapter 2). As part of good laboratory practice, the accuracy of the analytical results from the graphite furnace were tested. Some subsamples of a homogenized sample of the stone loach were analysed in a different laboratory (Central Veterinary Institute (CDI), Lelystad, the Netherlands). The single difference in method was that 10 µl of a matrix modifier, a 1:1 mixture of 40 g l^{-1} $NH_4H_2PO_4$ and 5 g l^{-1} $Mg(NO_3)_2$, was injected with the sample into the graphite chamber of the atomic absorption spectrophotometer at CDI, but not at Monks Wood.

No cadmium was detected in two samples at Monks Wood (limit of detection 5.5 ng) and no lead was detected in two other samples at CDI (limit of detection 20 pg). These results were excluded from calculations hereafter (figure 2). Results were transformed onto a logarithmic scale. The differences between the CDI and Monks Wood results for each sample were normally distributed, and in general, results from the two laboratories were comparable, although there was a significant, relatively small mean difference for lead (table 3).

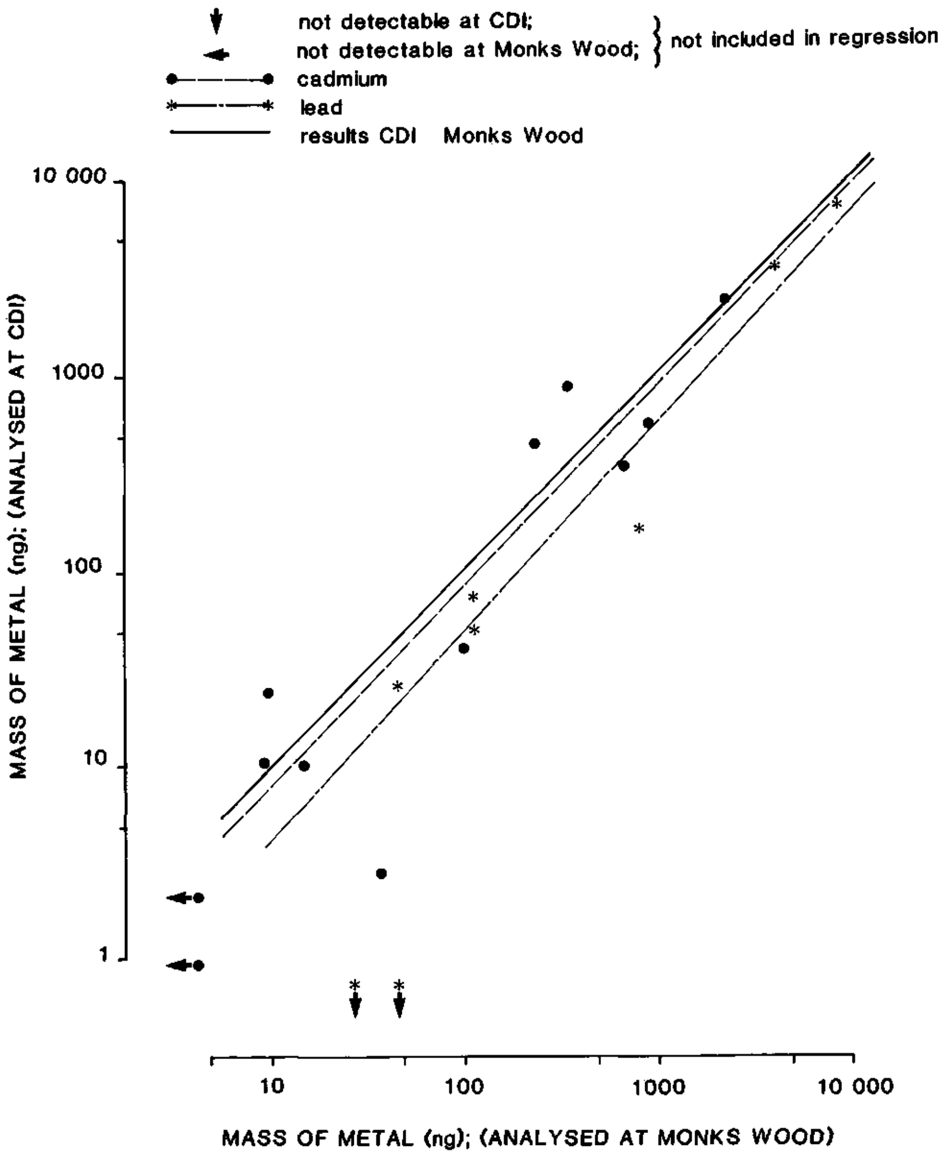

Fig. 2 Inter-laboratory comparison of chemical analyses for cadmium and lead in stone loach: regression of cadmium (—— ——) and lead (—— – ——) results from CDI on those for Monks Wood.

Table 3 Results for mass of cadmium and lead (ng) determined in two laboratories. In 2 samples metal levels were below the limit of detection and these samples were excluded from computations. Metal levels were transformed onto a logarithmic scale.

	Metal (ng)	
	Cadmium	Lead
Number of samples analysed with detectable levels	10	6
Limit of detection	5.5[a]	0.02[b]
Mean of difference[c] ± s.e.	0.09 ± 0.14	0.26 ± 0.10

[a] refers to analyses at Monks Wood;
[b] refers to analyses at CDI;
[c] indicates the difference in metal levels for each sample as determined by the two laboratories.

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PART II LABORATORY EXPERIMENTS

CHAPTER 2: Uptake and Elimination of Water-borne Cadmium by the Fish *Noemacheilus barbatulus* L. (Stone Loach).

Arch. Environ. Contam. Toxicol., in press.

UPTAKE AND ELIMINATION OF WATER-BORNE CADMIUM BY THE FISH *NOEMACHEILUS BARBATULUS* L. (STONE LOACH).

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ABSTRACT

Fish *Noemacheilus barbatulus* L. (stone loach), were exposed to cadmium in water to study rates of uptake and loss in three experiments: one during which they were exposed for up to 4 days to 1.0 mg/L cadmium and subsequently kept in clean water for up to another 8 days at 8°, 16° and 18° C; a second one during which fish were exposed to a range of cadmium concentrations in water (0.08 - 0.93 mg/L) and a third one during which they were starved or fed with *Tubifex* while some were exposed to 0.067 mg/L cadmium. All levels were well below those that are acutely toxic. Results showed that fed fish did not change weight while all starved fish lost weight, at a higher rate for exposed fish than for control fish. Size of the fish affected rates of uptake and loss of cadmium. These rates increased with temperature. Bioconcentration factors decreased with size of fish, increased with temperature up to about 16 °C and decreased as the concentration of cadmium in water increased. Feeding appears to increase the rate of cadmium intake from the water. The data indicate that metabolic rate governed rates of uptake and loss to an appreciable extent.

INTRODUCTION

Knowledge of the exposure-dose relationship is important for understanding and predicting effects of pollutants on wildlife, and physiological processes play an important role in the rates at which an organism takes up and gets rid of contaminants (DeFreitas and Hart, 1975; Niimi, 1983). Therefore, studie were made of the effect of body weight, temperature and starvation - all factors that influence metabolic rate in fish - on the intake and loss of cadmium from ambient water by the stone loach (*Noemacheilus barbatulus* L.), a fairly common fish in British rivers.

MATERIALS AND METHODS

Sampling and Maintenance of Fish

The stone loach were caught on three occasions by electric fishing from the Suttonbrook, a small stream in Derbyshire (National Grid Reference SK 222 342). This site had low concentrations of heavy metals in the sediment (Nichol *et al.*, 1970; Webb *et al.*, 1978). Fish were kept in storage tanks at about 16 °C for at least one month during which period they were fed on a mixture of live food (*Tubifex* spp.) and pellets of fish food, to adapt the fish to domestic water.

The Exposure System and Cadmium Solution

Fish were exposed to cadmium in water in a continuous-flow system, developed from one described by Benoit *et al.* (1982). The water came from the mains supply (for water quality see Table 1) and cadmium sulphate was added from a stock solution in distilled water. Preliminary experiments (Table 2) showed no detectable precipitation or colloidal formation of cadmium had occurred (Benes and Major, 1980; de Mora and Harrison, 1983).

The fish were kept singly, to avoid interaction (Street and Hart, 1985) and to enable valid comparisons to be made between results obtained for individual fish, in aquaria with 6 L of water and a flow-rate of about 1.8 L/hr. Cadmium concentration was checked daily (Table 3). The temperature of the water was kept at the nominal value ± 0.1 °C. The light-dark regime was 12 hr light : 12 hr dark during all experiments.

Experimental Procedure

Table 3 summarizes the three experiments. During the first and the second experiment, fish were starved to estimate uptake of cadmium from water only and to avoid any uptake from food (Taylor, 1983). Effect of feeding and interaction with water-borne exposure were studied in the third experiment.

Table 1 Aspects of quality of mains water used during the three experiments described in table 3; mean, standard error (s.e.) and number of measurements (n), cation and anion concentrations in mg/L.

variable	mean	±	s.e.	n
pH	7.7	±	0.1	18
Conductivity (μS)	779	±	11	18
Ca^{2+}	121.6	±	3.4	45
Mg^{2+}	8.87	±	0.31	45
K^{+}	9.72	±	0.10	45
Na^{+}	54.1	±	1.0	45
total - P	0.25	±	0.02	18
PO_4^{3-} - P (dissolved)	0.18	±	0.01	18
Org. N + NH_4^+-N	0.45[a]	±	0.04[a]	17[a]
$NO3^-$ - N (dissolved)	3.53	±	0.70	18
NH_4^+ - N (dissolved)	[b]			
Cl^-	66.8	±	0.8	18
SO_4^{2-} - S	56.8	±	0.8	4

[a] the concentration in one sample was below the limit of detection of 0.10 mg/L and was excluded from computations;
[b] the concentration in all samples was below the limit of detection of 0.10 mg/L.

Table 2 Concentration of cadmium, before and after centrifugation at two different speeds, in water samples taken from experimental aquaria.

sample no.	concentration of cadmium in stock solution (mg/L)	degree of dilution with tap water	nominal cadmium concentration (mg/L)	observed cadmium concentration in water samples (mg/L)		
				untreated	after centrifugation at 3000 g	30 000 g
1	187	1 : 360	0.52	0.48	0.45	0.45
2	195	1 : 360	0.54	0.49	0.46	0.45
3	106	1 : 180	0.59	0.59	0.60	0.60
4	108	1 : 180	0.60	0.59	0.60	0.61
5	223	1 : 180	1.24	1.15	1.15	1.12
6	220	1 : 180	1.22	1.21	1.21	1.21

Table 3 Summary of details of the experiments during which stone-loach were exposed to water-borne cadmium.

Experiment	Temperature (°C)	Feeding regime	Cadmium concentration during exposure (mg/L) nominal	measured mean	± s.e.	no. of samples	Date fish caught in Derbyshire	Total no. of fish used per experiment	Range of dry weights of fish used (mg)
1	8	starved	0.0	n.d.[a]		10			
			1.0	1.01	± 0.04	50	07.04.86	55	
	16	starved	0.0	n.d.[a]		10			
			1.0	1.03	± 0.06	36	07.04.86	55	
	18	starved	0.0	n.d.[a]		10			
			1.0	1.08	± 0.02	75	09.09.86	55	
									25 - 1814
2	16	starved	0.00	0.0028	± 0.0002[b]	10			
			0.10	0.08	± 0.00	20			
			0.22	0.18	± 0.00	10			
			0.46	0.41	± 0.01	10			
			1.00	0.93	± 0.01	10	15.05.87	40	58 - 2001
3	16	fed/	0.00/	0.0007	± 0.0001[b]	12			
		starved	0.08	0.067	± 0.009	30	15.05.87	50	137 - 1228

[a] not detectable on flame AA; limit of detection 0.02 mg/L;
[b] samples analysed on electrolyte furnace.

Before an experiment, temperature in the storage tanks was gradually changed from 16 °C towards the experimental temperature, thus allowing the fish to acclimatize further for two to three days (five days for change towards 8 °C). Fish were then caught individually in a net, which was dried on the outside with a slightly damp tissue paper for about five seconds. The fish were weighed by transferring them to a tared vessel containing water on a balance. More accurate measurements would have killed the fish. They were arrayed in order of weight and allocated at random within successive blocks of fish to each treatment to give five fish per treatment (in the third experiment, ten fish per treatment). The fish were transferred to the experimental room for final acclimatization for one day (Solbé and Flook (1975); Solbé and Cooper (1976)).

In the first experiment, fish were sampled on days 1,2 and 4 after which exposure to cadmium ceased and those fish remaining were kept in clean water. In the second experiment, covering a range of cadmium concentrations in the water, the loach exposed to the lowest concentration (0.08 mg/L) were sampled as in the first experiment, all others on day 4 only. In the third experiment fish were sampled at the start of the experiment and after 4 days, while feeding took place just before darkness when the loach became active (Welton *et al.*, 1983). During the experimental period fish were left undisturbed to avoid any outside interference.

After sampling, fish were killed and weighed, this time directly on a balance on aluminium foil after blotting dry for about five seconds. Consequently, even if there had not been any actual change in weight, the final weight of any individual fish was less than its estimated initial weight because of inevitable loss of moisture (see Fig. 1 for experiment 1).

The gills were dissected to analyse them separately from the rest of the body for purposes outside the scope of this paper. The samples were weighed, dried at 85°C for 3 days and then reweighed. No deaths occurred in any experiment.

Metal analysis

The fish were digested in chemically-clean boiling tubes with 1 ml 'Ultrar' concentrated nitric acid. If necessary, especially with big fish, small additional volumes of acid were added. The samples were completely digested when no brown fumes appeared at a temperature of 120°C. The volume of acid was made up to 1 ml after digestion. The contents of each boiling tube were decanted into a measuring cylinder, rinsed with deionized water and made up to a volume of 20 ml with 5% nitric acid. The solution was poured into a glass vial with polythene screwtop, ready for cadmium determination by an atomic absorption spectrophotometer (electrolyte furnace with background noise correction).

Statistical Computations and Mathematical Modelling

Wet weight of each fish before the start of the experiment and at the time of sampling were used to estimate the proportional change of wet weight for fish individually during the experiment. These estimates were compared by analysis of covariance to evaluate the effect of treatment on change of weight. The results are therefore expressed on a wet weight basis. Changes of compartmental volumes can affect concentration of pollutant, quite independent of any transfers of pollutants between compartments (Moriarty, 1975). It is therefore more appropriate, when fish weight changes during an experiment, to measure mass rather than concentration of pollutant (Moriarty, 1984).

The total metal burden in the fish was obtained by adding together the results for the gills and those for the rest of the body. The data were then transformed onto a logarithmic scale so that the residual variation in the data conformed more nearly to a normal distribution.

For each experiment the effect of treatments on cadmium burden were tested by analysis of covariance (Sokal and Rohlf, 1981) with dry weight of the body as the independent variable (covariate) and with temperature, concentration of cadmium in the water, time of sampling and feeding regime (where appropriate) as factors. Analysis of covariance is based on assumptions of homogeneity of residual variances about the individual regressions and similarity of slopes. These assumptions were tested in each instance. The effect of body weight on cadmium burden was then estimated, for each experiment, by using the common regression coefficient. The effects of different treatments were assessed by comparisons of the regression intercepts. The results for the different treatments are given as means adjusted for weight: the cadmium burden for a fish of standard weight. The standard weight of fish was taken as the mean weight of all the fish in each of the three experiments separately.

To use the experimental data to obtain estimates of the parameters in the one-compartment model, data from the uptake and elimination experiment were adjusted for different weights according to equation 1 and then back-transformed to an arithmetic scale according to equation 2:

$$\text{Alog Cd}_i = \log \text{MCd}_i - \hat{b} * (w_i - \bar{w}) \qquad (1)$$

$$\text{ACd}_i = 10^{(\text{Alog Cd}_i + \sigma^2/2)} \qquad (2)$$

in which: $\bar{w}$ = mean logarithmic weight of all fish in the analysis of covariance;

w_i = logarithmic weight of the i-th fish;

$\hat{b}$ = estimated common slope of the regression of cadmium on weight after logarithmic transformation;

MCd_i = mass of cadmium (ng) in the i-th fish;

Alog Cd_i = logarithmic value of the mass of cadmium of the i-th fish adjusted for weight;

σ^2 = residual variance in the analysis of covariance;

ACd_i = adjusted mass of cadmium (ng) of the i-th fish on an arithmetic scale.

The results of the three temperatures for each period (during or after exposure) were fitted to the equation for a one-compartment model (Atkins, 1969).

RESULTS

One fish from experiment 1 at 8°C sampled on day 8, had an anomalously high cadmium content of 38.4 μg, 3.54 standard deviations from the predicted value. Therefore this fish was excluded from further computations.

Loss of Weight

Analysis of covariance for the proportional loss of wet weight on the initial weight for each of the three experiments shows that the residual variances about the regressions lack homogeneity in experiments 1 and 3 (Table 4a). However, the number of observations for each treatment is the same in all groups in the third experiment and in all but one group in the first experiment. Therefore, this lack of homogeneity is relatively unimportant (Scheffé, 1959). The slopes of the regressions are not significantly dissimilar. The assumptions of this analysis appear to be adequately satisfied.

Some apparent loss of wet weight is inevitable in fish sacrificed on day 0, because of the different weighing methods used for initial allocation to treatments and when sacrificed. Despite this technical point, the data show that, as expected, starved fish lose weight with time (Fig. 1, similar results were found in the second and third experiment). The regression coefficient for weight loss is greater at 16° than at 8°C (Table 5). Comparison of loss of weight by control fish kept at 18°C cannot be readily made as the fish sampled on day 0 have lost more weight than at the other temperatures.

In the first experiment, fish at all three temperatures lose weight significantly during exposure ($P<0.05$ for each temperature) while after

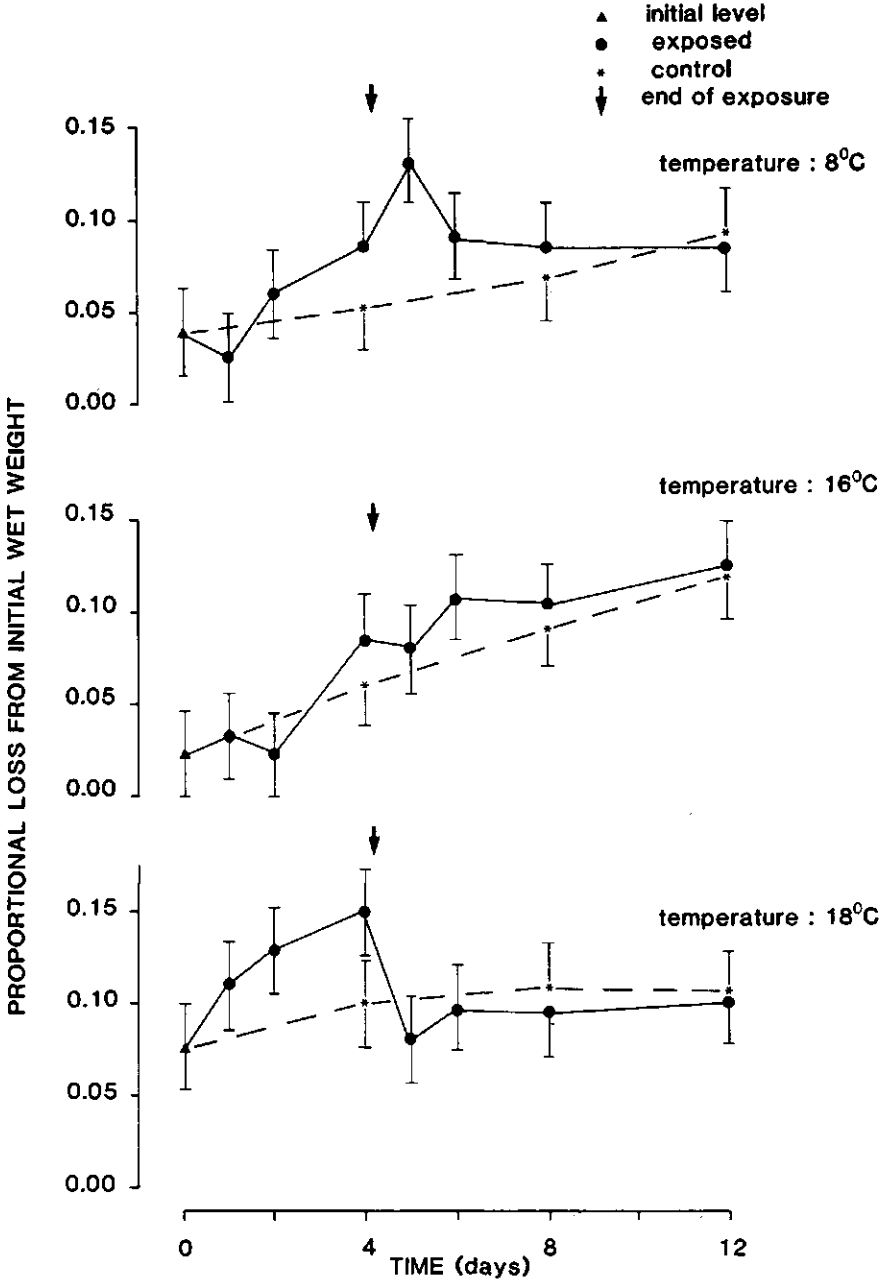

Fig. 1. Proportional loss with time from initial wet weight and standard errors of stone loach of 2679.2 mg initial wet weight; experiment 1. ▲ indicates apparent loss of weight at the start of the experiment.

exposure (between day 4 and 12) there is no significant loss in any group (Table 5). However, there appears to be an effect of temperature on the regression coefficient ($F_{2,56}$ = 3.86, P<0.05) resulting in significantly less weight loss at 18° than at 8° and 16°C.

In the second experiment there is a difference in loss of weight between the groups (P<0.10, Table 4a). Control fish lose weight during the 4 days' period (P<0.05) similarly to those in the first experiment. Also, loss of weight by fish exposed to 0.08 mg/L cadmium concentration is significant (P<0.05) but there is no effect of concentration on the proportional loss of weight by fish all sampled after 4 days (Table 5). The latter result is confirmed by the results of the third experiment by the two groups of starved fish. The weight of fed fish does not change significantly during the experiment. It is therefore desirable to express the amount of cadmium in the loach in units of mass rather than of concentration to evaluate the effect of the different treatments.

Effect of Body Size on Cadmium Burden

The residual variances about the regressions are homogeneous except in experiment 1 (Table 4b) but as only 1 out of 33 groups does not contain five observations, this lack of homogeneity can be ignored (Scheffé, 1959). The slopes of the regressions of cadmium burden on body weight are compared for the groups of five fish used for each treatment. There is little evidence, in any of the three experiments, for significant differences in the regression coefficients. In the first experiment, the common slopes for each temperature are greater at 8 and 16°C (0.38 ± 0.14 and 0.39 ± 0.18 respectively) than at 18°C (0.25 ± 0.10), although the differences are not significant ($F_{2,128}$=0.30).

Table 4 Summary of results of analysis of covariance for a. the proportional loss of weight on initial wet weight, b. the logarithmic amount of cadmium on the logarithm of the dry weight of stone loach used in three different experiments.

a. proportional loss of weight on wet weight

Experiment no.	Tests for homogeneity of residual variance about regression χ^2	df	Tests for dissimilarity of individual slopes	Estimate for common slope	±s.e.	Variance ratio for effect of treatments
1	$\chi^2 = 53^{*}$	32	$F_{32,98} = 1.15$	-0.008	±0.002	$F_{32,130} = 1.88^{**}$
2	$\chi^2 = 9.04$	7	$F_{7,24} = 0.90$	-0.014	±0.004	$F_{7,31} = 2.07^{+}$
3	$\chi^2 = 12^{*}$	4	$F_{4,40} = 0.75$	-0.016	±0.008	$F_{4,44} = 2.45^{*}$

b. logarithm of the mass of cadmium on the logarithm of dry weight

Experiment no.	Tests for homogeneity of residual variance about regression χ^2	df	dissimilarity of individual slopes	Estimate for common slope	±s.e.	Variance ratio for effect of treatments
1	$\chi^2 = 51^{*}$	32	$F_{32,98} = 1.01$	0.34	±0.08	$F_{32,130} = 12.30^{***}$
2	$\chi^2 = 11.8$	7	$F_{7,24} = 1.01$	0.66	±0.08	$F_{7,31} = 12.26^{***}$
3	$\chi^2 = 3.99$	4	$F_{4,40} = 0.34$	1.15	±0.17	$F_{4,44} = 12.13^{***}$

$^{+}$, *, ** and *** indicate significance at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively.

Table 5 Regression of the proportional loss of wet weight of stone loach, adjusted for difference in weight, on either time of sampling t, both during and after exposure to water-borne cadmium (a. experiment 1 and b. experiment 2), or on the concentration of cadmium in water when sampled at day 4 (b. experiment 2).

a. experiment 1.

Temperature (°C)	Cadmium in ambient water (mg/L)	Period (days)	Estimates for coefficient b	±	s.e.
8	0.0047	0 - 4	0.0032	±	0.0032
		4 - 12	0.0050^{+}	±	0.0025
	1.01	0 - 4	0.0132^{*}	±	0.0048
		$4 - 12^{\#}$	-0.0024	±	0.0038
16	0.0047	0 - 4	0.0095^{*}	±	0.0038
		4 - 12	0.0072^{*}	±	0.0028
	1.03	0 - 4	0.0152^{*}	±	0.0063
		4 - 12	0.0050	±	0.0047
18	0.0038	0 - 4	0.0059	±	0.0086
		4 - 12	0.0006	±	0.0034
	1.08	0 - 4	0.0171^{*}	±	0.0083
		4 - 12	-0.0026	±	0.0046

b. experiment 2.

Fixed parameter	Estimates for coefficient b	±	s.e.
time	0.0371	±	0.0337
cadmium concentration of 0.08 mg/L	0.0214^{*}	±	0.0078
cadmium concentration of 0.0027 mg/L	0.0187^{*}	±	0.0080

Notes: $^{+}$ and * indicate significant difference from zero at the 0.10 and 0.05 probability levels respectively;
$^{\#}$ denotes that one fish sampled at day 8 was excluded, see text for details.

The common slope for the three temperatures combined, representing the body weight exponent, equals 0.34 ± 0.08.

In the second experiment the slopes are not different from each other ($F_{7,24}$ = 1.01) and the body weight exponents for the cadmium burden in the total fish equals 0.66 ± 0.08. For the third experiment the value for the common slope is 1.15 ± 0.17 while there is no effect of treatment ($F_{4,40}$ = 0.34).

Temperature Effect on Cadmium Burden

The rapid increase and, where observed, decline in the cadmium burden of the fish in the first and second experiment (Figs. 2 and 3) indicate high rates of loss of cadmium from the fish. The loss after exposure follows a curvilinear (i.e. biphasic) pattern for fish kept at 16° and 18°C (Table 6). The lack of curvilinearity at 8°C may be a result of the effect of lower temperature on metabolism. Control fish also lost a significant amount of cadmium (Table 6), although unlike the exposed fish and despite the larger period of observation, there were no significant departures from a linear regression.

This biphasic pattern indicates that the cadmium in the loach is not homogeneously distributed and that, consequently, a two-compartment model is appropriate for describing the body burden of cadmium after exposure. However, data fitted to a two-compartment model do not reduce the residual variation to a significant extent when compared to a one-compartment model. Therefore a one-compartment model appears to be adequate to describe the cadmium burden both during and after acute exposure. Estimates from a one-compartment model for the rate of cadmium intake during exposure show that the value of I for fish exposed to 1 mg/L is rather small compared to that for fish exposed to 0.08 mg/L (Table 7).

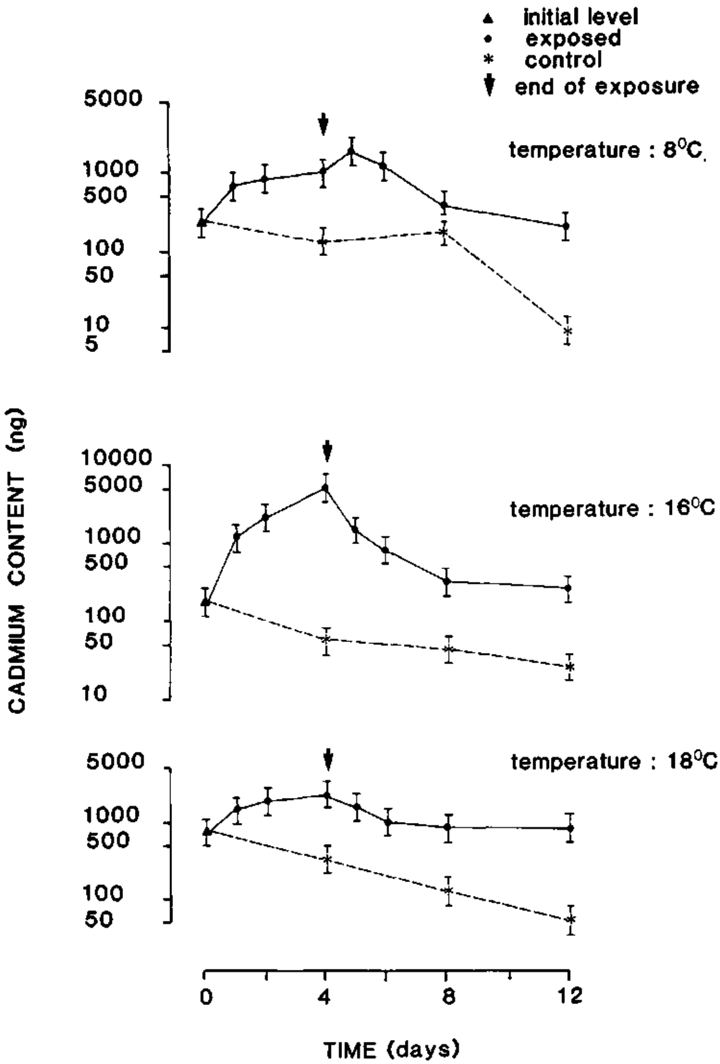

Fig. 2. Mean cadmium content (ng) and standard errors of stone loach of a standard weight of 473.7 mg dry weight at the start of the experiment (Δ), used as controls (*) or during and after exposure (●) to water-borne cadmium (1 mg/L) for a maximum of 4 days after which the remaining fish were kept in clean water. Experiment 1 performed at three different temperatures. Note: One fish sampled at t=8 days at 8° C excluded; see text for details.

Results for fish kept at 18°C cannot readily be compared with those for fish kept at 8° and 16°C, because they had a relatively high initial body burden of cadmium. Random variation for the estimate of initial body burden on day

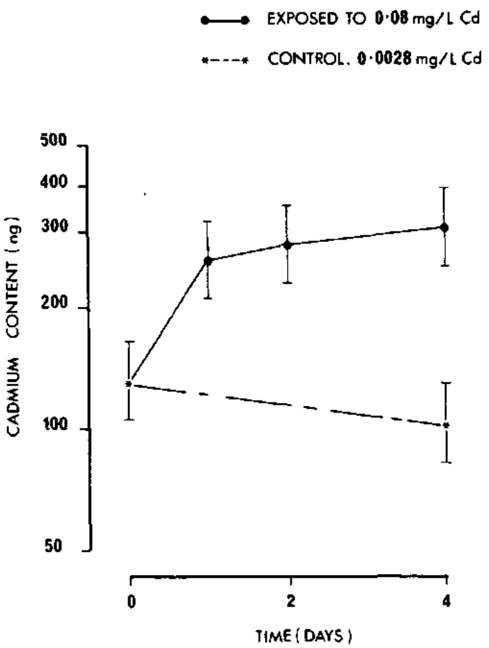

Fig.3a.

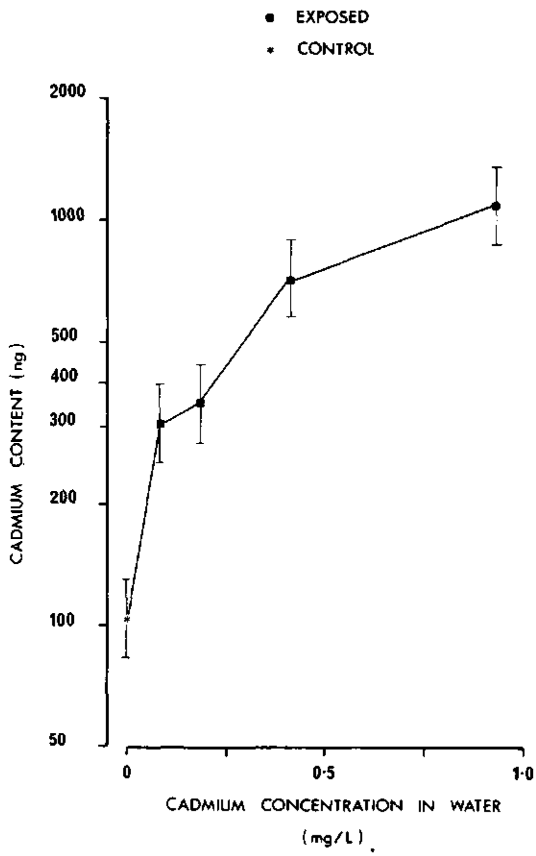

Fig.3b.

Fig. 3. Mean cadmium content (ng) and standard errors of stone loach of a standard weight of 590.1 mg dry weight exposed to (a) 0.08 mg/L cadmium in water and sampled at different times or (b) exposed to a range of cadmium concentrations in water during 4 days; experiment 2 performed at 16° C.

0 could therefore appreciably increase the error associated with the estimate of k, the elimination rate constant at 18°C. From the equation for a one-compartment model it can be deduced that a lower starting value would have reduced the estimated elimination rate constant and the rate of intake for the same asymptotic body burden.

Table 6 Regression analysis for the first experiment of the logarithm of the adjusted cadmium burden (ng) in stone loach sampled on time (t) after exposure of 4 days to 1.00 mg/L cadmium in water or used as controls.

Treatment	Temperature (°C)	Period (days)	Estimates for regression coefficients (linear) b	± s.e.	(quadratic) c	± s.e.
exposed	8	4 - 12	-0.113***	± 0.027	not applicable	
	16	4 - 12	-0.724***	± 0.240	0.036*	± 0.015
	18	4 - 12	-0.292**	± 0.108	0.015*	± 0.007
control	8	0 - 12	-0.101**	± 0.033	not applicable	
	16	0 - 12	-0.065**	± 0.018	not applicable	
	18	0 - 12	-0.097***	± 0.013	not applicable	

*, ** and *** indicate significant difference from zero at the 0.05, 0.01 and 0.001 probability levels respectively.

Table 7 Estimates for rates of intake (I), body burden at day 4 and asymptotic burden, and elimination rate constant k during and after exposure of stone loach to water-borne cadmium at three different temperatures. Estimates derived from a one-compartment model.

Experiment no.	Temperature (°C)	Cadmium in ambient water (mg/L)	Rate of intake I (ng/day)	Body burden (ng) on day 4 (geometric mean)	asymptotic	Elimination rate constant k (/day) during exposure	after exposure
2	16	0.08	446	315.6	348	1.28	not applicable
1	8	1.00	1163	999.5	1472	0.79	0.18
1	16	1.00	1618	5374.8	7705	0.21	0.43
1	18	1.00	3428	2255.7	2857	1.20	0.16

The fish kept at 16°C acquire cadmium more rapidly than at 8°C during exposure and, after exposure, they also lose it more rapidly. The data suggest that the elimination rate constant k is lower at 16°C than at 8°C during exposure to 1.00 mg/L cadmium in water. However, the elimination rate constants estimated separately for the periods during and after exposure appear not to be the same. The estimated value of k is higher during exposure to 0.08 mg/L than to 1.00 mg/L cadmium in water (Table 7).

The results of the second experiment (Fig. 3) show how the dose of cadmium in the loach changes with exposure and time. The highest level is found in fish exposed to the highest concentration of water-borne cadmium but the net rate of intake (ng/day/mg cadmium in water/L) decreases as the concentration of cadmium in the water increases.

Bio-concentration factor (BCF) at day 4 approaching steady state levels of cadmium are highest at 16 °C (10.9), then at 18 (4.4) and lowest at 8 °C (1.5). BCFs decrease with increase of concentration of cadmium in water (see Fig. 3).

Effect of Starvation

The food (*Tubifex spp.*) on which the fish were fed contained 0.26 ± 0.03 mg cadmium/kg dry weight (based on 8 samples). The fish were offered daily a maintenance ration of food just enough to keep their weight constant: 56.5 mg wet weight (about 8.2 mg dry weight) of *Tubifex* for the standard weight of fish of 404.6 mg dry weight.

The control fish in all experiments lost cadmium from their body (Figs. 2, 3 and 4). The results of the third experiment show that both groups of fed fish have higher cadmium levels than the comparable starved ones although the differences are not significant (Fig. 4). However, for fish exposed to 0.067 mg cadmium/L in the water, the magnitude of this difference is approximately 10 times the intake of cadmium via the food. For fish exposed to 0.0007 mg Cd/L in the water, this difference is approximately twice the intake of cadmium via the food. There is clearly no interaction between feeding regime and degree of exposure to cadmium in water.

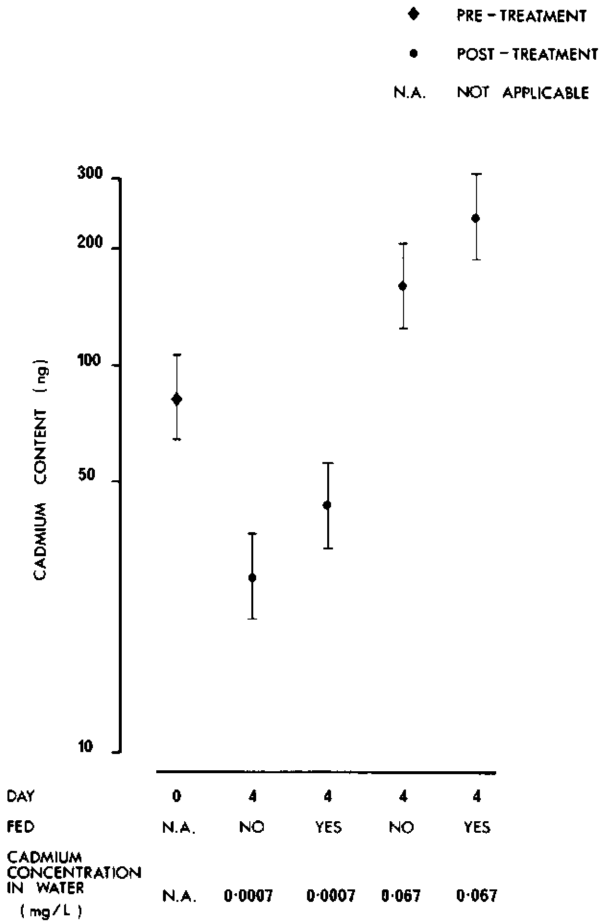

Fig. 4 Mean cadmium content (ng) and standard errors of stone loach of a standard weight of 404.6 mg dry weight before (♦) and after (●) four different treatments; experiment 3 performed at 16° C.

DISCUSSION

Fish were exposed to cadmium at levels well below those that are acutely toxic. Solbé and Flook (1975) found a 7-day LC_{50} value of 4.5 mg/L cadmium, with 100% survival after 66 days of exposure at 1.2 mg/L. They also found that no change in the normal behaviour to hide during daylight had occurred at cadmium concentrations and periods similar to the ones in this study. Some evidence was found to suggest that exposed fish lose weight more rapidly during exposure than did control fish (Fig. 1, Table 5).

The apparent loss of weight by fish sacrificed at day 0 was consistent in all three experiments although the difference between initial and final wet weight of fish kept at 18°C was greater. All starved fish lost weight with time. The tendency of fish kept at 18°C to lose weight rapidly during exposure is presumably exceptional, because the mean values of loss after exposure are lower. Although the water content of the fish from different groups within the first experiment decreases with temperature ($F_{2,161}$= 10.13, P<0.01) the dry weight is not affected ($F_{2,161}$= 1.80, P>0.10).

The proportional loss of weight appears to be independent of body weight i.e. the body weight exponent as in the classical equation for growth (Von Bertalanffy, 1938) is not different from unity. All losses are attributed to the different treatments. The results suggest that there is an effect of temperature presumably as a result of affected metabolic processes.

Because the body weight of the fish change significantly during the course of the experiments, changes in the mass rather than concentration of cadmium are considered to be the most appropriate unit to evaluate the effect of different treatments (Moriarty, 1984). Estimates of biological half-life among studies and between species based on body burden provide a better basis for comparison (Niimi, 1987).

The results show that body size is important in assessing uptake and loss of a pollutant. This can explain part of the fluctuation in pollutant levels as suggested by Martin and Coughtrey (1982). The body weight exponent which describes these two processes for fish of different weight is different from unity in all three experiments and, therefore, expressing the body burden as concentration has to include information on body weight (Moriarty *et al.*, 1984). Pollutant levels expressed as concentrations do not necessarily account completely for differences in body weight.

The results of the first experiment are consistent with the standard pattern of uptake and loss of heavy metals during and after exposure: net intake during exposure decreases with time, and the rate of loss after exposure also decreases with time (Nordberg, 1976; Pascoe *et al.*, 1986).

Most of the published data suggests that concentration of cadmium in fish exposed to cadmium in water increases for several weeks before equilibrium is established (McCracken, 1987). The results herein suggest that, during the first few days, an equilibrium is established rapidly. A wide range of values has been found for elimination rate constants. The stickleback (*Gasterosteus aculeatus*) and rainbow trout (*Salmo gairdneri*) had comparable rates (Oronsaye, 1987; Pascoe and Shazili, 1986), but much lower rates have been reported for brook trout (Benoit *et al.*, 1976) and rainbow trout (Kumada *et al.*, 1980). There could be, at least, three reasons for these differences. First, published results do not usually measure body concentrations until fish have been exposed for at least 2 weeks (Brown *et al.*, 1986; Benoit *et al.*, 1976). If there is a first plateau reached within the first few days after which body concentrations rise again, then this would be missed by taking the first sample after 2 weeks' exposure. It has been suggested that cadmium becomes irreversibly bound to low-molecular-weight proteins (perhaps including metallothionein) in the liver and kidney (McCracken, 1987; Thomas *et al.*, 1985) after relatively long-term exposures, which could account for an initial plateau before a second longer phase of net cadmium intake. Binding of cadmium to protein would also give a slower elimination rate. Secondly, loach were usually starved to avoid uptake from the food. Long-term experiments (Brown *et al.*, 1986) require feeding of the fish. Starvation affects metabolic rate and, consequently, uptake of metal (see below). Results for sticklebacks during and after exposure (Oronsaye, 1987) were similar to mine for stone loach. Thirdly, hardness of the water is likely to affect rates of uptake and loss of cadmium (Pärt *et al.*, 1985; Pascoe et al., 1986). Many of the published studies have been performed in soft water (e.g. Benoit *et al.*, 1976; Kumada *et al.*, 1980), whereas mine used hard water (table 1). Although the underlying mechanism is not yet fully understood, there is general consensus that as water hardness increases, uptake of cadmium decreases (McCracken, 1987).

Increase in BCF with temperature, as found noted herein, is in accordance with the review by Taylor (1983) and follows the same trend as the effect of temperature on respiration: a maximum in the rate of oxygen consumption was measured at 16 °C. The field observations on cadmium levels in stone loach from streams yield much higher values for BCFs, comparable to those derived from other studies under both laboratory (Benoit *et al.*, 1976; Sullivan *et al.*, 1978) and field circumstances (Adams *et al.*, 1980; Murphy *et* *al.*, 1978). These low BCF values in the present laboratory studies probably relate to the use of high concentrations of cadmium in the water and of relatively small fish. BCFs decrease as the concentration of cadmium increases (Benoit *et al.* (1976) for trout and Pascoe and Mattey (1977) for stickleback). Both the laboratory experiments described in this paper, and field data show that the BCF in stone loach decreases as size increases. Although the range of weight of fish used is not always indicated in the literature, the heavier fish in my experiments (Table 3) are comparable to the loach described by Brown *et al.* (1986) and, in general, of similar size as the sticklebacks (*Gasterosteus aculeatus*) described by Pascoe and Mattey (1977) and Woodworth and Pascoe (1983). These results yielded values for BCF that are comparable to the present study.

Several studies report the effect of temperature and feeding regime on metabolism of fish species (Beamish, 1974; Beamish and Mookherjii, 1964; Eccles, 1985). All authors conclude that metabolic rate increases with temperature. In this paper it is shown that temperature and size of the organism are important for evaluating rates of uptake and elimination of water-borne cadmium by stone loach.

The exchange of cadmium between the water and the fish takes place mainly at the surface of the gills (Calamari *et al.*, 1980; Niimi, 1983). The data indicate that the rates of uptake and elimination of cadmium increase with temperature, which confirms Niimi (1983). The saturation concentration of oxygen in water decreases when temperature increases. At the same time, the fish's demand for oxygen is likely to increase with rise of temperature (Niimi, 1987), so that, unless the efficiency of uptake of oxygen from the water increases greatly, the volume of water passing over the gills will increase with temperature. It has been proposed that differences in volume of water passing over the gills are responsible for differences in uptake of contaminants (Neely, 1979; Norstrom *et al.*, 1976). Hence, the temperature of the water is likely to govern the rates of uptake and loss of cadmium by the fish to a large extent. Comparison of the results at 8° and 16°C suggests that temperature has an effect on both the rate of uptake and elimination constant: rate of uptake being less influenced than the elimination constant. Jimenez *et al.* (1987) found for benzo(a)pyrene in the bluegill sunfish that temperature had a greater effect on the rate of uptake than on the rate of elimination.

Opperhuizen and Schrap (1987) concluded that, for hydrophobic chemicals such as polychlorinated biphenyls, the aqueous oxygen concentration affects uptake efficiencies rather than the uptake constants. They found no effect of lowered oxygen concentration.

Bruggeman *et al.* (1981) assume that the rate constant for loss of pollutant from an organism is the same during and after exposure. Moriarty (1984) concludes however (from a three-compartment model) that such an assumption needs to be tested in each instance. There is circumstantial evidence that this rate constant is not the same during and after exposure. Niimi (1983) supports the view that models of contaminants should view the elimination component as a response distinct from the uptake phase. Particularly the uptake component would be related to the energetic requirements of the fish.

Inevitably, the data contain a degree of experimental error, even after the cadmium burden has been adjusted for different weights. Standard deviations of the estimates for parameters in compartment models cannot be readily used to determine confidence limits (Atkins, 1969).

Starvation reduces metabolic rate (Beamish, 1974, Collvin, 1984). Feeding rate affects the volume of water that passes through the gill chamber, thereby affecting the uptake of metal from both food and water (Luoma, 1983). Also, Rodgers *et al.* (1987) suggest that the higher levels of pollutant found in fed fish reflect their higher metabolic rate. The results in the third experiment suggest that cadmium intake from the water is greater in fed than in starved fish or that elimination rate is reduced. An increased intake seems more probable: it could well result from a higher metabolic rate and larger volume of water passing over the gills. Jimenez *et al.* (1987) found that fed bluegill sunfish (*Lepomis macrochirus*) have a higher rate of uptake and also of elimination than do starved fish. Riisgård *et al.* (1987) conclude that fed mussels have a higher rate of uptake of cadmium from the water because of a higher rate of filtration.

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Chapter 3: Metabolic rate and uptake and loss of cadmium from food by the fish *Noemacheilus barbatulus* L. (stone loach).

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METABOLIC RATE AND UPTAKE AND LOSS OF CADMIUM FROM FOOD BY THE FISH *NOEMACHEILUS BARBATULUS* L. (STONE LOACH).

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ABSTRACT

Fish, *Noemacheilus barbatulus* (stone loach), of different body weights were used to study rates of uptake and loss of cadmium during and after dietary exposure. Fish were kept singly in a continuous-flow system, and fed tubificid worms. The worms had a range of cadmium levels, but all levels were below that needed to cause acute lethal toxicity in the fish. Body weight affected both the maintenance ration and the amount of food consumed *ad libitum*, but the exponent for body weight (0.78 ± 0.04), relating body weight to food consumption, was unaffected by either temperature or the size of feeding ration. The cadmium content of the worms did not affect the size of the maintenance ration. Metal burden in fish changed rapidly both during and after exposure. After exposure, the cadmium burden of starved fish usually declined more rapidly than in fed fish. A 58-fold increase in cadmium content in the food produced a 28% increase of body burden in the fish, and there was no evidence for bio-magnification. Maintenance ration and ration *ad libitum* and rates of uptake and loss of cadmium increased with temperature within the range 8 - 18 °C, but exposure to cadmium at 16 °C yielded a higher asymptotic body burden than either 8 °C or 18 °C. Rate constants for loss of cadmium after exposure appear to be lower than for loss during exposure. Rates of uptake and loss of cadmium vary with metabolic rate. A maximum in the rate of oxygen consumption was measured at 16 °C, above which the rate dropped, presumably due to stress. The exponent for body weight was unaffected by activity or temperature. Body weight of fish appeared to affect both the rates of uptake and loss of cadmium, and feeding rations and respiration to the same extent : body weight exponents were not dissimilar.

INTRODUCTION

The relationship between the concentration of a contaminant in the environment and the body burden of an organism plays a central role in ecotoxicological problems. There has been a long debate about the importance of different routes of entry of contaminants, including heavy metals, for the body burden of aquatic organisms. Fish can acquire pollutants via three possible sources: food, water and sediment. The latter route of entry may be through ingestion of sediment (Tessier and Campbell, 1987). Different fish have different feeding habits (Maitland, 1965) which influence the relative contribution of metals originating from different sources (Ney and Van Hassel, 1983).

The fish used in this study, *Noemacheilus barbatulus* L. (stone loach), is a bottom-feeder (Maitland, 1965), and sometimes buries itself in the mud (Hyslop, 1982). Benthic organisms have been shown to take up metal from the sediments either via interstitial water or directly via ingested sediment (Duffus, 1986; Lewis and McIntosh, 1986). Dietary exposure may also contribute considerably to the cadmium burden of loach. Uptake of waterborne cadmium has already been described for this species (Douben, 1989a).

Knowledge of the routes and rates of uptake and loss of a pollutant is important for the assessment of the relative contributions of different sources of pollutant to the body burden of an organism. Additionally, physiological processes, which also affect body burden (DeFreitas and Hart, 1975; Niimi, 1983) need to be taken into account. For example, perch *Perca fluviatilis* kept at 15 °C had higher body burdens of cadmium than those kept at 5°C during exposure to 22 μg litre^{-1} (Edgren and Notter, 1980). Cadmium burden in stone loach was highest at 16 °C compared with those kept at 8 and 18 °C due to changes in rates of uptake and loss (Douben, 1989a).

If any growth occurs, the concentration of pollutant within an organism will increase less than the total burden due to 'growth dilution' (Norstrom *et al.*, 1976). Interpretation of data is therefore simplified if a maintenance ration is fed: i.e., that amount of food that is just sufficient to keep the body weight stable.

Four experiments were set up:

1. to estimate the maintenance and maximum feeding rations for fish of different weights at 8°, 16° and 18° C;
2. to measure the cadmium burden of loach after feeding three different concentrations of cadmium in the food at 18 °C;
3. to quantify the effects of starvation, temperature and of different concentrations of cadmium in the food on the uptake and elimination of cadmium by the stone loach;
4. to estimate metabolic activity of the loach by the amount of oxygen consumed in respiration;
5. to quantify the effect of body weight on metabolic rate and cadmium burden by studying fish of different sizes.

The results of this and my other work are intended to evaluate the contribution of cadmium originating from different parts of the physical environment to the cadmium burden in stone loach under field conditions.

MATERIALS AND METHODS

Sampling and Keeping of the Fish
Stone loach were caught on four occasions by electric fishing from the Suttonbrook, a small stream in Derbyshire (National Grid Reference SK 222 342). This site had low concentrations of heavy metals in the sediment (Nichol *et al.*, 1970; Webb *et al.*, 1978). Fish were then kept in storage tanks for at least one month, during which period they were fed on a mixture of live food (*Tubifex* spp.) and pellets of fish food.

The Exposure System
During dietary experiments, fish were kept in a continuous-flow system, developed from one described by Benoit *et al.* (1982). The water came from the mains supply (for aspects of water quality see Table 1). The fish were kept singly, both to avoid interaction (see Street and Hart, 1985) and to study differences between individual fish, in aquaria with 6 litres of water and a flow-rate of about 1.8 litre h^{-1}. The temperature of the water was kept at the nominal value ± 0.1 °C. All experiments had a 12 h light : 12 h dark regime.

Food
Stone loach were fed on tubificid worms. For experiments in which *Tubifex* with an enhanced cadmium content were used, the worms were exposed to a

Table 1 Aspects of quality of mains water used during the three experiments described in Table 3; mean, standard error (s.e.) and number of measurements (n), ionic concentrations in mg $litre^{-1}$.

variable	mean	±	s.e.	n
pH	8.1	±	0.1	9
Conductivity (μS)	779	±	22	9
Ca^{2+}	95.2	±	7.9	11
Mg^{2+}	8.89	±	0.12	11
K^{+}	10.0	±	0.1	11
Na^{+}	47.0	±	3.5	11
total - P	0.27	±	0.01	12[a]
PO_4^{3-} - P (dissolved)	0.27	±	0.02	14
Org. N + NH_4^+-N	0.49	±	0.03	14
$NO3^-$ - N (dissolved)	4.14	±	0.25	14
NH_4^+ - N (dissolved)	[b]			14
Cl^-	70.6	±	0.8	14
SO_4^{2-} - S	54.3	±	2.4	14

[a] the concentrations in two samples were below the limit of detection of 0.10 mg $litre^{-1}$ and were excluded from computations;
[b] the concentration in all samples was below the limit of detection of 0.10 mg $litre^{-1}$.

concentration of 1 or 2 mg litre^{-1} cadmium (as cadmium sulphate) for 20 h and then offered to the fish. *Tubifex* with background levels of cadmium are referred to as control. Subsamples of *Tubifex* were taken daily to check the cadmium concentration (see Table 2). This procedure enabled a range of cadmium concentrations in *Tubifex* to be obtained, of similar magnitude to concentrations found in food items from rivers (0.01 - 100 mg kg^{-1} dry weight; see Douben, 1989c). Stone loach eat this type of food as soon as it is offered, and the amount of cadmium lost from the food before consumption is therefore minimal. Thus the intake of cadmium can be estimated reliably (see page and Welton *et al.*, 1983). During dietary exposure, loach were defined as having a good appetite when the maintenance ration was offered and consumed.

<u>Experimental Procedure for Feeding Experiments</u>
Table 2 summarizes the three feeding experiments that were carried out.

Two to three days before an experiment the temperature in the storage tanks was gradually changed towards the experimental temperature, thus allowing the fish to acclimatize. At the same time the fish were starved to empty their guts. Fish were then caught individually in a net, which was dried on the outside with a slightly damp tissue paper for about five seconds. The fish were then weighed by transferring them to a tared vessel containing water on a balance. They were arrayed in order of weight and allocated at random within successive blocks of fish to each treatment, to give five fish per treatment for each experiment (ten fish per treatment in the second experiment). The fish were then transferred to the experimental room for further acclimatization for one day.

In the first experiment, for each of the six groups with five fish of similar weight, fish were randomly allocated to five different feeding regimes. The feeding regimes ranged from no food (starvation) to one in which they were fed *ad libitum* (maximum consumption). Fish were fed daily for 14 days, but if they had not eaten the previously-offered food by the next day, the amount of food was reduced for that day. The fish were killed after 14 days, weighed and dissected. For each individual fish the relative growth rate (RGR) was calculated:

$$\mathrm{RGR} = \frac{\log\text{ (final wet weight) - }\log\text{ (initial wet weight)}}{\text{observation period (days)}} \qquad (1)$$

The maintenance ration was then estimated separately, for each of the six groups of five fish of similar weight, from a linear regression of the relative growth rate on the weight of *Tubifex* consumed. The maximum rate of food consumption was also estimated for each of the six groups.

In the second experiment, loach were fed daily a maintenance ration of *Tubifex* with three concentrations of cadmium (Table 2). After 14 days of feeding, the fish were starved for one day to allow them to digest the food and were then sampled.

Table 2 Summary of details of three experiments with stone loach. Number of observations in parentheses.

Experiment no.	Temperature (°C)	Concentration of cadmium in water (µg litre^{-1}) mean ± s.e.	in diet (mg kg^{-1}) treatment	logarithmic mean ± s.e.	geometric mean	Date fish caught in Derbyshire	Range of weight of fish (mg dry weight)
1	8		control	0.64 ± 0.03 (4)	4.39		
	16		control	1.13 ± 0.03 (4)	13.5		
	18		control	0.55 ± 0.12 (5)	3.56		
		2.1 ± 0.3 (15)				16.07.86	43.8 - 1421.4
2	18		control	-0.06 ± 0.09 (27)	0.87		
			exposed[a]	1.31 ± 0.09 (13)	20.34		
			exposed[b]	1.70 ± 0.10 (12)	50.31		
		4.7 ± 2.7 (26)				22.09.86	45.2 - 2314.2
3a	8		control	n.d.[c] (16)	[c]		
			exposed[b]	1.82 ± 0.02 (8)	66.0		
		1.1 ± 0.2 (24)				09.07.87	69.0 - 2578.3
3b	16		control	0.36 ± 0.07 (14)	0.44		
			exposed[b]	2.23 ± 0.03 (10)	169		
		1.3 ± 0.2 (12)				23.03.87	84.6 - 1606.7
3c	18		control	-0.35 ± 0.06 (14)[d]	0.45		
			exposed[b]	1.91 ± 0.07 (8)	81.9		
		0.3 ± 0.1 (23)				09.07.87	121.7 - 1675.4
3d	16		control	0.003 ± 0.098 (3)	1.006		
			exposed[a]	1.60 ± 0.02 (8)	39.57		
			exposed[b]	1.97 ± 0.06 (8)	93.67		
		0.7 ± 0.2 (20)				09.07.87	63.5 - 1945.7

[a] Tubifex were kept for 20 h in water with 1 mg litre^{-1} cadmium;
[b] Tubifex were kept for 20 h in water with 2 mg litre^{-1} cadmium;
[c] not detectable, limit of detection 0.65 mg kg^{-1};
[d] 3 samples with cadmium levels below the limit of detection of 0.35 mg kg^{-1} excluded.

Table 3 Concentration of cadmium in *Tubifex* exposed to two different concentrations of cadmium in water for 20 h and subsequently kept in clean water for one day. Number of observations in parentheses.

Cadmium in water ($mg\ litre^{-1}$)	Cadmium concentration in *Tubifex* ($mg\ kg^{-1}$ dry weight) after 20 h exposure logarithmic mean ±s.e.	geometric mean	one day later logarithmic mean ±s.e.	geometric mean
1.0	1.31 ±0.09 (13)	20.34	1.22 ±0.02 (4)	16.5
2.0	1.70 ±0.10 (12)	50.31	1.62 ±0.06 (4)	41.9

In the third experiment, fish were fed daily with their maintenance ration with *Tubifex* either with control (background) or with enhanced cadmium content. After 4 days of exposure some fish were fed control food while others were starved up to 14 days. Given the results of the second experiment, fish were sampled in the morning, having been fed for the last time at the end of the day before.

In all experiments, the first feeding took place on day 0 just before the onset of darkness, when loach become active (Welton *et al.*, 1983), so that loss of cadmium from the food was minimized. On all occasions, the fish were sampled in the morning; after sampling, the fish were killed and weighed again, this time directly on a balance on aluminium foil after blotting dry for about five seconds.

Any undigested food and faeces were thoroughly squeezed out from the gut. The gut contents of fish used in the third experiment at 16°C, were analyzed to compare the cadmium concentrations with those in subsamples of *Tubifex*, to estimate whether the gut was emptied. Little was found in these samples and thus, given the weight of these samples (mean dry weight of 2.3 mg ± 0.3 for 25 samples). Gut contents will have contributed little to the estimated cadmium burden of the fish. There was no point in collecting faeces from the aquaria for cadmium determination because they could have been excreted for some time, partly disintegrated and cadmium could have leached out.

The gills were dissected out to be analysed separately from the rest of the body. These data were intended for other work which will be published separately. The samples were weighed, dried at 85°C for 3 days and then reweighed to compare results based on dry weight (Kay, 1985).

Altogether, 7 fish out of 285 died: 3 fish, one in each treatment group, died before the end of the second experiment: 4 fish died during the third experiment: one in the control group on day 12 at 8°, and three, one in each treatment group, on day 13 at 16° C, thus suggesting that deaths were not caused by the treatments. These fish were excluded from the computations.

Metal analysis

The fish were digested in chemically-clean boiling tubes with 1 ml 'Ultrar' concentrated nitric acid. If necessary, especially with big fish, small additional volumes of acid were added. The samples were completely digested when no brown fumes appeared at a temperature of 120°C. The volume of acid was made up to 1 ml again after digestion. The contents of each boiling tube were decanted into a measuring cylinder, rinsed with deionized water and made up to a volume of 20 ml with 5% nitric acid. The solution was poured into a glass vial with a polythene screwtop, ready for cadmium determination by an atomic absorption spectrophotometer (electrolyte furnace with background noise correction).

The total metal burden in the fish was obtained by adding together the results for the gills and those for the rest of the body. Whole body burden was used, not concentration, to avoid any bias from differences of individual fish (Moriarty *et al.*, 1984) (see also below).

Statistical Computations and Mathematical Modelling

Weight is a prime source of variability and if this source of variation is not taken into account, then differences between treatments can be masked and results can be misleading. Moreover, one of the specific aims of this study was to quantify the effect of body weight. Therefore, all groups included fish of different body weights, but with comparable mean weights. The original data had therefore to be adjusted for weight before the effect of different treatments (combination of factors) could be assessed. Analysis of covariance (see Sokal and Rohlf, 1981) rather than analysis of variance, allows for differences in weight by removing that part of the variation that is due to differences in body weight (see also equation 2 below), and, at the same time, provides information on the effect of body weight (e.g. Douben, 1989a and b).

The effect of treatment (described below) on food consumption was estimated by analysis of covariance of the logarithmic amount of food consumed on the logarithmic amount of food offered at maintenance ration, which depends on weight, (covariate) for control fish sampled at day 4 and 14 and for exposed fish sampled at day 2 and 4.

The wet weights of the fish before the start of the second and third experiments and at the time of sampling, were used to estimate the proportional change of wet weight for each fish during the experiment. These estimates were compared by analysis of covariance, with initial wet weight as covariate, to evaluate the effect of treatment on change of weight.

Changes of compartmental volumes can affect the concentration of pollutant, independent of any transfers of pollutants between compartments (Moriarty, 1975). In this study comparisons were made between fed and starved fish. However, even when volumes of some compartments do not change, mass rather than concentration of a pollutant provides a better basis for comparison of results from different studies (Niimi, 1987), particularly when body weight affects concentration (Moriarty *et al.*, 1984). Metal burdens were transformed onto a logarithmic scale so that the residual variation in the data conformed more nearly to a normal distribution.

For each experiment, the effect of treatment (combination of factors) on cadmium burden was tested by analysis of covariance, with dry weight of the body as the independent variable (covariate) and with temperature, concentration of cadmium in the food, feeding regime and time of sampling as factors. Analysis of covariance is based on assumptions of homogeneity of residual variances about the individual regressions and similarity of slopes. These assumptions were tested in each instance (for example see Table 4). The effect of body weight on cadmium burden (objective 5) was then estimated, for each experiment, by using the common regression coefficient, which provided the exponent for body weight. The results for the different treatments, after allowing for differences in weight, are given as means adjusted for weight: the cadmium burden for a fish of standard weight. The standard weight of fish was taken as the mean weight of all the fish in each of the three separate experiments ($\bar{w}$ in equation 2 below). For a similar approach see Douben (1989a).

In the third experiment, the efficiency of metal uptake by the stone loach was estimated by comparing the change in cadmium burden of fish with the amount of cadmium consumed (Hatakeyama and Yasuno, 1982; Niimi, 1983). Therefore, the amount of cadmium consumed by each individual fish was calculated separately, for each sub-experiment, from the mean dry weight of *Tubifex* and the mean concentration of cadmium in the worms. The fish were arrayed in order of increasing dry weight per treatment. Then, pairs of groups of fish were formed from samples taken on consecutive occasions. Thus, for controls, pairs were taken from the groups sampled on days 0 and 4, and another pair from those sampled on days 4 and 14, while for exposed fish, pairs were taken from groups sampled on days 0 and 2, and on days 2 and 4 (see Table 6). The difference between the cadmium burden of each fish in one group and the burden of the corresponding fish in the other group was then calculated. The efficiency of metal uptake by loach over that period of time was then estimated as the ratio of this difference in metal burden to the total amount of cadmium consumed. This comparison was made without adjusting for different body weights because no significant reduction in variation was obtained.

Data from the third experiment were used to estimate the parameters of a one-compartment model of metal burden (see Atkins, 1969). These data were first adjusted for different weights of fish (equation 2) and then back-transformed to an arithmetic scale according to equation 3:

$$\mathrm{Alog\ Cd}_i = \log \mathrm{MCd}_i - \hat{b} * (w_i - \bar{w}) \qquad (2)$$

$$\mathrm{ACd}_i = 10^{(\mathrm{Alog\ Cd}_i + \sigma^2/2)} \qquad (3)$$

where : $\bar{w}$ = mean logarithmic weight of all fish in the analysis of covariance;

w_i = logarithmic weight of the i-th fish;

$\hat{b}$ = estimated common slope of the regression of cadmium on weight after logarithmic transformation;

MCd_i = mass of cadmium (ng) in the i-th fish;

$\mathrm{Alog\ Cd}_i$ = logarithmic value of the mass of cadmium of the i-th fish adjusted for weight;

σ^2 = residual variance in the analysis of covariance;

ACd_i = adjusted mass of cadmium (ng) of the i-th fish on an arithmetic scale.

Oxygen consumption

Fish were starved for one day before the start of the measurements, to ensure an empty gut. Allocation of fish to treatments was by weight, as in the previous experiments. Fish were kept in either continuous light or continuous darkness, thus facilitating the measurement of two levels of oxygen consumption: routine oxygen consumption when fish make only spontaneous movements; active oxygen consumption when fish are more active (see also Beamish and Dickie, 1967).

Fish were placed in individual enclosed chambers, which were full of water with an oxygen content near to 100% of air saturation, to minimise anaerobic respiration. Water temperature was initially the same as in the storage tanks, but it was changed to the required temperature at a rate of about 1°C per hour, and it was then held constant (± 0.1 °C). The inlet and outlet of the chambers were closed when measurement started, so that, as the fish respired, the oxygen concentration of the water dropped. The fish were kept undisturbed in the chambers during the measuring period. The maximum oxygen depletion was 40%. After completion of the experiment the fish were killed and reweighed.

The oxygen content of the water was measured with a calibrated Radiometer oxygen electrode (E5046), and the output was read on an oxygen meter (PHM 71) as a percentage saturation. The mass of oxygen consumed was then calculated from the volume of the chamber. Although the presence of the fish reduced the volume of the water, the volume of the fish was always less than 0.2% of the volume of the chamber. This effect was therefore negligible.

RESULTS

Effect of Body Size on Maintenance and Maximum Rations

Analysis of covariance for the weight of *Tubifex* needed for maintenance and maximum rations on the wet weight of the fish shows that the residual variances about the regressions lack homogeneity at the 0.05 probability level (Table 4). This does not appreciably affect the contrast between the adjusted means for the different treatments as the samples are equal in size (Scheffé, 1959). The slopes of the regressions are not significantly dissimilar

($F_{5,24}<1.0$). The assumptions of this analysis appear therefore to be adequately satisfied.

The results for the mean wet body weight (W) show that there is no effect of either temperature or feeding regime on the exponent of body weight ($F_{2,27}=1.61$, $P>0.10$ and $F_{1,28}<1.0$ respectively):

$$f = a * W^{0.78} \qquad (4)$$

where : f is the wet weight of *Tubifex* (mg), for both maintenance and maximum ration, at 8°, 16° and 18 °C;

a is a coefficient influenced by temperature and feeding level.

The exponent has a standard error of 0.04. Both temperature and activity affect the amount of *Tubifex* required, i.e. affect coefficient a ($F_{5,29}=74.38$, $P<0.001$) (Fig 1). The results of (curvi)linear regression of the amount of food intake for a feeding regime on temperature (Table 5) indicate that the difference between the maximum food intake and the maintenance requirement is minimal at about 13 °C. However, data for only three temperatures are available and the effect of temperature on this difference is difficult to interpret in biological terms. Therefore, the linear regressions are presented in Fig. 1. Much of this difference will be available for growth.

<u>Effect of Body Size on Cadmium Burden</u>

In neither experiment 2 or 3 did cadmium in the diet affect food consumption by the stone loach; no treatment had a significant effect on wet weight of fish during either the second experiment ($F_{3,52}=1.78$, $P>0.10$) or the third

Table 4 Summary of results of analyses of covariance for: (experiment 1) the logarithmic weight of food consumed at maintenance and maximum feeding ration at three temperatures on wet weight of stone loach, and (experiments 2 and 3) the logarithm of the cadmium burden on the logarithm of the dry weight of stone loach.

Experiment no.	Tests for homogeneity of residual variances about regression χ^2	df	Tests for dissimilarity of individual slopes	Estimate for common slope ± s.e.	Variance ratio for effect of treatments
1	11.12^{*}	5	$F_{5,24} < 1.0$	0.78 ± 0.04	$F_{5,29} = 74.38^{***}$
2	1.009	3	$F_{3,49} = 1.33$	0.63 ± 0.11	$F_{3,52} = 4.36^{**}$
3[#]	60.69^{*}	39	$F_{39,116} < 1.0$	0.70 ± 0.06	$F_{39,155} = 6.97^{***}$
3[$]	49.50^{*}	30	$F_{30,134} < 1.0$	0.70 ± 0.06	$F_{30,164} = 9.37^{***}$

[#] indicates that previously exposed fish on the regimes starved and control food were kept separately in the analysis of covariance;

[$] indicates that previously exposed fish on the regimes starved and control food were combined into one group per sampling occasion in the analysis of covariance;

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 probability levels respectively.

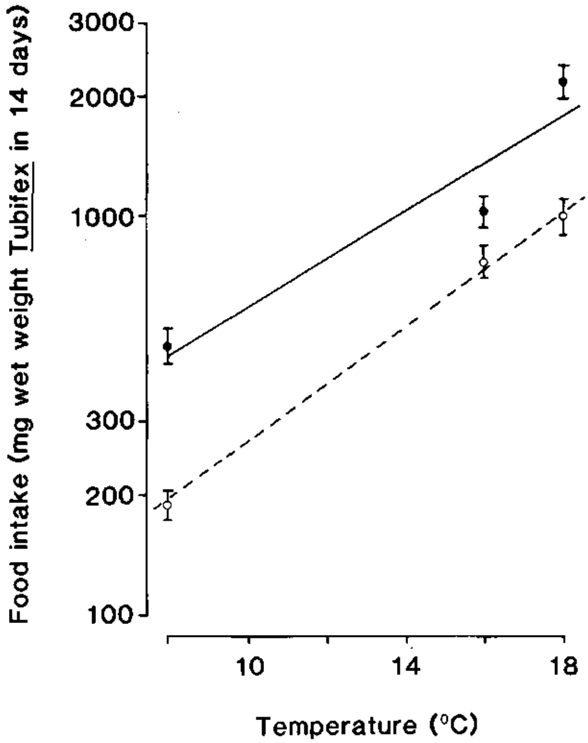

Fig. 1 Amount of *Tubifex* (with standard errors) eaten by stone loach of a standard weight of 1694.4 mg wet weight, at three different temperatures. Linear regressions indicated for maintenance ration (o---o) and when fed *ad libitum* (●——●).

Table 5 Regressions of the logarithm of the amount of food intake in two feeding regimes on temperature (see also Fig. 1).

Feeding ration	Estimates for regression coefficients (linear) b	±	s.e.	(quadratic) c	±	s.e.
Maintenance	0.0729***	±	0.0069	not applicable		
Maximum	-0.2479***	±	0.0463	0.0121***	±	0.0018

*** indicates significant difference from zero at the 0.001 probability level.

experiment (fish starved after 4 days of exposure excluded: $F_{30,120}=1.14$, $P>0.10$). These results confirm that the fish had received a maintenance ration. Although there was a significant ($P<0.05$) effect of body size on the proportional change of wet weight during feeding of fish ($7.5 \times 10^{-06} \pm 3.2 \times 10^{-06}$), for practical purposes this effect is negligible.

The residual variances for the regressions of cadmium content on dry weight of fish are homogeneous in the second but not in the third experiment (Table 4). However, as most, 36 of the 40, groups of fish contain five observations, there is little difference between the estimated and weighted variances. Thus inequality of variances has little effect on inferences about means (Scheffé, 1959). The slopes are not dissimilar in either the second or the third experiment (Table 4). There is no effect of temperature ($F_{2,125}<1.0$). From the common slopes for these experiments, the body weight exponents are estimated to be 0.63 ± 0.11 and 0.70 ± 0.06 respectively.

Effect of Dietary Cadmium on Body Burden

In the second experiment fish fed for 14 days on a maintenance ration of *Tubifex* with 3 different levels of cadmium contain more cadmium than those sampled at the start of the experiment (Fig 2) ($F_{3,52}=4.11$, $P<0.05$). The increase in body burden of cadmium was greater (28 %) in fish fed on the most heavily contaminated *Tubifex*, but this difference is clearly not significant.

The results of the third experiment (Fig. 3) show that the cadmium burden in all fish kept on a control diet had increased by, on average, 70% within 14 days, although this increase is not significant. The data suggest that almost all of the cadmium in the food was taken up by these fish (Table 6).

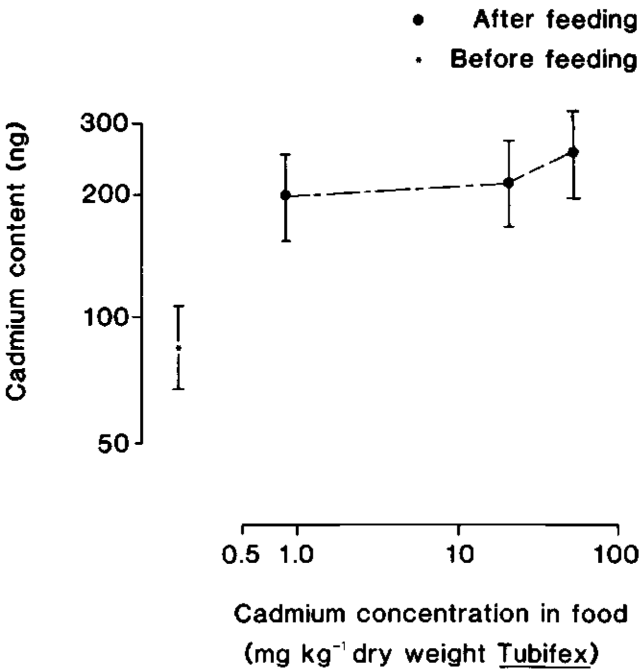

Fig.2.

Fig. 2 Mean cadmium content (ng) and standard errors of stone loach, of a standard weight of 434.0 mg dry weight, at the start of the experiment (*) and after 14 days of feeding at 18 °C on a maintenance ration of live *Tubifex* with three different levels of cadmium (●).

Cadmium levels rose more quickly in fish fed on exposed *Tubifex*, although, during the four days' exposure, the rate of increase in the body burden decreased with time. The net efficiency of cadmium uptake also decreased with higher concentrations in the food (Table 6, experiment 3d).

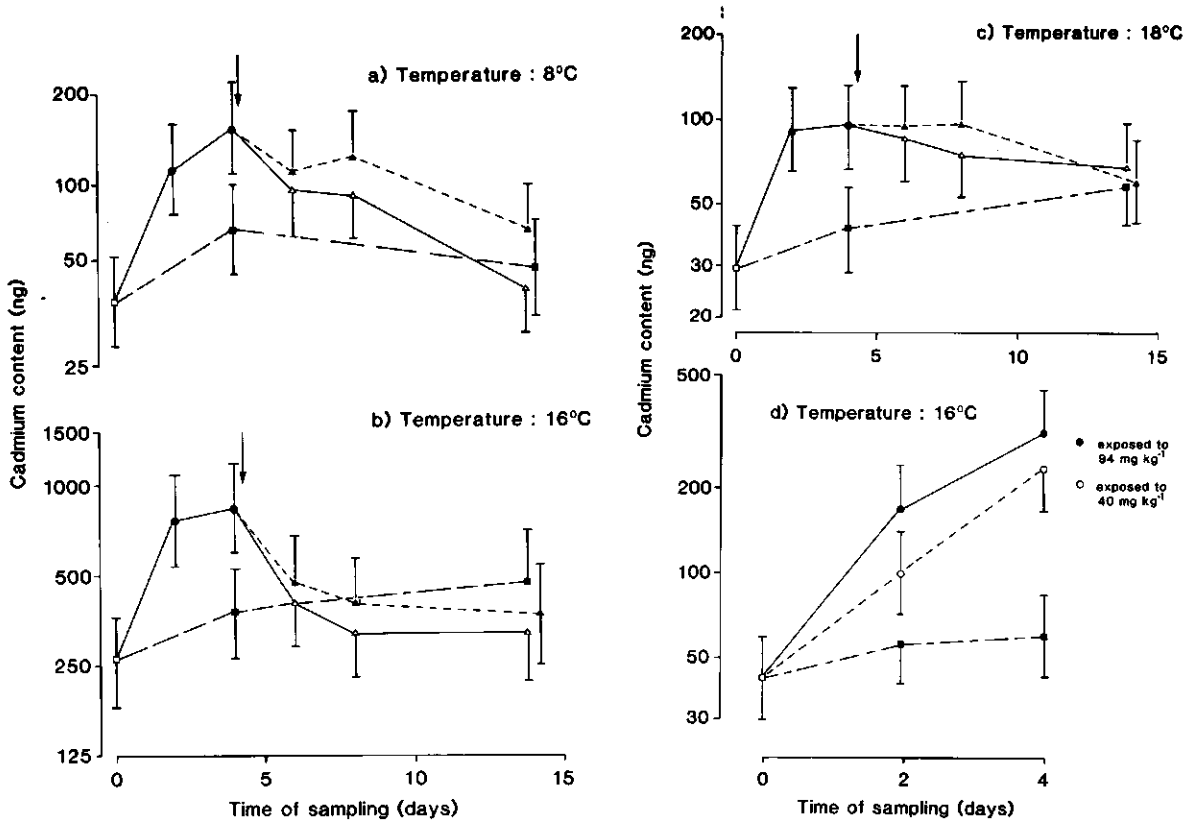

Fig. 3 Mean cadmium content (ng) and standard errors of stone loach, of a standard weight of 577.9 mg dry weight, at the start of the experiment (□), in controls (■), during two types of exposure (●) and (○) and after exposure when fed on control food (▲) or starved (△). ↓ indicates end of exposure. Experiment performed at three different temperatures (a) 8°, (b) 16°, (c) 18° and (d) 16°C.

Table 6 Efficiency of cadmium uptake by stone loach from *Tubifex* with different concentrations of cadmium (third experiment, see Table 3 for details).

Temperature (°C)	Experiment no.	Cadmium in diet (mg kg^{-1})	Period (days)	Efficiency means ± s.e.	No. of observations
8	3a	< 0.65	0 - 4	1.62 ± 3.25	5
			4 - 14	0.01 ± 1.26	4*
		66.0	0 - 2	0.29 ± 0.20	5
			2 - 4	0.08 ± 0.22	5
16	3b	0.44	0 - 4	7.59 ± 5.25	5
			4 - 14	1.98 ± 5.82	4*
		169	0 - 2	0.13 ± 0.03	5
			2 - 4	0.02 ± 0.03	5
	3d	1.006	0 - 2	0.57 ± 1.33	5
			2 - 4	0.17 ± 0.31	5
		39.57	0 - 2	0.07 ± 0.02	5
			2 - 4	0.06 ± 0.03	5
		93.67	0 - 2	0.06 ± 0.01	5
			2 - 4	0.03 ± 0.01	5
18	3c	0.45	0 - 4	0.21 ± 0.60	5
			4 - 14	0.14 ± 0.11	5
		81.9	0 - 2	0.03 ± 0.01	5
			2 - 4	0.00 ± 0.00	5

* one fish which died before the end of the experiment was excluded.

Effect of Temperature on Cadmium Burden

Within each experiment, the relative increase in cadmium burden (i.e. final burden/initial burden) of fish increased with concentration of cadmium in the *Tubifex* (Fig 4). For fish fed on control *Tubifex* all relative increases are of similar magnitude. Relative increases for fish fed on exposed *Tubifex* tend to increase with the exposure, except for experiment 3b, where the initial

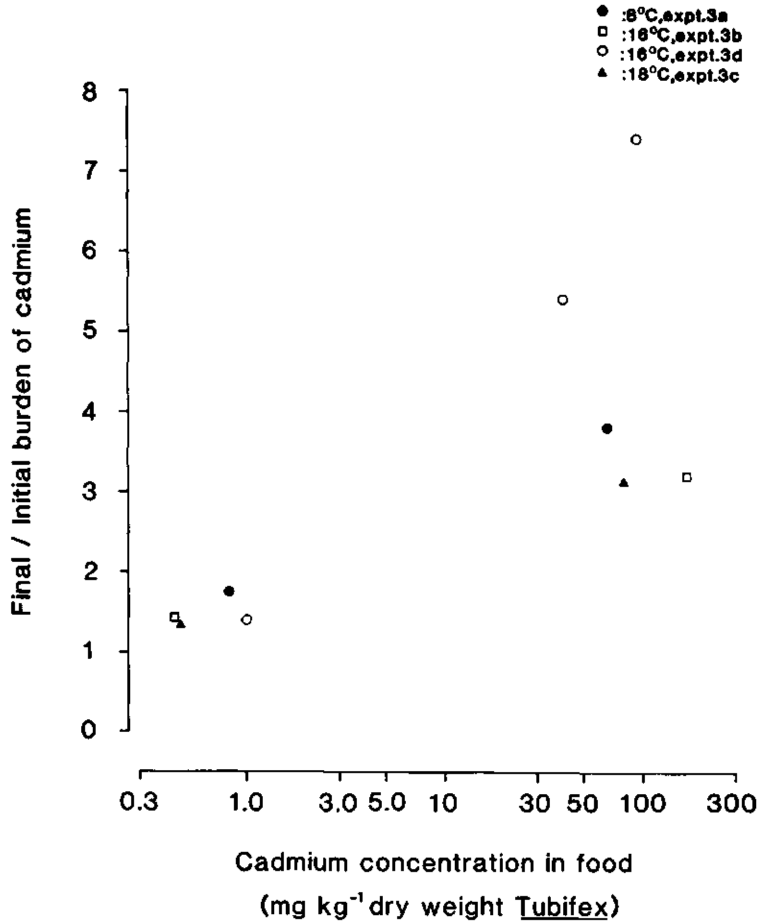

Fig. 4 Effect of cadmium content of food on the cadmium burden of stone loach fed for 4 days on control or exposed *Tubifex* (see Table 2 for details).

burden was much higher than in other experiments (see Fig 3). A possible effect of temperature is therefore obscured.

The rapid approach to a steady state of cadmium burden during exposure, and the rapid decline after exposure, indicate high rates of loss of cadmium from stone loach in the third experiment. Within 2 days after exposure, the levels were comparable to those in fish fed continuously on control food, at all three temperatures. By the end of each of the experiments (day 14), there was no significant difference in the cadmium burden of the fish fed on different diets. When the results for starved and fed fish are combined (see also Fig. 2), the loss after exposure follows a curvilinear (i.e. biphasic) pattern for fish kept at 16° C only, while a linear relationship is adequate to describe the data obtained for the other temperatures (Table 7). This biphasic pattern at 16 °C indicates that the cadmium in the loach is not homogeneously distributed, and that, consequently, a two-compartment model is needed to describe the body burden of cadmium after exposure. Given the necessity to use the same model for all three different experimental temperatures, a one-compartment model has been used to describe the cadmium burden both during and after acute exposure in these experiments.

Estimates from a one-compartment model for the rate of cadmium intake (I) show that the values of I and of the elimination rate constant (k) are highest at 18° C for control and exposed fish during exposure (Table 8). Results for

Table 7 Regression analysis for the third experiment of the logarithm of the adjusted cadmium burden (ng) in stone loach on time (t) after exposure to cadmium; combined results for fish fed on control food and starved.

Temperature (°C)	Experiment no.	Estimates for regression coefficients (linear) b	±	s.e.	(quadratic) c	±	s.e.
8	3a	-0.038*	±	0.021	not applicable		
16	3b	-0.200*	±	0.095	0.009*	±	0.004
18	3c	-0.017	±	0.012	not applicable		

* indicates significant differences from zero at the 0.10 probability level.

fish kept at 16° C (in experiment 3b) cannot readily be compared with those for fish kept at 8° and 18° C, because the former had a relatively high initial burden of cadmium. However, fish used in experiment 3d had initial body burdens comparable to those of fish kept at 8° and 18° C (experiments 3a and 3c respectively). From estimates for I and k, it can be deduced that the asymptotic body burden of control and exposed fish will be highest at 16 °C (see also Fig 3); the two negative estimates for k were presumably the effect of random variation. The estimated body burden at steady state for exposed fish kept at 16°C (experiments 3b and 3d) are similar and highest (about 1100 ng) in spite of differences in initial levels of cadmium (Table 8).

The levels in cadmium burden of fish kept after exposure on control food or starved tend to be lower in starved fish than in fed fish (Fig. 3), but the difference is clearly not significant. The error associated with the estimates for k after exposure were obtained by linear regression (Table 8): the estimates for fed and starved fish appear not to be different for each temperature. Assuming that similar errors apply to the estimates for k during exposure, then the estimated values for k for the periods during and after exposure appear to be different: at 16° and 18° C these rate constants are lower after than during exposure.

Oxygen consumption

Fish in the experimental chambers were resting most of the time when kept in continuous light, while they were actively swimming at the end of the experiments in continuous dark when the lights were switched on, which confirms the view that stone loach are most active in the dark (Welton *et al.*, 1983). The measurements in light and darkness estimate routine and active oxygen consumption, respectively.

Table 8 Estimates for rates of intake (I), body burden at day 4 and asymptotic burden, and elimination rate constant k during and after exposure of stone loach to dietary cadmium at three different temperatures. Estimates derived from a one-compartment model based on adjusted body weights.

Experiment no.	Temperature (°C)	Cadmium in diet ($mg\ kg^{-1}$) during exposure	Rate of intake I ($ng\ day^{-1}$)	Body burden (ng) (geometric mean) on day 4 (exposed)	Body burden (ng) (geometric mean) on day 14 (control)	Body burden (ng) asymptotic	Elimination rate constant k (day-1) for control fish	Elimination rate constant k for exposed fish during exposure	after exposure treatment	after exposure value ± s.e.[a]
3a	8	< 0.65	299.6	-	52.85	80.97	3.70	-		n.a.
		66.0	57.66	155.3	-	n.a.	-	-0.05	fed	0.12 ± 0.06
									starved	0.22 ± 0.06
3b	16	0.44	132.58	-	484.19	779.9	0.17	-		n.a.
		169	694.37	842.3	-	1102.2	-	0.63	fed	0.10 ± 0.02
									starved	0.15 ± 0.02
3c	18	0.45	500.0	-	59.43	63.86	7.83	-		n.a.
		81.9	1073	95.0	-	109.94	-	9.76	fed	0.03 ± 0.04
									starved	0.02 ± 0.03
3d	16	1.006	32.49	n.a.	60.82[b]	87.81	0.37	-		n.a.
		39.57	35.84	231.8	n.a.	n.a.	-	-0.01		n.a.
		93.67	101.27	318.5	n.a.	1125.2	-	0.09		n.a.

Notes: n.a. = not applicable;
[a] standard errors estimated by linear regression;
[b] this estimate applies to control fish sampled at day 4.

Analysis of covariance showed that the residual variances about the regressions lack homogeneity at the 0.05 probability level. This does not appreciably affect the contrast between the adjusted means, as the number of observations in each combination of temperature and light regime is four (Scheffé, 1959). More important, the 14 regression coefficients differ no

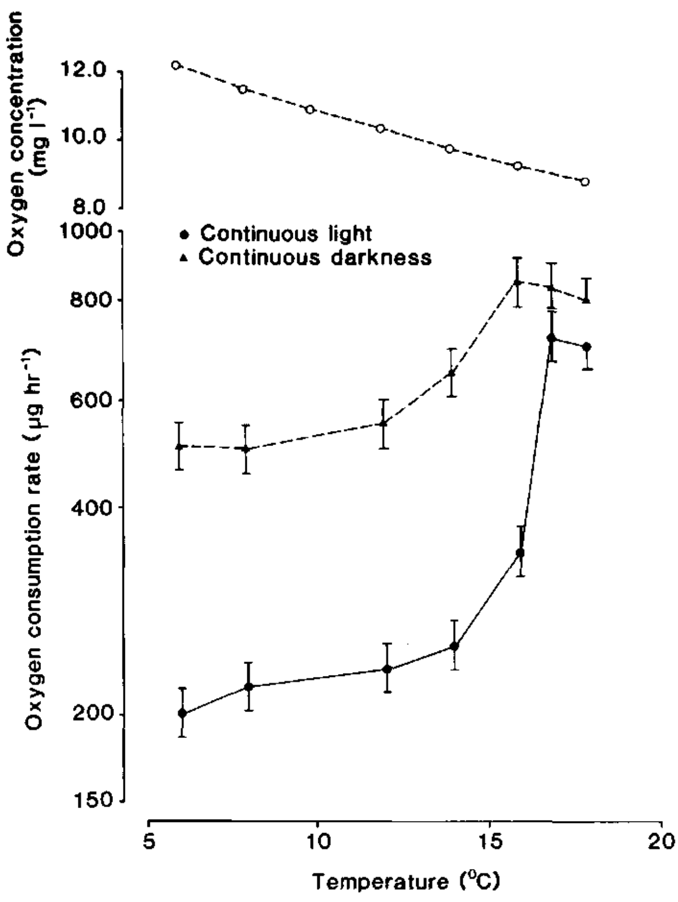

Fig. 5 Effect of temperature on the saturation concentration of oxygen in water, effect on oxygen consumption rate (µg h^{-1}) with standard errors of stone loach, of a standard weight of 495.2 mg dry weight, in both the light and the darkness.

more than random variation would suggest ($F_{13,28}$<1.0). The body weight exponents for the two light regimes do not differ ($F_{1,40}$<1.0):

$$O = a\,W^{0.81} \qquad (5)$$

where O : oxygen consumption rate ($\mu g\ h^{-1}$);

a : a coefficient influenced by temperature and light regime;

W : dry body weight (mg).

The exponent has a standard error of 0.03. Oxygen consumption increases with temperature until an optimum is reached, above which respiration rate decreases again. The difference between routine and active oxygen consumption decreases at higher temperatures, which suggests that activity decreases as the temperature rises above a certain level (Fig. 5).

DISCUSSION

In the present study, total body burden was inclusive of cadmium levels in the gut of stone loach. However, about one-third of the fish had empty guts at the time of dissection and the amount of faeces recovered from the guts of the other fish was small (2.3 mg dry weight corresponding, on average, to 15.3 mg wet weight, and which can be compared with the daily ration of 71.2 mg wet weight *Tubifex*, for fish of 577.9 mg dry weight). These results indicate that most of the food remains had already been evacuated within about 18 h of the fish being fed. There is little evidence in the literature (see e.g. Harrison and Klaverkamp, 1989; Kumada *et al.*, 1980) to suggest that adsorption of cadmium from the gut lumen onto the gut wall constitutes a significant part of the total body burden. However, since in the present study the gut was not analysed separately, adsorption onto the gut wall has to remain a possibility, with concomitant effects on the results obtained. The consistent increase in cadmium burden of loach with time (third experiment) corresponds with the results for trout and whitefish (Harrison and Klaverkamp, 1989).

The final concentrations of cadmium in the *Tubifex* worms fed to stone loach were, for seven separate exposures in five experiments, 74.4 mg kg^{-1} (dry weight), with a range of 20.3-169 mg kg^{-1} (Table 2). These values are comparable with the upper end of the range of cadmium concentrations found in food items from the River Ecclesbourne (0.01 - 100 mg kg^{-1} dry weight; see Douben, 1989c). In addition, *Tubifex* lose cadmium slowly (table 3). Given that *Tubifex* are readily digested by loach, all the cadmium associated with *Tubifex* should be available to the fish (Kay, personal communication). It is, therefore, unlikely that differences in distribution of the cadmium within the tissues of *Tubifex* artificially exposed in the present experiments, compared to *Tubifex* exposed in the wild, would significantly affect the results.

Although estimates of efficiency in uptake show great variation at low concentrations of cadmium in the diet, there is a clear trend between efficiency and cadmium levels in *Tubifex*: the efficiency drops considerably as the concentration of cadmium in the food increases (see also Hatakeyama and Yasuno, 1982); a higher proportion of the metal passes through the gut unassimilated (Dallinger and Kautzky, 1985; Luoma, 1983). Thus in spite of the higher concentration in *Tubifex*, the dose in loach does not increase to the same extent. Efficiency of cadmium uptake from exposed worms by the loach is 8%, on average, after four days of exposure. Results show that, as feeding continues, the efficiency of cadmium uptake by the fish is reduced (see also Hatakeyama and Yasuno, 1982; Niimi, 1983); this estimate is comparable with those by guppies of about 4% after 10 days (Hatakeyama and Yasuno, 1982) and by trout and whitefish of about 1% after 72 days (Harrison and Klaverkamp, 1989).

Diet can be an important source of a contaminant, in addition to water (Kay, 1985; Rodgers *et al.*, 1987). Cadmium concentrations in the water were low (Table 2) and were therefore unlikely to act as a significant source for the loach (Douben, 1989a). Cadmium in the *Tubifex* did not affect the appetite of the stone loach, and moreover, assimilation of energy was not affected by cadmium in the *Tubifex*. This is in contrast to the reduced intake of food contaminated with either copper (Lanno *et al.*, 1985) or zinc (Farmer *et al.*, 1978) for two salmon species. Fish showed an initial depression in growth, but caught up with control fish as experiments continued. Rodgers and Beamish (1982) found that the degree to which intake of food and growth of trout was depressed by methylmercury in the diet was proportional to the concentration of methylmercury, and that the exposed fish did not subsequently catch up with the control fish. Presumably many factors, such as type of food, species of fish, and type of contaminant, determine the effect on food consumption and growth, and any generalisation is, therefore, difficult to infer (see also Alabaster and Lloyd, 1982; Dallinger *et al.*, 1987; Rodgers *et al.*, 1987).

Laboratory studies on food consumption and growth can provide reliable estimates of food consumption in nature (Warren and Davis, 1967). My data suggest that in equation (4), for food consumption, and in equation (5), for respiration, the exponent for body weight is independent of ration and of level of activity, respectively; a conclusion also reached by Paloheimo and Dickie (1966) for several fish species. Webb (1978) suggested an exponent of between 0.75 and 0.8 for food consumption, which is consistent with my estimate of 0.78. The effect of body weight on respiration yields an estimate of 0.81 for the exponent, which is consistent with those found by several authors (e.g. Morris and North, 1984; Cech *et al.*, 1985; Eccles, 1985) and similar to the estimate from feeding experiments (Paloheimo and Dickie, 1966).

Body size affected cadmium burden and feeding ration to a similar extent: estimates of the exponent for cadmium intake (0.63 ± 0.11 and 0.70 ± 0.06) and for food intake (0.78 ± 0.04) were not significantly different. So far as I am aware, no results comparing these two types of experiments have been reported previously. Body weight exponents of 0.34 ± 0.08 and 0.66 ± 0.08 were found for stone loach exposed to water-borne cadmium (Douben, 1989a). Younger tropical fish (*Hyphessobrycon serpae*) had higher concentrations of zinc and lead than older fish, when fed tubificid worms (Patrick and Loutit, 1978), thus suggesting that the exponent for body weight was at least less than unity.

Higher temperatures increase both respiration, at routine and active levels, and the maintenance and maximum rations, i.e. metabolic rate increases with temperature (e.g. Beamish, 1974; Niimi and Beamish, 1974). The degree of effect depends on the temperature (Brett *et al.*, 1969). However, above a certain optimum temperature, which depends on the fish species (Hellawell, 1986), the fish become stressed, food consumption drops drastically, and eventually the fish die (Brett *et al.*, 1969). The results for oxygen consumption obtained at higher temperatures are in parallel with this: the increased demand for oxygen cannot be met because the maximum amount of oxygen in solution decreases and the volume of water passing over the gills is limited (Fig. 5) (see also Saunders, 1962). In addition, loach kept in continuous light were resting at all temperatures, i.e. the rise in oxygen consumption at, and above, 16 °C is not due to increased movement.

Temperature also affects the rate of intake of cadmium from the food: at higher temperatures fish eat more and are thus exposed to higher levels of cadmium. There is some suggestion that the relative increase of cadmium burden is highest at 16 °C: that the drop in relative increase at 18 °C is due to stress is supported by the results of the respiration experiment. The data suggest that temperature also affects the elimination rate constant (k). However, rate of uptake seems to be more influenced than rate of loss. This corresponds with results found by Jimenez *et al.* (1987) for the bluegill sunfish during water-borne exposure and contrasts with results found by Douben (1989a) for loach exposed to water-borne cadmium, where the rate of uptake was affected less by temperature than rate of loss. It is possible that there is a fundamental difference in the effect of temperature on cadmium uptake by stone loach according to the route of exposure e.g. water or food. There is no trend in change of the value of k with temperature for control fish. The value of k appears to be different during and after exposure. This supports the premise that the elimination phase should be regarded as a distinctive component from the uptake phase (Niimi, 1983; Moriarty, 1984). High rates of loss have also been found in rainbow trout (*Salmo gairdneri*) after dietary exposure (Kumada *et al.*, 1980). Cadmium is probably relocated between different tissues. In other words, cadmium in fish is not homogeneously distributed (as the results for after exposure of experiment 3b suggested) and the trend of relocation is affected by the route of exposure (e.g. Sangalang and Freeman, 1979).

Bio-magnification, defined as the occurrence of a substance at successively higher concentrations in successive trophic levels in food chains (Taylor, 1983), receives constant attention (see review by Connell, 1988). Cadmium in food webs was reviewed by Kay (1985). An approach to the question of bio-magnification which includes uptake, metabolism and loss of contaminants by individual species, is required to study transfer of pollutants through the food web (Moriarty and Walker, 1987). The ratio of rate of metal uptake to the rate of loss and metabolism (e.g. detoxification) determines whether or not the concentration of a pollutant is higher in the predator than in the prey. Assimilation of ingested pollutant is an additional variable (Moriarty, 1985), but this does not affect the principle. The data presented here show that temperature affects food consumption by the stone loach and efficiency of cadmium uptake from *Tubifex* declines with higher concentrations in the prey. Comparison of the highest concentration of cadmium of 1.47 mg kg^{-1} dry weight of loach (based on 851.1 ng for fish of a standard dry weight of 577.9 mg) with the highest concentration of cadmium in *Tubifex* of 169 mg kg^{-1} dry weight (both in experiment 3b), reveals that concentration of metal in loach is far lower than in the worms.

Many interacting factors influence an organism's metal burden. In field conditions, even in the unlikely event of a constant exposure, the intake of cadmium by the stone loach will be influenced by factors associated with metabolism.

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CHAPTER 4: Effect of Sediment on Cadmium and Lead in the Stone Loach (*Noemacheilus barbatulus* L.)

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EFFECT OF SEDIMENT ON CADMIUM AND LEAD IN THE STONE LOACH (*NOEMACHEILUS BARBATULUS* L.)

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ABSTRACT

Stone loach (*Noemacheilus barbatulus* L.), a common species of fish in Britain, of different body sizes were kept with different types of material on the bottom of their aquaria. One type was sediment taken from the River Ecclesbourne, Derbyshire, a site with high levels of cadmium and lead. Another type was acid-washed sand (a.w.s.). A third group was kept without any bottom-covering material (control). Fish were starved during the experimental procedure and therefore lost some weight, which was independent of treatment. Body size affected both cadmium and lead burden: the exponent for body weight was 0.88 ± 0.13 for cadmium and 0.59 ± 0.18 for lead ($t=1.32$). Fish with sediment had enhanced body burdens of both cadmium and lead on all occasions while those kept with a.w.s. usually had lower metal levels than control fish. In the presence of sediment from the River Ecclesbourne, by applying a one-compartment model, rate constants for loss of both cadmium and lead were high which resulted in a rapid approach of body burden to a steady state. It was suggested that uptake of metal from sediment was more important as a route of entry for lead than for cadmium under the described conditions.

INTRODUCTION

Aquatic organisms can acquire contaminants, and heavy metals in particular, from their environment. There has been a long debate about the importance of different parts of the physical environment (e.g. food, water, sediment) as sources of persistent pollutants, such as heavy metals, for aquatic animals. The role of sediments in aquatic systems is complex; they play a crucial role in the distribution of heavy metals in the aquatic environment (Allan, 1986). They can act as a repository and release metals into the rest of the environment e.g. water (Salomons, 1985; Salomons *et al.*, 1987). High metal concentrations in the sediment and low concentrations in the water indicate the chronic nature of pollution (Dallinger and Kautzky, 1985).

This paper describes a study on the importance of exposure to cadmium and lead in sediment for the metal burden of the stone loach (*Noemacheilus barbatulus* L.), a common fish in British rivers. It forms part of a larger study on the exposure-dose relationship of heavy metals in stone loach. Laboratory studies covering exposure of the loach to water-borne (Douben, 1989a) and dietary cadmium (Douben, 1989c) and field studies covering lead and cadmium burden of loach and fluctuations in concentration of these heavy metals in water have already been discussed (Douben, 1989b and d).

Two experiments are described, which were intended to:

1. assess the effect of body size on metal burden;
2. determine the importance of sediment for the metal burden;
3. quantify rates of uptake and loss of both cadmium and lead by the stone loach in the presence of sediment.

The results of this study were compared with lead and cadmium burden of stone loach under field circumstances (Douben, 1989b).

MATERIALS AND METHODS

Sampling and Keeping of the Fish

Stone loach were caught on three occasions by electric fishing from the Suttonbrook, a small stream in Derbyshire (National Grid Reference SK 222 342). This site had low concentrations of heavy metals in the sediment (Nichol *et al.*, 1970; Webb *et al.*, 1978). Fish were then kept in storage tanks for at least one month at about 16°C (the temperature of all experiments) during which period they were fed on a mixture of live food (*Tubifex* spp.) and pellets of fish food. This allowed the fish to acclimatize to mains water.

The Exposure System

Fish were kept in a continuous-flow system, developed from one described by Benoit *et al.* (1982). The water came from the mains supply (for aspects of water quality see Douben 1989a). The fish were kept singly, both to avoid interaction (see Street and Hart, 1985) and to enable valid comparisons to be made between results obtained for individual fish, which were kept in aquaria with 6 l of water and a flow-rate of about 1.8 l h^{-1}. The temperature of the water was kept at 16 ± 0.1 °C. All experiments had a 12 hr light : 12 hr dark regime.

Sediment

Two types of bottom-covering material were used: one type was sediment from the River Ecclesbourne (National Grid Reference SK 288 505), a site with high concentrations of cadmium and lead (Webb *et al.*, 1978; Moriarty and Hanson, 1988), and the other was acid-washed sand (a.w.s). The use of these two sediments was to distinguish between sediment as a source of metal and as a physical object. Representative sediment samples were taken from the River Ecclesbourne with a stainless steel scoop. They were sieved at the site through a 2 mm sieve and transported to the laboratory in covered polythene buckets.

Water

During both experiments water samples were taken to determine the metal concentration. There were no significant differences between the results for individual aquaria: data were therefore pooled for each experiment (table 1). Given the low metal concentration, it was assumed that no uptake occurred from the water (for cadmium see Douben, 1989a).

Experimental Procedure

Table 1 summarizes the two experiments that were carried out. Fish were starved to estimate uptake of cadmium and lead from sediment only and to avoid any uptake from food (see also Taylor, 1983).

Table 1 Details of two experiments with stone loach and sediment. Number of observations in parentheses.

Experiment no.	Concentration of metal in water ($\mu g\ l^{-1}$)		Concentration of metal in sediment ($mg\ kg^{-1}$)				
	cadmium mean ± s.e.	lead mean ± s.e.	type of sediment[a]	cadmium logarithmic mean ± s.e.	cadmium geometric mean	lead logarithmic mean ± s.e.	lead geometric mean
1	0.96 ± 0.41 (23)	14.21 ± 4.26[b] (18)	none	n.a.		n.a.	
			R. Ecc[c]	1.42 ±0.05 (12)	26.36	3.67 ± 0.03 (12)	4677
			a.w.s.	-1.70 ±0.10 (12)	0.02	0.69 ± 0.02 (12)	4.90
2	0.17 ± 0.03[d] (10)	3.05 ± 1.35[e] (4)	none	n.a.			
			R. Ecc[c]	1.43 ±0.04 (13)	26.86	3.68 ± 0.01 (13)	4792
			a.w.s.	-1.65 ±0.16 (12)	0.02	0.66 ± 0.01 (12)	4.56

n.a. indicates not applicable;
[a] a.w.s. (acid-washed sand) is classified as sediment for practical purposes;
[b] lead levels in 5 samples were below the limit of detection of 0.4 $\mu g\ l^{-1}$ and excluded;
[c] indicates that sediment from the River Ecclesbourne was used;
[d] cadmium levels in 14 samples were below the limit of detection of 0.1 $\mu g\ l^{-1}$ and excluded;
[e] lead levels in 20 samples were below the limit of detection of 1.5 $\mu g\ l^{-1}$ and excluded.

For all experiments sediments or acid-washed sand (a.w.s.) were added to the aquaria two days before the start of an experiment and left to settle down. Then after settling there was a layer of about 2 cm of bottom-covering material. At the same time, fish in the storage tanks were starved for 24 hours to empty their guts. They were then caught individually in a net, which was dried on the outside with a slightly damp tissue paper for about five seconds. The fish were then weighed by transferring them to a tared vessel containing water on a balance. They were arrayed in order of weight and allocated at random within successive blocks of fish to each treatment to give five fish per treatment for each experiment (ten fish per treatment in the second experiment). The fish were then transferred to the experimental room for final acclimatization for one day.

In the first experiment, groups of fish were exposed to either sediment or a.w.s. A third group of fish in aquaria with no sediment or sand served as controls. Five fish in each group were sampled at the start and after 2, 4 and 8 days. The second experiment was performed to confirm the results of the first; sampling took place after 8 days only.

After sampling, fish were killed and weighed again, this time directly on a balance on aluminium foil after blotting dry for about five seconds. Any gut content was squeezed out. Care was taken to ensure that no sediment particles were left behind on the skin. The gills were dissected out to analyse them separately from the rest of the body. These data were intended for other work which will be published separately. The samples were weighed, dried at 85°C for 3 days and then reweighed. The total metal burden in the fish was obtained by adding together the results for the gills and those for the rest of the body.

Metal analysis

Fish

Metal analyses were carried out as described by Douben (1989a). Total metal burden in the fish was estimated as described above.

Sediment

A subsample of the sediment from the aquaria was ground from which approximately 1 g was weighed into a 50 ml boiling tube, digested with 5 ml 'Ultrar' concentrated nitric acid, left overnight at room temperature,and then heated until no brown fumes appeared at 120 °C.

The solution was then topped up with fresh acid to 5 ml, decanted and rinsed with distilled water into a 25 ml volumetric vessel and made up to volume to give an approximately 20% acid solution. These solutions were analysed for cadmium and lead by atomic absorption spectrophotometry.

Statistical Computations and Mathematical Modelling

Mass of body burden was used because body weight changed during the course of the experiments (see Douben, 1989a and b; Moriarty *et al.*, 1984). Metal burdens were transformed onto a logarithmic scale so that the residual variation in the data conformed more nearly to a normal distribution.

Weight is a prime source of variability. If this source of variation is not taken into account, then differences between treatments can be masked and results misleading by this particular type of variability. Moreover, one of the specific aims of this study was to quantify the effect of body weight. Therefore, all groups included fish of different body weight but with comparable mean weights. The original data had therefore to be adjusted for weight before the effect of different treatments (combination of factors) could be assessed on, for example, metal burden in stone loach. This type of statistical analysis of the results, analysis of covariance (see Sokal and Rohlf, 1981) rather than analysis of variance, allows for differences in weight by removing part of the variation that is due to differences in body weight, and, at the same time, provides information on the effect of body weight (e.g. Douben, 1989a).

The data for wet weight of the fish before the start of the experiments and at the time of sampling were used to estimate the proportional change of wet weight for each fish during the experiment. These estimates were compared by analysis of covariance (see Sokal and Rohlf, 1981), with initial wet weight as covariate, to evaluate the effect of treatment on change of weight. Assumptions of the analysis were tested in each instance (see Douben, 1989a). Analysis of covariance for the proportional loss of wet weight on the initial weight for each of the two experiments shows that the residual variances about the regressions lack homogeneity in the second experiment (table 2a). However, as all groups of fish contain the same number of observations, there is little difference between the estimated and weighted variances. Thus inequality of variances has little effect on inferences about the means (Scheffé, 1959).

For both experiments the effects of treatments (combination of factors) on lead and cadmium burden were tested by analysis of covariance, with dry weight of the body (ranging from 71.5 to 1972.9 mg) as the independent variable (covariate) and with type of sediment and time of sampling as factors. The results for the different treatments are given as means adjusted for weight: the cadmium burden for a fish of standard weight. The standard weight of fish was taken as the mean weight of all the fish in both of the separate experiments.

Data from the first experiment were adjusted for differences in weights of fish (Douben, 1989a) and then used to estimate the parameters of a one-compartment model of metal burden (see Atkins, 1969). Models with and without a component for intake of metal (I) were fitted and the best fit was used for comparisons. Standard deviations of the estimates for parameters in compartment models cannot readily be used to determine confidence limits (Atkins, 1969) except when a linear regression is appropriate for net loss (after 8 days).

RESULTS

Loss of weight

The slopes of the regressions are not dissimilar in both experiments (table 2a), thus the assumptions of the analysis of covariance appear to be adequately satisfied. There was a significant ($P<0.001$) effect of body size on the proportional change of wet weight (-2.05E-04 ± 0.26E-04 and -3.35E-04 ± 0.80E-04 for the first and second experiment respectively), but for practical purposes this effect is negligible. All fish lost weight and, although there was a significant effect of treatment in the first experiment (table 2a), the amount of weight lost was not different between the groups of fish sampled at the same time.

Effect of body size on metal content

One fish from the first experiment had an anomalously high lead content; the value was 11.8 μg, 3.2 standard deviations from the predicted value. Therefore this fish was excluded from computations with lead.

The relationship between body weight and metal content can be expressed as:

$$M = a * W^b \qquad (1)$$

where : M is the metal content (ng);

W is dry body weight (mg);

a is a coefficient;

b is the body weight exponent.

The body weight exponent has, of course, a value of 1.0 if concentration of metal in the body is independent of body weight. The residual variances about the regression of metal content on dry weight of fish are homogeneous for cadmium (table 2b) but not for lead (table 2c). The slopes are not dissimilar in either of the experiments. For cadmium the two experiments also

Table 2 Summary of results of analyses of covariance for a. the proportional loss of weight on initial weight, b. the logarithmic amount of cadmium and c. the logarithmic amount of lead on the dry weight of stone loach in two experiments.

a: proportional loss of weight

Experiment no.	Tests for homogeneity of residual variance about regression χ^2	df	dissimilarity of individual slopes	Estimate for common slope ± s.e.	Variance ratio for effect of treatments
1	11.48	9	$F_{9,30} = 1.18$	-2.05 ± 0.26 E-04	$F_{9,39} = 16.53^{***}$
2	9.91^{*}	3	$F_{3,32} = 1.60$	-3.35 ± 0.80 E-04	$F_{3,35} < 1.0$

b: cadmium

Experiment no.	Tests for homogeneity of residual variance about regression χ^2	df	dissimilarity of individual slopes	Estimate for common slope ± s.e.	Variance ratio for effect of treatments
1	10.45	9	$F_{9,30} = 1.20$	0.69 ± 0.12	$F_{9,39} = 3.28^{**}$
2	3.43	3	$F_{3,32} = 1.36$	1.14 ± 0.23	$F_{3,35} = 4.76^{**}$
all	20.21^{+}	13	$F_{13,62} = 1.15$	0.88 ± 0.13	$F_{13,75} = 3.69^{***}$

- continued -

- continuation -

c: lead

Experiment no.	Tests for homogeneity of residual variance about regression χ^2	df	Tests for dissimilarity of individual slopes		Estimate for common slope ± s.e.	Variance ratio for effect of treatments
1	18.37^{*}	9	$F_{9,29}$	<1.0	0.44 ± 0.22	$F_{9,38} = 5.14^{***}$
2	8.36^{*}	3	$F_{3,32}$	<1.0	1.02 ± 0.28	$F_{3,35} = 13.99^{***}$
all	26.80^{*}	13	$F_{13,61}$	<1.0	0.59 ± 0.18	$F_{13,74} = 7.24^{***}$

$^{+}$, *, ** and *** indicate significance at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively.

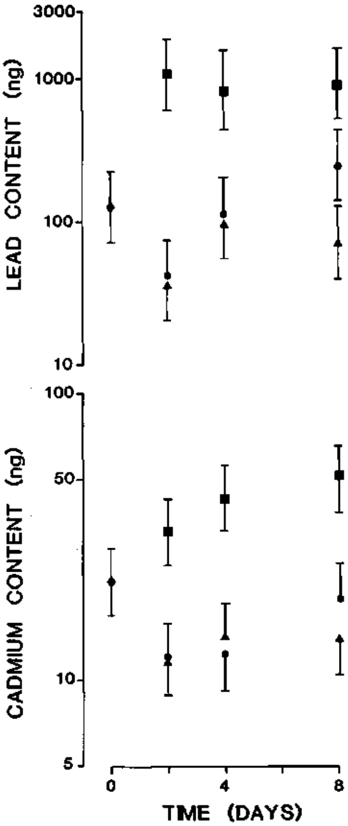
had similar slopes ($F_{1,74}$=3.29, P<0.10), but they differed for lead ($F_{1,73}$=7.95, P<0.01). More important, all individual slopes showed no significant variation: $F_{13,62}$=1.15, P>0.10 for cadmium and $F_{13,61}$<1.0 for lead; the way body burden changes with weight is not affected by exposure. Combining the results of the two experiments, then the body weight exponents are estimated, from the common slopes, to be 0.88 ± 0.13 for cadmium and 0.59 ± 0.18 for lead which are not significantly different from each other (t=1.32, P>0.10).

Effect of sediment and acid-washed sand on metal burden

The results of the second experiment confirmed the first: fish kept with sediment from the River Ecclesbourne always had higher metal burdens than both control fish and fish kept with a.w.s. (figs 1 and 2). Fish kept with a.w.s. in their aquaria had always lower lead levels than control fish sampled at the same time. Cadmium levels followed a similar trend, but none of these differences were significant (figs 1 and 2).

A one-compartment model has been used for describing loss of metal from fish. To facilitate comparisons between the different groups of fish, a one-compartment model has also been used to estimate rates of intake of metal by fish exposed to sediment from the River Ecclesbourne. Estimates for rate of intake (I) and the elimination rate constant (k) are given in table 3. The error associated with the estimates for the elimination rate (k) were obtained by linear regression only when there was net loss of metal from the fish.

Given the effect of type of metal on the body weight exponent, the effect of sediment and a.w.s. on metal burden of loach has to be assessed separately for cadmium and lead.



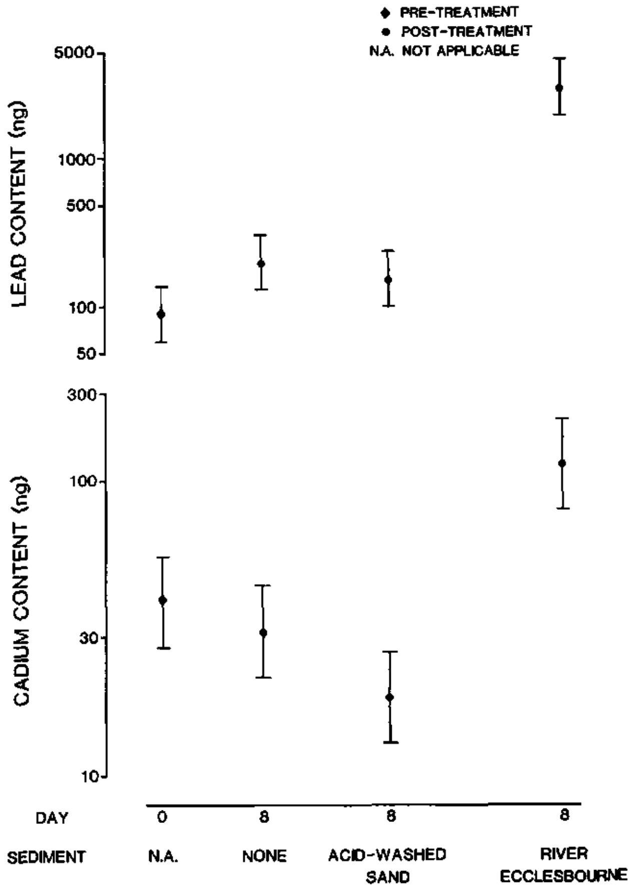


Fig. 1 Mean lead and cadmium content (ng) and standard errors of stone loach at the start of the experiment (♦) and in the presence of 3 types of sediment in the aquaria. (■) indicates presence of sediment from the River Ecclesbourne, (●) indicates control (no sediment) and (▲) indicates presence of acid-washed sand. Standard weight of fish was 490.1 mg dry weight for lead results and 479.7 mg dry weight for cadmium results. First experiment, see table 1 for details.

Fig. 2 Mean lead and cadmium content (ng) and standard errors of stone loach of a standard weight of 500.4 mg dry weight at the start of the experiment (♦) and after exposure to 3 types of sediment in the aquaria (●). Second experiment, see table 1 for details.

Table 3 Estimates for rates of intake (I), elimination rate constant (k), body burden at day 8 and asymptotic burden for cadmium and lead of stone loach with two types of sediment in the aquaria and one control. Estimates for first experiment were derived from a one-compartment model based on adjusted body weights of fish. See text for details.

Metal	Type of sediment[a]	Rate of intake I ($ng\ day^{-1}$)	Elimination rate constant k (day^{-1})[b]	Body burden (ng) on day 8 (geometric mean) (measured)	(estimated)	asymptotic
Cadmium	none	n.a.	0.002 ± 0.058	19.69	20.91	n.a.
	a.w.s.[c]	n.a.	0.07 ± 0.07	13.75	13.80	n.a.
	R. Ecc[d]	35.66	0.55	50.51	64.1	64.8
Lead	none	16626	70.3	247.3	236.4	236.4
	a.w.s.[c]	n.a.	0.04 ± 0.03	72.2	130.0	n.a.
	R. Ecc[d]	152660	32.5	935.6	4701	4701

Notes:
n.a. = not applicable;
[a] for practical reasons a.w.s. is classified as sediment;
[b] standard errors, where indicated, estimated by linear regression in case of net loss;
[c] indicates acid-washed-sand;
[d] indicates sediment from the River Ecclesbourne.

Cadmium

Body burden in control fish and in fish kept with a.w.s. tended to decrease although estimated values of k were not significantly different from zero (table 3). However, fish kept with sediment from the River Ecclesbourne have higher levels of cadmium than those sampled at the start of the experiment. The value of k appears to be highest in the presence of this type of sediment. A steady state burden of cadmium was approached rapidly. None of the cadmium levels in loach used in the second experiment were significantly different (P>0.10) from those in fish used in the first experiment with the same treatment.

Lead

Fish kept with sediment from the River Ecclesbourne had significantly higher lead burden than those at the start of the experiment (fig 1).

The one-compartment model suggests uptake of lead by control fish (table 3). However, random variation was such that there was no significant difference in lead burden after 8 days in comparison with the start of the experiment (fig 1). Also, the estimate of the asymptotic body burden based on these results is within the range of the metal burden of fish sampled at the start of the experiment. The model suggests that already after 8 days this highest burden is reached (table 3). Analysis of covariance of both experiments combined showed that lead levels in control fish and fish kept with a.w.s. sampled after 8 days in the two experiments were similar (P>0.10); differences between lead levels in fish exposed to River Ecclesbourne sediment were relatively insignificant (P<0.10).

DISCUSSION

The results of the second experiment have confirmed the outcome of the first one: fish exposed to sediment with relatively high concentration of metal have enhanced cadmium and lead burden in comparison with initial levels. Also levels in fish that had received the other two treatments were similar. Comparable effects have been shown for invertebrates (Lewis and McIntosh, 1986). Entry of metal from sediment into fish may depend on the type of fish (Maitland, 1965). Ney and Van Hassel (1983) have shown for different species of fish that benthic fish had higher metal levels than mid-water fish. Stone loach buries itself in the mud (Hyslop, 1982).

Although there were no significant differences between metal levels of control fish and of those kept with acid-washed sand, there was a trend that way. For lead, on all four occasions fish with acid-washed sand had lower body burden than control fish. If there were no real differences, the probability of these results is 1 in 16. For cadmium, fish with acid-washed sand had lower body burdens on three of the four occasions. Possibly, the physical presence of sediment reduces body burden slightly perhaps through change in behaviour and metabolism of the fish.

Estimates of rate constant for loss (k) of cadmium during exposure obtained in this study (0.55) is closer to the value of k obtained during dietary exposure (0.63) than the value of k obtained during water-borne exposure (0.21 for exposure to 1 mg l^{-1} and 1.28 for exposure to 0.08 mg l^{-1} cadmium) (see Douben, 1989a and c). The estimated value of k for cadmium is presumably lower than the one for lead during exposure to sediment, suggesting that the biological half-life is greater for cadmium than for lead. Average lead burden, in the first experiment, was highest in fish sampled on day 2. Calculation of half-life based on the entire curve may unduly be influenced by this high burden, leading to an underestimate of the true half-life. It is noteworthy that a wide range of half-lives have been found, even for the same metal (see review by Niimi, 1983). Half-life depends on the route of exposure; for example lead taken up from water is more readily lost than lead taken up from food (Vighi, 1981). In this study estimates of k for control fish and fish kept with a.w.s. were not different from zero (table 3).

The results clearly demonstrate the effect of body size on cadmium burden. If body weight affects respiration in the same way as it affects metal burden, the exponents for body weight will be similar. Other studies concerning oxygen consumption rate, maintenance ration and uptake of cadmium from food, have shown values between 0.63 and 0.81 (all with relatively small standard errors) for the exponent of body weight (see Douben, 1989c). Also, field observations on cadmium levels have shown a similar value (0.79 ± 0.06) (Douben, 1989b). The exponent relating body weight to cadmium levels, obtained in this study, has a value of 0.88 ± 0.13. The considerable standard error leads to no significant difference between this value and other exponents of body weight. However, it should be pointed out that those others were all different from unity.

For lead in stone loach, no data from other laboratory studies are available on the effect of body size on metal burden. Comparison of the value of the exponent for body weight in this study (0.59 ± 0.18) with one from field investigations (0.13 ± 0.21) (Douben, 1989b) shows that there is relatively little difference between these two estimates ($t=1.66$, $P<0.10$). Given the value of exponents of body weight for oxygen consumption rate and maintenance ration, there is presumably no direct link between the effect of body weight on these processes and on lead burden. Although the exponent for body weight for cadmium and lead found in this study are not different, the estimate for lead is lower than the estimate for cadmium; a similar trend has been found for cadmium and lead in stone loach during field observations (Douben, 1989b).

There is little information from laboratory experiments with which the present study can be compared. It has been shown for some fish that they can take up pollutant directly from the sediment (Gillespie, 1972).

There are numerous field observations usually dealing with the relationship between contaminated sediment and metal burden in fish (e.g. Bradley and Morris, 1986; Ney and Van Hassel, 1983), including stone loach (Douben, 1989b). From the site in the River Ecclesbourne where the sediment was sampled, data on metal burden in the bullhead (*Cottus gobio* L.) are published (Moriarty *et al.*, 1984). The last two studies report high levels of cadmium and lead in fish from locations with high metal concentration in the sediment. The question arises whether or not these observed levels are due to uptake from the sediment. Comparison of the observed metal levels for fish exposed to sediment from the River Ecclesbourne with levels in fish of similar weight caught in the same river during field observations (Douben, 1989b) shows that cadmium levels in this study are lower and lead levels are of similar order (table 4). Fish can acquire heavy metals from different sources: food, water and sediment. Douben (1989b) suggested that, based on the exponent for body weight, water-borne cadmium presumably did not

Table 4 Mean lead and cadmium burden (ng) and standard errors of stone loach of similar weight (from the Suttonbrook) after 8 days' exposure to sediment from the River Ecclesbourne (first experiment) and of stone loach taken from the River Ecclesbourne on two occasions. (see Douben, 1989b for details).

Origin of fish	Mean dry weight (mg)	Metal burden (ng)			
		Lead logarithmic mean ± s.e.	geometric mean	Cadmium logarithmic mean ± s.e.	geometric mean
Experimental fish	490.1[a]	2.97 ± 0.25	933	n.a.	
	479.7[a]	n.a.		1.70 ± 0.13	50.12
Field fish	436.5[b]	3.15 ± 0.12	1413	2.11 ± 0.19	128.8
	549.5[c]	2.96 ± 0.25	912	1.98 ± 0.08	95.5

n.a. indicates not applicable;
[a] mean dry weight of fish differed for lead and cadmium because one fish was excluded for lead computations, see text for details;
[b] fish were caught on 17 July 1985;
[c] fish were caught on 14 August 1985.

contribute substantially to the burden of stone loach. The difference between the observed cadmium levels in this study and those obtained during field investingations may therefore indicate an additional uptake from the diet. For lead, assuming that only lead in solution is available for uptake and given that the concentration of lead in solution is low (up to 0.6 $\mu g\ l^{-1}$) because most of it is associated with particles (Douben, 1989d), entry from the water is unlikely to occur. Then comparison of these laboratory results with the field data suggests that there is virtually no additional uptake of lead from the food. Douben (1989b) suggested already that uptake of metal via the food was less important for lead than for cadmium. This hypothesis is supported by the outcome of this study.

The results of this study indicate that some of the cadmium and lead in sediments can be taken up by fish. Bio-availability of particulate-associated contaminants can be assessed in two ways (Allan, 1986): biological uptake and chemical extraction (e.g. sequential extraction), but there is a serious lack of studies that compare results from these two methods (Calmano and Förstner, 1983).

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PART III FIELD STUDIES

CHAPTER 5: Lead and Cadmium in Stone Loach (*Noemacheilus barbatulus* L.) from three rivers in Derbyshire

Ecotoxicology and Environmental Safety, in press.

LEAD AND CADMIUM IN STONE LOACH (*NOEMACHEILUS BARBATULUS* L.) FROM THREE RIVERS IN DERBYSHIRE

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ABSTRACT

Fish *Noemacheilus barbatulus* L. (stone loach) were caught at about 4-weekly intervals from single sites in three Derbyshire rivers, with different concentrations of cadmium and lead in sediments and water, during a one-year's sampling programme. Fish were classified by age, site and sampling occasion. Growth was allometric and affected by temperature. A steady state of cadmium burden was reached by fish of two years old or more but not by younger fish. For lead, fish rarely showed an increase in body burden. Differences in body size accounted for most of the variation in cadmium levels between loach of different agegroups but less important for lead levels. The exponent for body weight was not affected by age of fish and was about 0.79 ± 0.06 for cadmium and for lead 0.13 ± 0.21. There was some correlation between cadmium levels in fish of different agegroups taken at the same time from any site; levels of significance were higher when differences due to body size were discounted. Then, sampling time did not explain a significant part of the residual variation. Fluctuations in the cadmium and lead burdens for fish in the same agegroup within each of the sites were correlated for some comparisons. Loach from sites with higher metal concentrations had higher levels of both cadmium and lead. It is suggested that cadmium uptake from food contributed considerably to the body burden of loach.

INTRODUCTION

This paper describes a one-year survey on cadmium and lead levels in the fish *Noemacheilus barbatulus* L. (stone loach), a common fish in British rivers, from three different sites in Derbyshire. It forms part of a larger study on the exposure-dose relationship of heavy metals in this species. Exposure of the loach to water-borne and dietary cadmium in laboratory experiments, and changes in concentrations of heavy metals in water from these sites, have already been discussed (Douben, 1989a, b and c).

The objectives were to:

1. select a measure of size of fish;
2. assess the effect of body size on metal burden;
3. study changes of cadmium and lead levels in fish with time;
4. evaluate whether amounts of metal in fish reflect the concentrations in the environment (e.g. water, sediment).

MATERIALS AND METHODS

Sampling of fish

Fish were caught by electric fishing from single sites in three Derbyshire rivers at about 4-weekly intervals between 22 May 1985 and 28 May 1986. The concentrations of cadmium and lead in water (see Douben (1989c) for details) and sediments (Moriarty and Hanson, 1988; Webb *et al.*, 1978) differed between sites. High rainfall during a few days before the intended sampling days in April and May 1986 made the water muddy, resulting in poor visibility, and actual sampling took place one week later. A total number of 417 fish were caught (see table 1), with non-detectable levels of lead and cadmium in 26 and 13 fish respectively. Fish were transported to the laboratory in river water in closed polythene bags and kept overnight in aerated river water. They were killed the next day by cutting the spinal cord just behind the head. They were then weighed after blotting dry for about five seconds and their length was measured. Sex was determined by inspection of the gonads. Any gut content was squeezed out.

The gills were dissected out to analyse them separately from the rest of the body. These data were intended for other work which will be published separately. The samples were weighed, dried at 85 °C for 3 days and then reweighed.

At the sampling sites maximum and minimum temperatures since the previous sampling occasion were recorded, pH (at least twice) and conductivity were measured and water samples were taken to measure aspects of water quality (table 2).

Metal analysis

The fish were digested with concentrated nitric acid (see Douben (1989a) for details). Cadmium and lead were determined by an atomic absorption spectrophotometer (electrolyte furnace with background noise correction). The total metal burden in the fish was obtained by adding together the results for the gills and those for the rest of the body.

Determination of age

The age of the stone loach can be determined by the number of opaque rings in the otoliths (Smyly, 1955). However, the otoliths are very small in the loach, even in big fish. Dissecting the fish for otoliths destroys the head, increases the risk of contamination of the sample and reduces the number of fish that can be handled in one day enormously. However, length of fish can be used to distinguish different age groups. This was confirmed by ageing some fish by both methods (see also Mills and Eloranta, 1985; Kännö, 1969). 0+ fish (fish less than one year old) and I+ fish (fish between one and two years old) can be distinguished by length with almost complete certainty (see fig 1). It is virtually impossible to distinguish between fish of two years old or more by length. Individual fish were therefore allocated to one of three agegroups: 0+, I+ and two or more years old (II+).

Statistical computations

Metal levels were measured in units of mass in preference to units of concentration (see Moriarty, 1984; Moriarty *et al.*, 1984; Niimi, 1987).

Statistical analyses were based on samples, defined as a group of fish in one agegroup sampled at one site at one time. When fish contained non-detectable levels of metal, then the mean metal content, with standard deviation, was calculated for all fish with detectable levels in that sample. When the difference between the limit of detection and the mean value was greater than 3 standard deviations, values for those fish with non-detectable levels were discarded. However, when this difference was less than 3, then the metal level of that fish was taken to be half the value of the limit of detection, in order to minimize the maximum error, and the value for that fish was included in both the calculations of the mean for the total sample and in all further computations.

Table 1 Details of fish *Noemacheilus barbatulus* (stone loach) taken from three rivers in Derbyshire between 22 May 1985 and 28 May 1986

Sampling time		Sampling site								
		River Ecclesbourne			Brailsford Brook South			Suttonbrook		
	Age-group:	II+ or more	I+	0+	II+ or more	I+	0+	II+ or more	I+	0+
220585		6	0	0	8	4	0	8	7	0
180685		6	4	0	5	8	0	4	6	0
170785		6	6	0	2	8	0	1	10	0
140885		5	6	1	6	1	9	0	6	7
110985		6	8	4	1	8	5	0	5	6
091085		5	3	1	4	8	3	1	4	8
061185		0	0	2	3	7	6	0	6	9
041285		4	3	0	4	2	4	0	5	9
020186		1	2	0	0	0	0	0	4	11
290186		4	2	0	0	1	0	0	0	0
260286		3	2	2	11	2	5	0	0	0
260386		4	4	0	4	4	4	0	3	0
300486		4	3	0	3	5	1	0	6	0
280586		6	3	0	1	11	1	2	14	0
	Total	60	46	10	52	69	38	16	76	50
	Grand total per site		116			159			142	

Table 2 Mean values and standard errors (s.e.) of aspects of water quality at three sites. Measurements were made on 14 occasions at 4-weekly intervals during the period May 1985 - May 1986; ionic concentrations in mg l^{-1}.

Variable	Site					
	River Ecclesbourne		Brailsford Brook South		Suttonbrook	
	mean	s.e.	mean	s.e.	mean	s.e.
pH	7.3	0.1	7.6	0.1	7.5	0.1
Conductivity (µS)	373	17	431	13	478	14
Ca^{2+}	65.7	8.0	76.1	9.8	81.9	9.5
Mg^{2+}	9.3	0.9	18.2	1.4	19.7	1.5
K^{+}	6.3	0.9	11.2	1.8	9.6	1.0
Na^{+}	33.9	11.6	11.2	1.2	11.3	1.4
total - P	1.35[a]	0.61	0.19[a]	0.03	0.21	0.04
PO_4^{3-} - P (dissolved)	0.92[a]	0.42	0.14[a]	0.03	0.16	0.04
Org. N + NH_4^+-N	0.37[b]	0.09	0.52[b]	0.13	0.55[b]	0.12
NO_3^- - N (dissolved)	4.28	0.74	6.93	1.76	7.42	2.05
NH_4^+ - N (dissolved)	0.52[c]	0.36	0.23[c]	0.09	0.20[c]	0.07
Cl^-	54.1	2.0	25.2	2.3	26.3	2.3
SO_4^{2-} - S	20.0	1.1	20.3	1.4	23.8	1.5

[a] On five occasions the concentration PO_4^{3-} - P exceeded the concentration of total - P; repetition of analyses at the same time confirmed these inconsistencies;
[b] On five occasions levels were below the limit of detection of 0.10 mg l^{-1};
[c] On ten occasions levels were below the limit of detection of 0.10 mg l^{-1}.

Data for total metal burden were transformed onto a logarithmic scale so that the residual variation in the data conformed more nearly to a normal distribution.

Slopes of individual regressions of metal burden on dry weight were compared between samples to test the variation if any on the exponent for body weight (see equation 2 page 148).

Changes in metal content with time were evaluated in two ways: first, without adjusting for differences in weight, by applying analyses of variance to the measured levels of metal in samples, and secondly, after adjusting for differences in weight, due mostly to growth, by applying analyses of covariance (see Sokal and Rohlf, 1981). When the analysis of variance yielded a significant difference between times in amount of metal, then a regression analysis with backward elimination was performed to test whether there was a significant trend in metal levels with time of sampling.

To evaluate whether metal levels in fish of different agegroups from one site were correlated, measured mean levels (thus not adjusted for differences in weight) of both cadmium and lead in fish of different ages were compared. Mean values were used rather than individual data in a model to avoid assumptions about the relationship between time and metal level in fish of different agegroups. Consequently, multiple numbers of fish caught at one time were regarded as replicates used to improve the estimate of the mean values.

Mean metal burden were then adjusted for differences in weight of all fish within the same agegroup i.e. both within and between sampling times. For pairs of agegroups the differences between their mean metal burden were compared to test whether any trend with time in these differences was apparent. Additionally, the correlation between cadmium and lead levels in all fish of the same age from one site were compared to test whether fluctuations in the body content of these two metals were correlated.

The effect of site on metal burden was evaluated by analyses of covariance, with dry weight as covariate.

RESULTS

Temperature and Length

In all rivers growth was greatest when the temperature of the water was highest (fig. 1a, b and c), and was restricted to the period April to September. Small fish (I+ and 0+) grew faster than bigger and older fish (II+ or more). Fish sampled in the winter tended to be lighter.

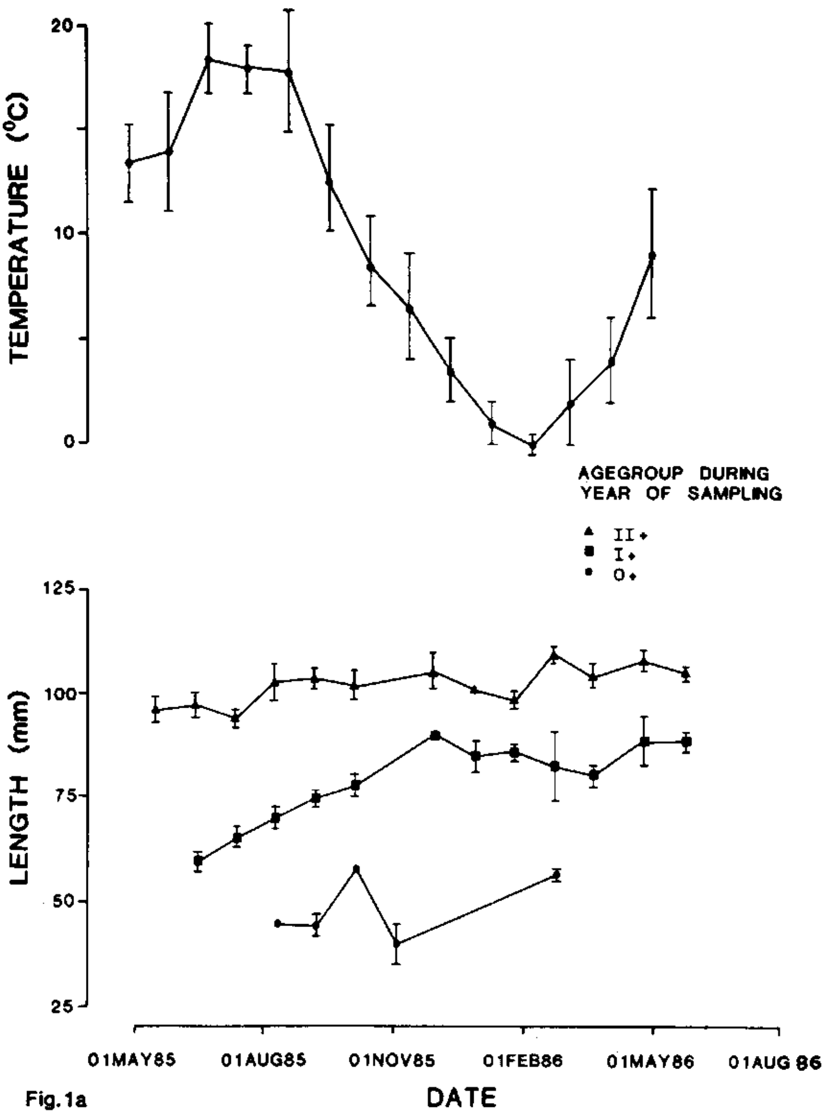
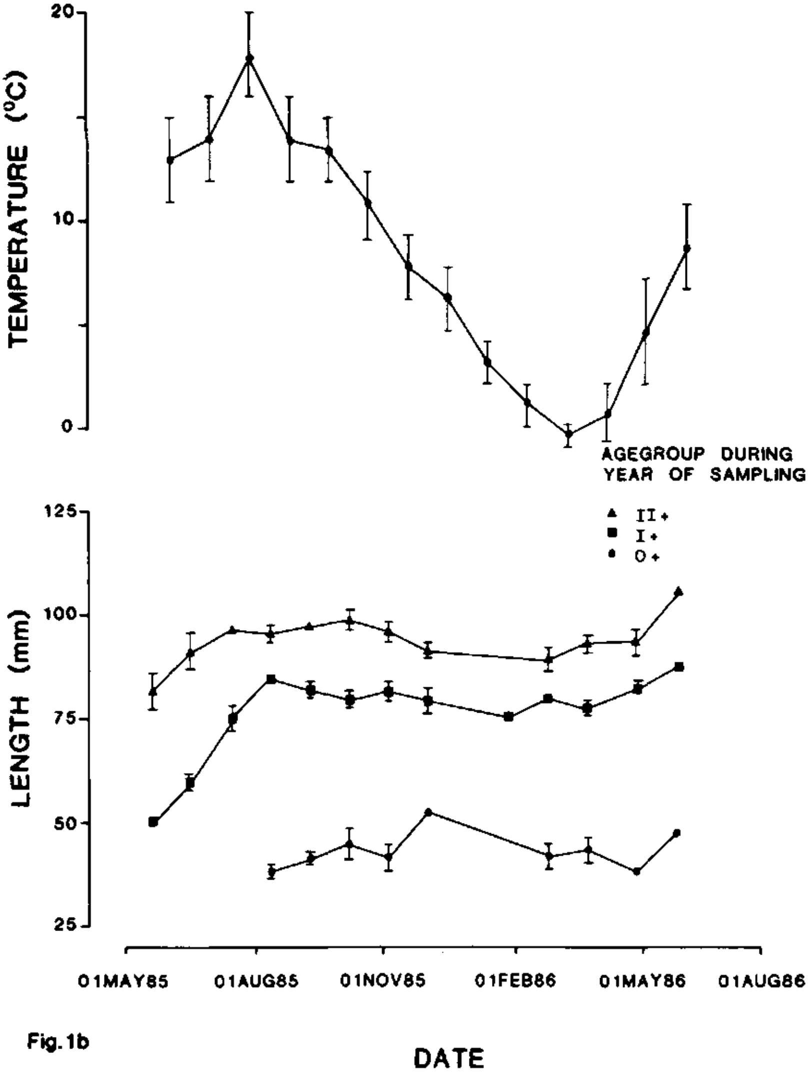


Fig. 1 Mean water temperatures (°C) and range during intervals of 4 weeks, and mean length (mm) with standard errors, of stone loach from three different agegroups sampled from three sites in Derbyshire: (a) River Ecclesbourne (b) Brailsford Brook South (c) Suttonbrook. For sample sizes see table 1.



TEMPERATURE (°C)
20
10
0
AGEGROUP DURING
YEAR OF SAMPLING
▲ II+
■ I+
● O+
LENGTH (mm)
125
100
75
50
25
01MAY85
01AUG85
01NOV85
01FEB86
01MAY86
01AUG86
DATE

Fig. 1b

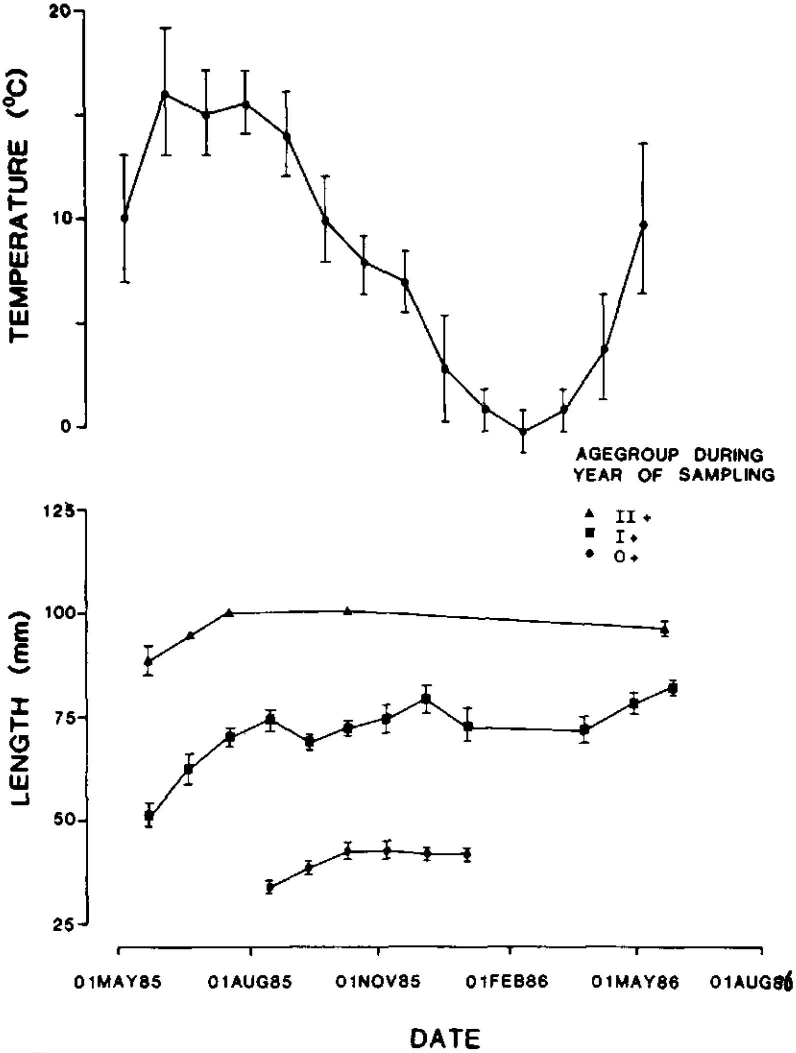

20
10
0
TEMPERATURE (°C)
AGEGROUP DURING
YEAR OF SAMPLING
II +
I +
0 +
125
100
75
50
25
LENGTH (mm)
01MAY85
01AUG85
01NOV85
01FEB86
01MAY86
01AUG86
DATE

Fig. 1c

Water Content

The percentage water content of fish decreased with increase in length and weight at all sites and all sampling times. Slopes of the regressions of the percentage water content on the logarithm of length (mm) sometimes differed significantly for individual sites within each agegroup at different times and between agegroups at all times. However, despite the drop in percentage water content with length, no significant difference was found between the slopes of the regressions of the logarithm of fresh weight (weight of fish before dissection), total wet weight (weight of gills and of rest of body after dissection combined) and total dry weight (all measurements in mg) on the logarithm of length. Moreover, there were no differences between these slopes for different agegroups or sites.

Given that some moisture is inevitably lost during dissection and that time for dissection for all fish was not the same, dry weight seemed the best measure of size of fish and, therefore, all further comparisons are based on dry weight.

Type of Growth

There was no significant effect of sampling time on the dry weight of II+ loach except for those from the River Ecclesbourne, where dry weight increased significantly with time of sampling (fig 2 and table 3). Dry weights of 0+ and I+ fish from Suttonbrook increased more slowly than from the other two sites (fig 2).

Growth of fish can be expressed as:

$$y = a^*x^b \qquad (1)$$

where y represents dry weight (mg) of loach, x represents length (mm) of fish and a is a coefficient. The value of b (3.131 ± 0.063), was not affected by site ($F_{2,326}<1.0$). and is significantly greater than 3 ($P<0.05$), i.e. growth is allometric.

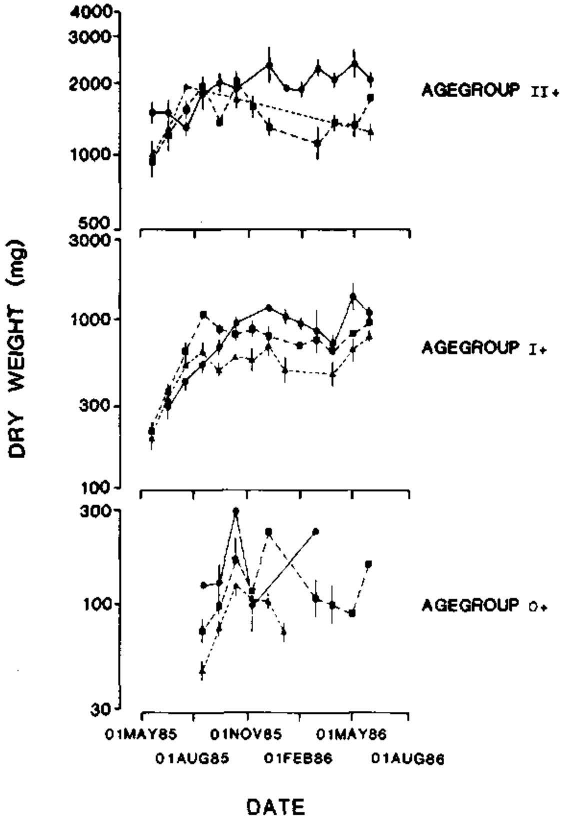


Fig. 2 Mean dry weight (mg) with standard errrors of stone loach sampled at about 4-weekly intervals from three sites in Derbyshire, for three agegroups: II+, I+ and 0+. For sample sizes see table 1. ● indicates fish from the River Ecclesbourne, ▲ those from the Suttonbrook and ■ those from Brailsford Brook South. For sample sizes see table 1.

Table 3 Results of analyses of variance of the logarithm of dry weight (mg) of stone loach and regression analyses of the logarithm of dry weight on sampling time (t = intervals of 4 weeks since 24 April 1985) from three sites in Derbyshire.

Site	Age-group	Analysis of variance: Source of variance	df	Mean Square	F-value	Estimates for regression coefficients (linear) b	± s.e.	(quadratic) c	± s.e.	(cubic) d	± s.e.
River Eccles-bourne	II+ or more	Sample	12	0.0318	3.19^{**}	0.039^{**}	± 0.013	-0.002^{+}	± 0.001	n.a.	
		Residual	47	0.0100							
	I+	Sample	11	0.1477	10.61^{***}	0.330^{***}	± 0.075	-0.034^{***}	± 0.011	0.0011^{*}	± 0.0004
		Residual	34	0.0139							
	0+	Sample	4	0.0615	2.34	n.a.		n.a.		n.a.	
		Residual	5	0.0263							
Brails-ford Brook South	II+ or more	Sample	11	0.0414	1.70	n.a.		n.a.		n.a.	
		Residual	40	0.0244							
	I+	Sample	12	0.2017	18.46^{***}	0.388^{***}	± 0.038	-0.049^{***}	± 0.006	0.0019^{***}	± 0.0003
		Residual	56	0.0109							
	0+	Sample	8	0.1044	3.62^{**}	n.a.		n.a.		n.a.	
		Residual	29	0.0288							

- continued -

- continuation -

Sutton-brook	II+ or more	Sample	4	0.0303	1.35	n.a.	n.a.	n.a.
		Residual	11	0.0224				
	I+	Sample	11	0.2033	9.77^{***}	$0.332^{***} \pm 0.050$	$-0.044^{***} \pm 0.008$	$0.0018^{***} \pm 0.0003$
		Residual	63	0.0208				
	0+	Sample	5	0.1880	10.17^{***}	$0.662^{***} \pm 0.100$	$-0.048^{***} \pm 0.008$	n.a.
		Residual	44	0.0185				

$^{+}$, *, ** and *** indicate significance (analysis of variance) or a significant difference from zero at the 0.10, 0.05, 0.01 and 0.001 probability level respectively;
n.a. indicates not applicable

Effect of Body Size on Metal Content

The relationship between body weight and metal content can be expressed as:

$$M = a * W^{b} \qquad (2)$$

where M is the metal content (ng);

W is dry body weight (mg);

a is a coefficient;

b is the body weight exponent.

The body weight exponent has, of course, a value of 1.0 if concentration of metal in the body is independent of body weight. For each site the body weight exponent of fish was unaffected by either sampling time within agegroup or agegroup. Moreover, individual slopes showed relatively little significant variation: $F_{75,252}<1.0$ for cadmium, $F_{75,251}=1.30$ ($P<0.10$) for lead. Analyses of covariance of the logarithmic amount of metal on the logarithm of the dry weight (covariate), with all sampling times combined for each of the three agegroups, showed that within each site, the residual variances about the regressions were homogeneous and that there was no significant effect on the slopes of the regressions for agegroups within sites or for sites (table 4). This suggests that the effect of body weight on both the cadmium and lead burden is independent of age of loach. Type of metal affects the value of b: 0.79 ± 0.06 for cadmium and 0.13 ± 0.21 for lead.

Table 4 Summary of results of analyses of covariance, of the logarithm of amount of metal (ng) on the logarithm of dry weight (mg), for the effect of agegroup per site and all sites combined.

Site	Metal: Cadmium				Metal: Lead			
	Tests for homogeneity of variance		Tests for similarity of slopes	Estimate for common slope	Tests for homogeneity of variance		Tests for similarity of slopes	Estimate for common slope
	χ^2	df		mean ± s.e.	χ^2	df		mean ± s.e.
River Ecclesbourne	0.93	2	$F_{2,83} < 1.0$	0.80 ± 0.23	0.21	2	$F_{2,83} = 1.38$	0.19 ± 0.49
Brailsford Brook South	5.39*	2	$F_{2,121} < 1.0$	0.75 ± 0.18	5.05	2	$F_{2,121} = 2.45^{*}$	0.59 ± 0.36
Suttonbrook	2.26	2	$F_{2,115} < 1.0$	0.83 ± 0.14	4.65*	2	$F_{2,114} < 1.0$	-0.38 ± 0.29
All sites combined	1.46	2	$F_{2,325} < 1.0$	0.79 ± 0.06	1.71	2	$F_{2,324} = 2.22^{*}$	0.13 ± 0.21

* indicates significance at the 0.10 probability level.

Effect of Sampling Time on Metal Content

Analyses of variance of the logarithmic amount of metal (ng) in fish for each agegroup for each site showed that there was a significant effect of sampling time on cadmium levels in all sets of data (table 5 and fig 3) but usually not on lead levels (table 5 and fig 4).

Body burden of cadmium and lead of II+ fish from all three sites (excluding II+ fish from the Brailsford Brook South sampled on 28 May 1986 for cadmium) had reached steady state levels. For cadmium, levels were higher in fish from the River Ecclesbourne.

Given that metal burden is affected by body weight, much more for cadmium than for lead, and body weight changes with time, differences in metal burden were assessed after adjusting for differences in weight of fish within one agegroup by analysis of covariance. Residual variances about individual slopes lack homogeneity at the 0.05 probability level on some occasions (table 6). This does not appreciably affect inferences about the means, given the range of sample sizes (Scheffé, 1959). Slopes are sometimes dissimilar (table 6). Extrapolation about the effect of body weight on metal burden seems to be justified because the body weight exponent did not change with dry weight. Cadmium levels fluctuated significantly with time, but lead levels did not, except for 0+ and I+ fish from Suttonbrook (table 6). These results for lead are not surprising given the effect of sampling time on lead levels without adjusting for weight and the relatively small value of the body weight exponent for lead.

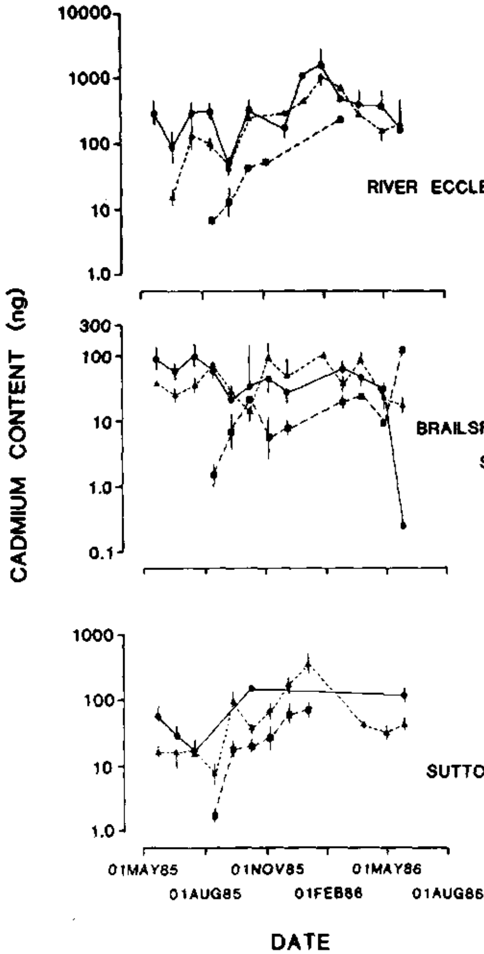


Fig 3 Mean cadmium content (ng) with standard errors of stone loach from three agegroups sampled at about 4-weekly intervals from three sites in Derbyshire: River Ecclesbourne, Brailsford Brook South and Suttonbrook. ● indicates II+ fish, ▲ I+ fish and ■ 0+ fish. For sample sizes see table 1.

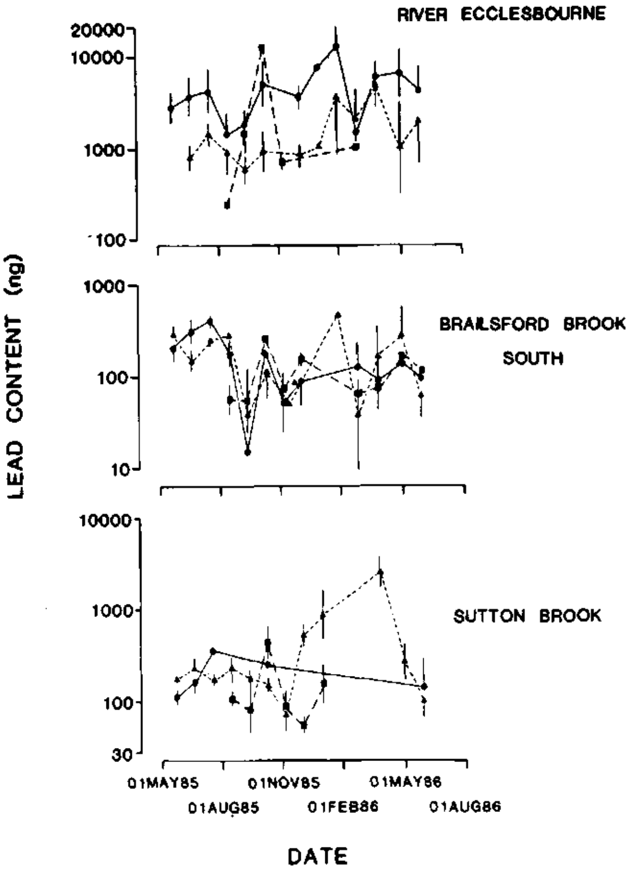


Fig 4 Mean lead content (ng) with standard errors of stone loach from three agegroups sampled at about 4-weekly intervals from three sites in Derbyshire: River Ecclesbourne, Brailsford Brook South and Suttonbrook. ● indicates II+ fish, ▲ I+ fish and ■ 0+ fish. For sample sizes see table 1.

Table 5 Results of analyses of variance of the logarithm of the amount of cadmium and lead in stone loach sampled between 22 May 1985 and 28 May 1986 from three sites in Derbyshire at about 4-weekly intervals (fish with non-detectable levels of metal excluded or value taken as half the limit of detection, see page for details).

Site	Age-group	Source of variance	Analysis of variance: Cadmium df	Cadmium Mean Square	Cadmium F-value	Lead df	Lead Mean Square	Lead F-value
River Ecclesbourne	II+ or more	Sample	12	0.7128	4.72***	12	0.3294	1.39
		Residual	47	0.1510		47	0.2367	
	I+	Sample	11	0.8696	8.94***	11	0.3175	1.24
		Residual	34	0.0973		34	0.2555	
	0+	Sample	4	0.6629	5.85*	4	0.4048	3.93+
		Residual	5	0.1134		5	0.1030	
Brailsford Brook South	II+ or more	Sample	11	0.6254	2.78**	11	0.2924	0.98
		Residual	40	0.2252		39	0.2985	
	I+	Sample	12	0.3894	2.99**	12	0.5644	1.50
		Residual	56	0.1302		56	0.3767	
	0+	Sample	8	1.0253	3.87**	8	0.2014	0.87
		Residual	28	0.2652		29	0.2311	
Suttonbrook	II+ or more	Sample	4	0.2474	1.47	4	0.0855	1.15
		Residual	11	0.1686		11	0.0743	
	I+	Sample	11	1.1016	7.56***	11	0.7585	3.97***
		Residual	63	0.1456		62	0.1909	
	0+	Sample	5	2.6831	16.99***	5	0.8372	3.57**
		Residual	44	0.1579		44	0.2347	

+, *, ** and *** indicate significance at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively.

Table 6 Summary of results of analyses of covariance of the logarithm of cadmium and lead burden (ng) on the logarithm of dry weight (mg) (covariate) of stone loach per agegroup. For details of samples see table 1.

Site	Age-group	Cadmium: Tests for homogeneity of variance χ^2	df	Cadmium: Tests for similarity of slopes	Cadmium: Variance ratio for effect of sampling time	Lead: Tests for homogeneity of variance χ^2	df	Lead: Tests for similarity of slopes	Lead: Variance ratio for effect of sampling time
River	II+	8.00	11	$F_{11,35} < 1.0$	$F_{12,16} = 5.22^{***}$	9.12	11	$F_{11,35} = 2.00^{+}$	$F_{12,46} = 1.49$
Eccles-	I+	16.26	11	$F_{11,22} < 1.0$	$F_{11,33} = 5.55^{***}$	7.24	11	$F_{11,22} = 2.52^{*}$	$F_{11,33} = 1.29$
bourne	0+	n.a.	2	$F_{2,2} < 1.0$	$F_{4,4} = 4.16^{+}$	n.a.	2	$F_{2,2} < 1.0$	$F_{4,4} = 2.40$
Brailsford	II+	20.36^{*}	9	$F_{9,30} < 1.0$	$F_{11,39} = 3.54^{**}$	18.02^{*}	9	$F_{9,29} < 1.0$	$F_{11,38} = 1.03$
Brook	I+	7.77	10	$F_{10,45} = 1.96^{+}$	$F_{12,55} = 3.29^{**}$	22.41^{*}	10	$F_{10,45} < 1.0$	$F_{12,55} = 1.12$
South	0+	8.87	5	$F_{6,21} < 1.0$	$F_{8,27} = 2.69^{*}$	9.17	6	$F_{6,22} < 1.0$	$F_{8,28} < 1.0$
Sutton-	II+	0.01	2	$F_{2,8} < 1.0$	$F_{4,10} = 3.00^{+}$	0.13	2	$F_{2,8} = 1.48$	$F_{4,10} < 1.0$
brook	I+	18.12^{+}	11	$F_{11,51} = 1.05$	$F_{11,62} = 7.53^{***}$	17.90^{+}	11	$F_{11,50} = 1.44$	$F_{11,61} = 4.04^{***}$
	0+	5.17	5	$F_{5,38} = 2.80^{*}$	$F_{5,43} = 12.89^{***}$	11.02	5	$F_{5,38} = 3.59^{**}$	$F_{5,43} = 3.38^{*}$

$^{+}$, *, ** and *** indicate significant difference at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively; n.a. indicates not applicable, only on 1 occasion more than 1 fish in this agegroup were caught.

Table 7 Correlation coefficients between the mean logarithmic amounts of metal (ng) in fish of three different agegroups sampled at three sites in Derbyshire between 22 May 1985 and 28 May 1986 at intervals of about 4 weeks (see table 1 for details). Results for lead towards the upper righthand side, for cadmium towards the bottom lefthand side. Number of sampling times in parentheses.

Metal	Site	Age-group	II+		I+		0+		Metal
Cadmium	River Eccles-bourne	II+	-----		0.386	(12)	0.835	(4)	Lead
		I+	0.809**	(12)	----		-0.146	(4)	
		0+	0.739	(4)	0.737	(4)	----		
	Brails-ford Brook South	II+	----		0.683*	(12)	0.489	(9)	
		I+	0.431 [a]	(12)	----		0.432	(9)	
		0+	-0.707**[b]	(9)	-0.591+	(9)	----		
	Sutton-brook	II+	----		-0.023	(5)	n.a.		
		I+	0.574	(5)	----		-0.394	(6)	
		0+	n.a.		0.957**	(6)	----		

n.a. indicates not applicable as at only one sampling time fish in both agegroups were caught;
+, * and ** indicate significance at the 0.10, 0.05 and 0.01 probability levels respectively;
[a] correlation coefficient equals 0.273 (11) when II+ fish sampled on 28 May is excluded;
[b] correlation coefficient equals -0.053 (8) when II+ fish sampled on 28 May is excluded.

Correlation in Metal Levels

Mean observed cadmium levels (i.e. not adjusted for differences in weight) in fish of different agegroups sampled at one time were correlated for II+ and I+ fish from the River Ecclesbourne and between I+ and 0+ fish from Suttonbrook (in both cases $P<0.01$, see also table 7 and fig 3). If one excludes the one II+ fish that was caught from Brailsford Brook South on 28 May 1986 the correlation between II+ and 0+ fish is not significant ($r=-0.053$ for 8 sampling times). In contrast to cadmium, the picture for lead showed only a significant positive correlation ($P<0.05$) between mean levels in II+ and I+ fish from Brailsford Brook South (table 7). The data suggest that when there is an adequate sample size, correlation between agegroups are significant despite the different trends with time for different agegroups.

Causes of the above correlations could include fluctuations due to factors such as time of sampling, change in exposure, sampling bias and differences in body weight. When the effect of body weight is excluded by adjusting for weight within each agegroup per site, regression of the difference in metal burden between fish of different age on time of sampling showed only a significant ($P<0.05$) decrease with time for lead between II+ and I+ fish from the River Ecclesbourne. There was some suggestion ($P<0.10$) of decrease with time for cadmium between I+ and 0+ fish from the Brailsford Brook South. The mean cadmium levels (adjusted for weight) showed significant positive correlations ($P<0.05$ and $P<0.01$) for 4 comparisons. There was some suggestion of positive correlation ($P<0.10$) for 2 comparisons for lead (table 8).

The correlation between cadmium and lead in each fish for each agegroup per site appeared to be significant for loach of II+ and I+ years old from the

Table 8 Correlation coefficient between mean logarithmic amount of metal (ng) in fish of three different agegroups (adjusted for differences in weight per agegroup) sampled at three sites in Derbyshire between 22 May 1985 and 28 May 1986 at intervals of about 4 weeks (see table 1 for details). Results for lead towards upper righthand side, for cadmium to the bottom left. Number of sampling times in parentheses.

Metal	Site	Age-group	II+		I+		0+		Metal
Cadmium	River Ecclesbourne	II+	----		0.353	(12)	0.650	(4)	Lead
		I+	0.794^{**}	(12)	----		0.119	(4)	
		0+	0.428	(4)	0.719	(4)	----		
	Brailsford Brook South	II+	----		0.558^{+}	(12)	0.492	(9)	
		I+	0.611^{*a}	(12)	----		0.620^{+}	(9)	
		0+	-0.706^{*b}	(9)	-0.542	(9)	----		
	Suttonbrook	II+	----		0.709	(5)	n.a.		
		I+	0.957^{*}	(5)	----		-0.486	(6)	
		0+	n.a.		0.957^{**}	(6)	----		

n.a. indicates not applicable as at only one sampling time fish in both agegroups were caught;
$^{+}$, * and ** indicate significance at the 0.10, 0.05 and 0.01 probability levels respectively;
^{a} correlation coefficient equals 0.607^{*} (11) when II+ fish sampled on 28 May is excluded;
^{b} correlation coefficient equals 0.012 (8) when II+ fish sampled on 28 May is excluded.

Table 9 Correlation coefficient between logarithmic amount of cadmium (ng) and the logarithmic amount of lead (ng) for individual stone loach caught at three sites in Derbyshire between 22 May 1985 and 28 May 1986 at intervals of about 4 weeks (see table 1 for details) (a) per agegroup and site; (b) per site.

(a)

Site	Agegroup	Number of fish	Correlation coefficient
River Ecclesbourne	II+	60	0.471***
	I+	46	0.462***
	0+	10	0.247
Brailsford Brook South	II+ [a]	51	0.306*
	I+	69	0.231^{+}
	0+	37	0.294^{+}
Suttonbrook	II+	16	0.037
	I+	74	0.073
	0+	50	0.040

(b)

Site	Number of fish	Correlation coefficient
River Ecclesbourne	116	0.504***
Brailsford Brook South	157 [b]	0.290***
Suttonbrook	140	0.088

$^{+}$, * and *** indicate significance at the 0.10, 0.05 and 0.001 probability levels respectively;

[a] correlation coefficient equals 0.364*** (number of fish equals 50) when the one II+ fish sampled on 28 May 1986 is excluded;

[b] correlation coefficient equals 0.300*** (number of fish equals 156) when the one II+ fish sampled on 28 May 1986 is excluded.

River Ecclesbourne ($P<0.001$) and for those in all agegroups from the Brailsford Brook South (table 9a). When all fish were pooled per site, then there was a highly significant correlation between the two metal levels in fish from the River Ecclesbourne and from the Brailsford Brook South (table 9b).

Effect of site on metal content

To assess the effect of site on metal burden of loach one has to discount the effect of body weight and/or sampling time. The effect of body weight on the exponent is evaluated above for both cadmium and lead.

Analyses of covariance were used to compare the effect of agegroup, discounted for differences in weight, on cadmium burden of fish caught on the same occasion. Residual variances about the regression occasionally lacked homogeneity at the 0.05 probability level (table 10). However, as the range of number of fish in different agegroups for each time are not appreciably dissimilar (table 1), there is little difference between the estimated and weighted variances. Thus the contrast between the adjusted means is not seriously affected (Scheffé, 1959). Individual slopes did not differ between agegroups for each sampling occasion (table 10). Individual elevations in cadmium levels did not differ for fish of different agegroup after allowing for the effect of body weight for each sampling time within site.

For lead, there is some effect of agegroup. Slopes of the regression of lead on dry weight for each site were not always similar ($P<0.10$ for 2 occasions from both the River Ecclesbourne and from the Suttonbrook) (table 10).

Cadmium

Results of the analyses of covariance, with dry weight as the covariate, show that the residual variances about the regressions lacked homogeneity on 2 occasions (table 11). However, at sampling time 4, sample sizes did not differ considerably (12, 16 and 13 for the River Ecclesbourne, Brailsford Brook South and Suttonbrook respectively), thus there is little difference between the estimated and weighted variances. Moreover, inequality of variances has little effect on inferences about the means (Scheffé, 1959). For sampling occasions 4 and 14 only the slopes differ between sites ($P<0.05$). Analysis of covariance showed a significant effect of site: fish from the River Ecclesbourne had higher levels than those caught at Brailsford Brook South and Suttonbrook, while there was hardly any difference between levels found at the two latter sites.

Lead

Given the lack of effect of time within agegroup and the significant effect of agegroup on lead levels, only comparisons in lead burden of fish within age-groups from the River Ecclesbourne and Brailsford Brook South can readily be made. For II+ fish however, those from the Suttonbrook can be included in this comparison. Lead burden increases slightly with age at both sites and loach from the River Ecclesbourne have always higher levels of lead than those from the Brailsford Brook South (fig 5). Body burden of II+ fish from the Suttonbrook do not differ significantly from those caught at Brailsford Brook South ($P>0.10$).

Table 10 Results of analyses of covariance of the logarithm of metal burden (ng) on the logarithm of dry weight (mg) of stone loach of different agegroups for each sampling time caught in three rivers in Derbyshire between 22 May 1985 and 28 May 1986 at about 4-weekly intervals (see table 1 for details). Summary of results of analyses of covariance of the logarithm of cadmium and lead burden (ng) on the logarithm of dry weight (mg) (covariate) of stone loach per agegroup. For details of samples see table 1.

Site	Sampling time	Cadmium: Tests for homogeneity of variance χ^2	df	Cadmium: Tests for similarity of slopes	Cadmium: Variance ratio for effect of agegroup	Lead: Tests for homogeneity of variance χ^2	df	Lead: Tests for similarity of slopes	Lead: Variance ratio for effect of agegroup
River Ecclesbourne	220585	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	180685	0.60	1	$F_{1,6} = 1.75$	$F_{1,7} < 1.0$	0.19	1	$F_{1,6} = 5.73^{+}$	$F_{1,7} = 1.11$
	170785	0.16	1	$F_{1,8} < 1.0$	$F_{1,9} = 0.21$	5.13^{*}	1	$F_{1,8} < 1.0$	$F_{1,9} = 4.61^{+}$
	140885	0.07	1	$F_{1,7} < 1.0$	$F_{2,8} = 1.69$	0.32	1	$F_{1,7} < 1.0$	$F_{2,8} = 5.78^{*}$
	110985	0.18	2	$F_{2,12} < 1.0$	$F_{2,14} < 1.0$	0.07	2	$F_{2,12} < 1.0$	$F_{2,14} = 3.11$
	091085	0.79	1	$F_{1,4} < 1.0$	$F_{2,5} < 1.0$	0.07	1	$F_{1,4} = 1.47$	$F_{2,5} = 3.11$
	061185	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	041285	2.33	1	$F_{1,3} < 1.0$	$F_{1,4} < 1.0$	0.01	1	$F_{1,3} < 1.0$	$F_{1,4} = 4.16$
	020186	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	290186	n.a.		$F_{1,2} = 1.03$	$F_{1,3} = 1.01$	n.a.		$F_{1,2} = 2.61$	$F_{1,3} < 1.0$
	260286	n.a.		$F_{2,1} = 2.62$	$F_{2,3} = 5.89^{+}$	n.a.		$F_{2,1} < 1.0$	$F_{2,3} = 7.27^{+}$
	260386	6.57^{*}	1	$F_{1,4} < 1.0$	$F_{1,5} < 1.0$	2.83^{+}	1	$F_{1,4} = 7.79^{*}$	$F_{1,5} < 1.0$
	300486	1.61	1	$F_{1,3} < 1.0$	$F_{1,4} < 1.0$	0.23	1	$F_{1,3} = 1.36$	$F_{1,4} < 1.0$
	280586	4.47^{*}	1	$F_{1,5} = 1.41$	$F_{1,6} = 1.32$	1.54	1	$F_{1,5} < 1.0$	$F_{1,6} = 4.02^{+}$

- continued -

- continuation -

Brails-	220585	3.81^{+}	1	$F_{1,8} = 1.29$	$F_{1,9} = 1.65$	2.72^{+}	1	$F_{1,8} < 1.0$	$F_{1,9} = 2.23$
ford	180685	0.02	1	$F_{1,9} < 1.0$	$F_{1,10} < 1.0$	0.20	1	$F_{1,9} < 1.0$	$F_{1,10} = 3.81^{+}$
Brook	170785	n.a.		$F_{1,6} < 1.0$	$F_{1,7} < 1.0$	n.a.		$F_{1,6} < 1.0$	$F_{1,7} = 3.21$
South	140885	2.73^{+}	1	$F_{1,11} = 2.05$	$F_{2,12} < 1.0$	0.04	1	$F_{1,11} = 1.32$	$F_{2,12} = 1.13$
	110985	3.22^{+}	1	$F_{1,9} < 1.0$	$F_{2,10} < 1.0$	2.16	1	$F_{1,9} = 1.08$	$F_{2,10} < 1.0$
	091085	8.85**	2	$F_{2,9} < 1.0$	$F_{2,11} < 1.0$	8.42**	2	$F_{2,8} < 1.0$	$F_{2,10} < 1.0$
	061185	0.79	2	$F_{2,10} < 1.0$	$F_{2,12} = 3.09^{+}$	7.30*	2	$F_{2,10} < 1.0$	$F_{2,12} = 1.85$
	041285	0.48	1	$F_{2,4} = 1.66$	$F_{2,6} = 3.93^{+}$	5.97*	1	$F_{2,4} = 1.08$	$F_{2,6} = 4.10^{+}$
	020186	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	290186	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	260286	0.01	1	$F_{2,12} = 1.58$	$F_{2,14} < 1.0$	2.54	1	$F_{2,12} < 1.0$	$F_{2,14} < 1.0$
	260386	0.93	2	$F_{2,5} < 1.0$	$F_{2,7} = 2.96$	1.20	2	$F_{2,6} = 1.90$	$F_{2,8} = 1.19$
	300486	0.29	1	$F_{1,4} = 5.28^{+}$	$F_{2,5} < 1.0$	5.59*	1	$F_{1,4} < 1.0$	$F_{2,5} < 1.0$
	280586**	n.a.		n.a.	$F_{1,9} < 1.0$	n.a.		n.a.	n.a.

- continued -

- continuation -

Sutton-brook	220585	4.78^{*}	1	$F_{1,11} < 1.0$	$F_{1,12} = 2.33$	4.73^{*}	1	$F_{1,11} < 1.0$	$F_{1,12} < 1.0$
	180685	0.68	1	$F_{1,6} < 1.0$	$F_{1,7} < 1.0$	0.01	1	$F_{1,6} < 1.0$	$F_{1,7} = 1.17$
	170785	n.a.		n.a.	$F_{1,8} = 1.61$	n.a.		n.a.	$F_{1,7} < 1.0$
	140885	1.06	1	$F_{1,9} = 5.74^{+}$	$F_{1,10} = 3.09$	3.27^{+}	1	$F_{1,9} < 1.0$	$F_{1,10} = 2.22$
	110985	0.08	1	$F_{1,7} < 1.0$	$F_{1,8} < 1.0$	0.59	1	$F_{1,7} = 4.89^{+}$	$F_{1,8} = 5.98^{*}$
	091085	4.52^{*}	1	$F_{1,8} = 1.39$	$F_{2,9} = 1.16$	4.45^{*}	1	$F_{1,8} < 1.0$	$F_{1,9} < 1.0$
	061185	0.16	1	$F_{1,11} = 4.44^{+}$	$F_{1,12} = 4.31$	0.01	1	$F_{1,11} = 7.21^{*}$	$F_{1,12} = 1.17$
	041285	0.72	1	$F_{1,10} = 1.66$	$F_{1,11} < 1.0$	0.12	1	$F_{1,10} < 1.0$	$F_{1,11} < 1.0$
	020186	0.02	1	$F_{1,10} < 1.0$	$F_{1,11} = 4.68$	0.12	1	$F_{1,10} < 1.0$	$F_{1,11} = 10.20^{**}$
	290186	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	260286	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	260386	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	300486	n.a.		n.a.	n.a.	n.a.		n.a.	n.a.
	280586	n.a.		$F_{1,12} < 1.0$	$F_{1,13} < 1.0$	n.a.		$F_{1,12} < 1.0$	$F_{1,13} = 1.22$

[a] indicates that fish in agegroup II+ has been discarded for cadmium;
n.a. indicates not applicable;
$^{+}$, *, ** and *** indicate significance at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively.

Table 11 Summary of results of analyses of covariance of the logarithm of cadmium burden (ng) on the logarithm of dry weight (mg) (covariate) of stone loach per sampling time for the effect of sampling site.

Sampling time	Tests for homogeneity of variance χ^2	df	Tests for similarity of slopes	Common slope b ± s.e.	Effect of site on cadmium burden	Logarithm of cadmium burden (ng) for adjusted mean dry weight: highest mean ± s.e.	middle mean ± s.e.	lowest mean ± s.e.
1	1.27	2	$F_{2,27} < 1.0$	0.91 ± 0.17	$F_{2,29} = 4.38^{*}$	2.13^{a} ± 0.15	1.85^{b} ± 0.10	1.61^{c} ± 0.09
2	4.31	2	$F_{2,27} = 1.57$	0.73 ± 0.22	$F_{2,29} = 1.11$	1.56^{a} ± 0.13	1.56^{b} ± 0.11	1.33^{c} ± 0.13
3	2.45	2	$F_{2,27} < 1.0$	0.87 ± 0.34	$F_{2,29} = 16.16^{***}$	2.26^{a} ± 0.12	1.60^{b} ± 0.13	1.30^{c} ± 0.13
4	6.12^{*}	2	$F_{2,35} = 3.44^{*}$	0.99 ± 0.11	$F_{2,37} = 14.60^{***}$	1.70^{a} ± 0.12	0.93^{b} ± 0.10	0.84^{c} ± 0.12
5	0.51	2	$F_{2,37} < 1.0$	0.60 ± 0.12	$F_{2,39} = 7.97^{**}$	1.80^{c} ± 0.11	1.39^{a} ± 0.09	1.21^{b} ± 0.09
6	13.88^{***}	2	$F_{2,31} = 1.05$	0.41 ± 0.22	$F_{2,33} = 11.63^{***}$	2.24^{a} ± 0.18	1.60^{c} ± 0.16	1.23^{b} ± 0.13
7	0.95	2	$F_{2,27} < 1.0$	1.05 ± 0.21	$F_{2,29} = 4.42^{*}$	2.20^{a} ± 0.38	1.75^{c} ± 0.14	1.24^{b} ± 0.14
8	3.13	2	$F_{2,25} = 1.93$	0.54 ± 0.18	$F_{2,27} = 19.71^{***}$	2.14^{c} ± 0.11	2.01^{a} ± 0.17	1.22^{b} ± 0.11
9	1.93	1	$F_{1,13} < 1.0$	0.64 ± 0.25	$F_{1,14} < 1.0$	2.20^{a} ± 0.30	2.13^{c} ± 0.10	n.a.
10					$F_{1,4} = 1.98$	3.07^{a} ± 0.17	2.28^{b} ± 0.51	n.a.
11	0.58	1	$F_{1,21} < 1.0$	0.47 ± 0.09	$F_{1,22} = 79.64^{***}$	2.55^{a} ± 0.08	1.67^{b} ± 0.05	n.a.
12	2.84	2	$F_{2,16} < 1.0$	0.36 ± 0.16	$F_{2,18} = 16.06^{***}$	2.41^{a} ± 0.09	1.72^{b} ± 0.08	1.70^{c} ± 0.15
13	0.12	2	$F_{2,16} = 1.40$	0.60 ± 0.28	$F_{2,18} = 8.59^{**}$	2.23^{a} ± 0.15	1.61^{c} ± 0.14	1.41^{b} ± 0.11
14	4.72^{+}	2	$F_{2,32} = 5.79^{**}$	-0.74 ± 0.46	$F_{2,34} = 14.89^{***}$	2.39^{a} ± 0.18	1.64^{c} ± 0.12	1.12^{b} ± 0.13
14^{d}	0.52	2	$F_{2,31} = 5.71^{**}$	-0.26 ± 0.37	$F_{2,33} = 13.27^{***}$	2.29^{a} ± 0.15	1.67^{c} ± 0.10	1.29^{b} ± 0.11

$^{+}$, *, ** and *** indicate significance at the 0.10, 0.05, 0.01 and 0.001 probability levels respectively;
^{a} indicates that the data apply to the River Ecclesbourne;
^{b} indicates that the data apply to the Brailsford Brook South;
^{c} indicates that the data apply to the Suttonbrook;
^{d} fish in agegroup II+ from Brailsford Brook South with non-detectable cadmium level excluded;
n.a. indicates not applicable.

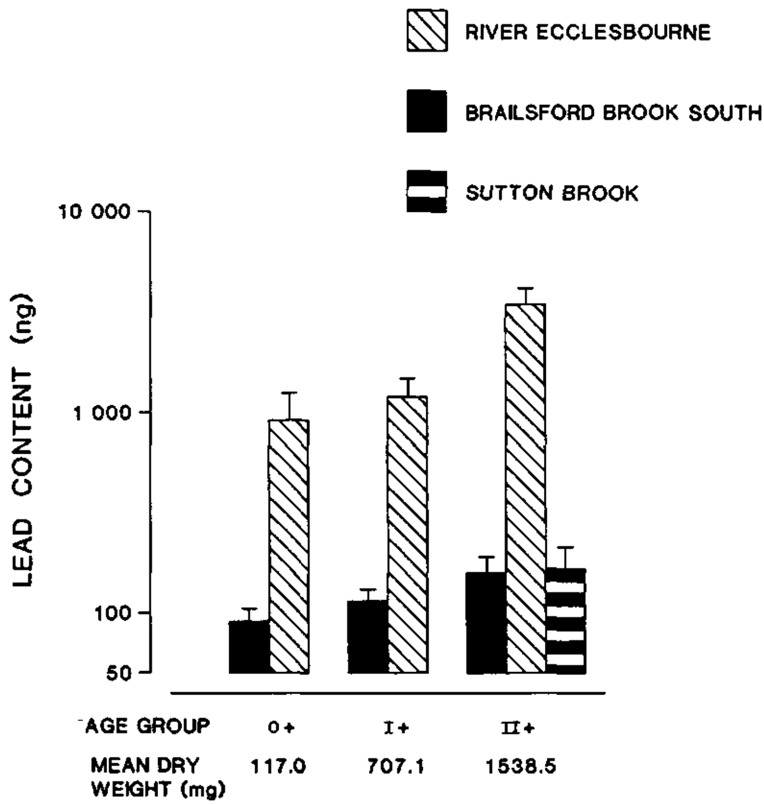


Fig. 5. Mean lead content (ng) with standard errors in stone loach in three different agegroups (adjusted for weight within agegroups) from the River Ecclesbourne and Brailsford Brook South caught between 22 May 1985 and 28 May 1986. II+ fish from Suttonbrook included. For sample sizes see table 1.

DISCUSSION

The age/length relationship of loach caught in Derbyshire are comparable with those found for stone loach from a Dorset chalk stream (Mills *et al.*, 1983) and from the River Ouzel, Buckinghamshire (Hyslop, 1982). The rate of fish growth increases with increase of temperature, due to increased metabolic activity (Robinson *et al.*, 1983), and is reduced as fish approach their maximum length even at high temperatures as data for the II+ agegroup for all streams indicate (see also Von Bertalanffy, 1938). Reduction in length during the winter was unexpected. A similar phenomenon has been found for loach from Dorset (Mills *et al.*, 1983) and for bullhead (*Cottus gobio*) from different sites in the R. Ecclesbourne (Moriarty, pers. comm.). Possible explanations include a bias in sampling or migration of bigger fish to other sites.

Allometric growth indicates that the different measures of body size are not directly proportional to each other. However, dry weight seems to be the best measure of body size as it does not change appreciably between sampling of fish and final weighing while other measures may change appreciably (Kay, 1985).

The results show that many factors, such as body size, site and date of sampling, affect the cadmium and lead burden of loach and that these two metals have to be considered separately.

Body size/age can affect pollutant levels (Phillips, 1980). Values for the exponents of body weight relating dry weight and metal burden are less than unity in this study: concentrations decrease as size increases. Comparisons of results based on concentration only can mislead when no information about body size is given, unless body sizes of the different specimens are alike. The extent to which metal levels change with body size is clearly different for cadmium and lead. The body weight exponent for cadmium (0.79 ± 0.06), but not for lead (0.13 ± 0.21), is similar to the values obtained during studies for exposure to dietary cadmium (0.63 ± 0.11 and 0.70 ± 0.06), food consumption studies (0.78 ± 0.04) and oxygen consumption rate (0.81 ± 0.03) (Douben, 1989b), but is different from those for exposure to water-borne cadmium (0.34 ± 0.08 and 0.66 ± 0.08) (Douben, 1989a). The values of these exponents for body weight suggest that metabolism of loach is important and that uptake of cadmium, far more than uptake of lead, is correlated with metabolism and that food can be an important source of cadmium.

The relative importance of dietary cadmium intake vs. uptake from water and/or sediments is poorly understood, and there is some controversy in recently published reviews about the relative contributions of food and water to the cadmium burden of fish (Kay, 1985, McCracken, 1987). Many factors can affect the importance of these two routes of entry: fish species, food quality, concentration and availability of metal in food and water (Alabaster and Lloyd, 1982; Dallinger *et al.*, 1987; Luoma, 1983).

Bio-concentration factors (BCF) are often used to compare the body burden of an organism with the degree of contamination in the water (Taylor, 1983). Comparison of the minimum and maximum metal levels in loach in this work with the lowest and highest concentrations of cadmium and lead in water samples from the three streams indicate that for cadmium BCF decreases with agegroup i.e. body size (table 12). Additionally BCFs are highest in fish from the River Ecclesbourne and of similar magnitude in fish caught at Brailsford Brook South and Suttonbrook. The estimated BCFs for cadmium are similar to those found in other studies (Adams *et al.*, 1980; Murphy *et al.*, 1978). However, these calculations do ignore any uptake from food, which may lead to errors (Taylor, 1983).

Table 12 Estimated bio-concentration factors for cadmium in stone loach of different agegroups caught in three rivers in Derbyshire between 22 May 1985 and 28 May 1986 at about 4-weekly intervals. Factors are based on minimum and maximum metal concentration measured in each agegroup and highest and lowest concentration determined in filtered water samples. (see Douben (1989c) for details of metal concentration in river water).

Site	Minimum and maximum cadmium concentration found in water ($\mu g\ l^{-1}$)	AGEGROUP					
		II+		I+		0+	
		min	max	min	max	min	max
River	0.10	200	8300	500	10300	500	9600
Eclesbourne	2.8	7.14	296	17.86	368	17.9	343
Brailsford	0.16	100[a]	625	100	1063	125	1438[b]
Brook South	1.4	11.4[a]	71.4	11.4	121	14.3	164[b]
Suttonbrook	0.10	100	900	100	7000	400	1000
	1.6	6.25	56.3	6.25	438	25	625

[a] fish in agegroup II+ from Brailsford Brook South with non-detectable cadmium level excluded;
[b] single fish in agegroup 0+ with cadmium concentration of 0.75 $\mu g\ g^{-1}$ caught on 28 May 1986 would have yielded a BCF of 536.

Bio-magnification appeared not to have occurred in the chain *Tubifex*-loach during laboratory studies (Douben, 1989b). Three variables determine metal uptake from the food: efficiency of uptake, concentration in the food and amount of food eaten, and they can interact (see Moriarty and Walker, 1987; Spacie and Hamelink, 1985). Although cadmium uptake from the diet varies and can be as low as 2%, cadmium burden of loach increased after dietary exposure to cadmium in *Tubifex* (Douben, 1989b). The concentrations of cadmium in food used in these experiments (range 0.45 - 170 mg kg^{-1} dry weight) were realistic (see table 13a). Some studies report increased cadmium burden of fish after dietary exposure; for example for mosquitofish (*Gambusia affinis*) (Williams and Giesy, 1978) and rainbow trout (*Salmo gairdneri*) (Kumada *et al.*, 1980). Food becomes an important pathway when water-borne concentrations are low particularly for more persistent (i.e. non-metabolizable) substances in the environment, such as heavy metals (Niimi, 1985). Although it is generally accepted that accumulation of cadmium from food is much less efficient than from water, uptake of cadmium from the diet may be of considerable importance under field circumstances since cadmium concentration in food items is usually higher than in the surrounding water (Taylor, 1983). Bio-magnification factors (BMF) of less than unity are usually found (Ferard *et al.*, 1983; Kay, 1985) including those for stone loach (Douben, 1989b). Comparison of concentration factors (BCF vs. BMF) is of little value in assessing the relative importance of the routes by which cadmium enters aquatic animals (Kay, 1985). Actual exposure and dose levels have to be compared, and also rates of loss.

Loach of 590 mg dry weight exposed to 80 $\mu g\ l^{-1}$ cadmium in water were estimated to reach a maximum body burden of 348 ng compared with approximately 100 ng for control fish (Douben, 1989a). If net intake of cadmium from water is directly proportional to the concentration of cadmium in the water, then, from levels of cadmium found in filtered water-samples (to estimate the exposure to water-borne metal) from the three sites (Douben, 1989c), one would expect body burdens of about 150 ng. Cadmium levels for loach of similar weight as measured in this study are usually in excess of those values. This supports the view that there is an additional pathway of cadmium intake, unless the efficiency of cadmium inake from water is higher at lower concentrations. Elimination rate constants after dietary exposure were also smaller than after water-borne exposure to cadmium for stone loach which implies that half-lifes were longer. In addition to similarity and dissimilarity of exponents of body weight, this supports the view that the role of food as a source of, at least, cadmium for stone loach must be taken into account.

The results indicate that differences in weight of fish in one agegroup explain only part of the variation. There were no significant reductions in the residual variances of the analyses of covariance in comparison with the analyses of variance although the residual variance for cadmium in the first analyses were consistently less than in the latter analyses for cadmium but not for lead. Analyses of covariance gave the same qualitative results as the analyses of variance for both metals although the variance ratio for sampling time is reduced when differences in dry body weight are taken into account (compare table 5 and 6) for both cadmium and lead.

Another factor that influences body burden is exposure. As exposure is by no means constant under field conditions (see Douben 1989c) it is therefore not surprising that metal levels in fish fluctuate. Given the few number of sampling times that could be used in some comparisons for correlation coefficients, fluctuations in cadmium burden of fish of different age from one site correlated reasonably well in spite of differences in actual body burden and size. Lead levels correlated to a lesser extent. After discounting for changes in weight (due to growth and possibly bias in sampling during the winter) the results (table 8) suggest that other factors than time cause fluctuations. Presumably environmental factors such as changes in metal concentration in water, affect the observed fluctuations of cadmium.

Table 13 Summary of range of mean concentrations of cadmium and lead (mg kg^{-1} dry weight) in (a) invertebrates and (b) sediments sampled from three different sites in Derbyshire. (see Douben 1989d for details).

(a)

Site	CONCENTRATION OF METAL ($mg\ kg^{-1}$ dry weight) Cadmium	Lead
River Ecclesbourne	0.01 - 100	1.0 - 10 000
Brailsford Brook South	0.01 - 10	0.1 - 100
Suttonbrook	0.01 - 10	0.1 - 100

(b)

Site	CONCENTRATION OF METAL ($mg\ kg^{-1}$ dry weight) Cadmium	Lead
River Ecclesbourne	40 - 75	6000 - 10 000
Brailsford Brook South	1 - 2	40 - 80
Suttonbrook	1 - 2	50 - 90

Site had a significant effect on both cadmium and lead burden of loach: metal levels in fish from the River Ecclesbourne were higher than in those caught at the Brailsford Brook South and Suttonbrook whilst there is no significant difference between the two latter sites (table 11 and fig 5). Higher concentrations of both cadmium and lead were found in water from the River Ecclesbourne (0.1-2.8 $\mu g\ l^{-1}$ for cadmium and 0.6-13.5 $\mu g\ l^{-1}$ for lead) than in water from the two other sites (0.1-1.6 $\mu g\ l^{-1}$ for cadmium and 0.6-3.0 μg

l^{-1} for lead; see also table 12) (Douben, 1989c). Concentration of both cadmium and lead in food items were highest in samples from the River Ecclesbourne (table 13a). Metal concentrations in sediments showed a similar trend (table 13b; Webb *et al.*, 1978). Similar results have been found for fish from contaminated waters (e.g. Adams *et al.*, 1980; Bradley and Morris, 1986). Metal burden of bullhead (*Cottus gobio*) caught at different sites in the River Ecclesbourne was higher at sites with higher metal concentrations in the sediment (Moriarty *et al.*, 1984).

CONCLUSIONS

Field data for stone loach exposed to cadmium and lead demonstrate that the relationship between exposure and body burden is not simple. Many variables, including body weight, time of year and site, can influence the relationship and interpretation of the data is therefore not straightforward.

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CHAPTER 6: Changes in concentration of heavy metals in river water

Environmental Pollution, submitted.

CHANGES IN CONCENTRATION OF HEAVY METALS IN RIVER WATER

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ABSTRACT

River water from three sites in different streams in Derbyshire was sampled during different periods within one year to evaluate fluctuations in cadmium and lead concentration. The results indicate that most of the cadmium was in solution while most of the lead was associated with particles at all sites. Period of sampling appeared to have a greater effect on the concentration of cadmium and lead than flow rate: metal levels were higher in the spring than in the autumn. Nevertheless the total lead concentration increased with flow rate, presumably because more particles were then brought into suspension, and the lead concentration in the filtrate was reduced at higher flow rates, presumably due to dilution. Dissolved cadmium concentration increased with rising flow rate at relatively low flow rates and was diluted at high flow rates. The data suggest that particles with which most of the lead is associated remain in suspension for considerable time even when flow rate decreases.

INTRODUCTION

The nature of the link between exposure to a contaminant in the environment and the dose received within an organism is a central problem in studies of the effects of pollutants in the environment: exposure determines the dose, followed by a response/effect of the organism (Doull *et al.*, 1980). Deeper knowledge of the exposure-dose relationship is important for understanding and predicting effects of pollutants on wildlife.

This paper is part of a larger study on the exposure-dose relationship of heavy metals in fish from the River Ecclesbourne, and other rivers in Derbyshire, and the contribution of different routes of entry to the body burden of fish (see Douben, 1989b). It deals with the degree of exposure and of fluctuations in exposure through the water under field circumstances. Studies were restricted to three sites because other related work has also been confined to the same sites (e.g. Douben, 1989a). The three sites with different concentrations of heavy metals in the sediment (Webb *et al.*, 1978) were: the River Ecclesbourne (national grid reference SK 288 505), the Brailsford Brook South (national grid reference SK 284 452) and a site in the Suttonbrook (national grid reference SK 222 342).

The three objectives were to:

1. assess the effect of flow rate on the total concentration of cadmium and lead in river water and in solution;
2. evaluate the variation between sampling times on the metal concentration for occasions with the same flow rate from different periods of the year;

3. study the change in metal concentration in water taken from one site on the same day at different positions in one stream when flow rate decreases.

Information on changes of metal concentration in water is important for assessing the likely fluctuation in exposure of aquatic organisms in river water. Uptake of heavy metals by fish from water takes place through the gills (Calamari *et al.*, 1980; Pärt *et al.*,1985). There is still some controversy over which forms of metal can be taken up: only free ions or complexes as well. Factors that can affect the amount of metal present in solution include pH (Davies, 1976), organic matter (Zitko, 1976), hardness of the water (Laxen, 1985; Winner & Gauss, 1986) and change in discharge (Grimshaw *et al.*, 1976). Earlier work had shown that pH and water hardness are relatively stable; for example, in water from the River Ecclesbourne pH and water hardness (Ca^{2+} mg l^{-1}) were 7.3 ± 0.1 and 65.7 ± 8.0 respectively (Douben, 1989a) (see also table 2).

MATERIALS AND METHODS

Nichol *et al.* (1970) reported high levels of lead and zinc in stream sediments from the valley of the R. Ecclesbourne, which probably originate from mineral veins at the head of the valley. Moriarty *et al.* (1982) reported high levels of cadmium in the R. Ecclesbourne, which commonly occurs as a substitute for zinc in the crystal lattice. The heavy metals in sediments in the other streams are not derived from any domestic or industrial effluent but occur naturally and, therefore, provide information on the normal background levels (Douben, 1989b).

By convention metals in solution are defined as those that pass through a filter of 0.45 µm pore size. In fact this type of filter permits the passage of a wide variety of forms e.g. ions, chelates, colloids (Allan, 1986;Stumm & Morgan, 1981). The filters (Millipore HAWP)[1] were made of cellulose acetate and were protected by a micro fibre-glass pre-filter (Millipore AP15)[1]. The sampling bottles, of high-density polyethylene, were thoroughly washed and

[1] brand name is given for reference purpose only and does not imply any commercial support by the N.E.R.C. or the C.E.C.

Table 1 Details of sampling programme of river water at three sites in Derbyshire during 4 periods: on each occasion 4 whole and 4 filtered samples were taken at each site.

Site	Period no.	Occasion	Sampling date	Estimated flow rate ($m\ s^{-1}$)	No. of samples with detectable levels* of metal ($\mu g\ l^{-1}$) Cadmium whole	Cadmium filtrate	Lead whole	Lead filtrate
Brailsford Brook South	1	1	07.04.87	0.49	4	3	4	0
		2	08.04.87	0.33	4	4	4	0
		3	09.04.87	0.28	4	4	4	0
		4	10.04.87	0.25	4	4	3	0
		5	16.04.87	0.23	4	4	0	0
	2	1	06.07.87	0.14	4	4	0	0
		2	07.07.87	0.17	3	3	0	0
		3	08.07.87	0.17	4	3	0	0
		4	09.07.87	0.14	4	4	3	0
	3	1	18.08.87	0.25	4	4	4	4
		2	19.08.87	0.25	4	4	2	4
	4	1	17.11.87	0.84	1	1	4	0
		2	18.11.87	0.71	4	1	4	0
		3	19.11.87	0.92	4	4	3	0
River Ecclesbourne	1	1	07.04.87	0.62	4	4	4	0
		2	08.04.87	0.79	4	4	4	3
		3	09.04.87	0.73	4	4	4	3
		4	10.04.87	0.62	4	4	4	0
		5	16.04.87	0.48	4	4	4	0

- continued -

- continuation -

River Eccles-bourne	2	1	06.07.87	0.35	4	4	4	4
		2	07.07.87	0.37	4	3	4	2
		3	08.07.87	0.35	4	3	4	4
		4	09.07.87	0.35	4	4	4	4
	3	1	18.08.87	0.21	4	4	4	2
		2	19.08.87	0.13	4	4	1	3
	4	1	17.11.87	0.44	1	0	3	1
		2	18.11.87	0.40	4	1	4	4
		3	19.11.87	0.72	4	4	4	1
Sutton-Brook	1	1	07.04.87	1.61	4	3	4	0
		2	08.04.87	1.47	4	4	4	0
		3	09.04.87	1.30	4	4	3	0
		4	10.04.87	1.13	4	4	3	0
		5	16.04.87	0.73	4	4	0	0
	2	1	06.07.87	0.33	4	4	2	0
		2	07.07.87	0.28	4	4	0	0
		3	08.07.87	0.21	4	2	0	0
		4	09.07.87	0.21	3	2	4	0
	3	1	18.08.87	0.21	4	4	4	3
		2	19.08.87	0.21	4	4	4	2
	4	1	17.11.87	1.07	1	0	2	0
		2	18.11.87	0.95	3	1	4	0
		3	19.11.87	1.14	4	4	3	0

* limit of detection for cadmium was 0.1 $\mu g\ l^{-1}$ and for lead 0.6 $\mu g\ l^{-1}$.

rinsed twice with distilled water. At the sampling site, the bottles were rinsed with either river water or with filtrate (see below). Samples were usually taken on consecutive days at the same position at a sampling site on 2 - 5 occasions in each of 4 periods (table 1). At each site 8 samples were collected per occasion: 4 filtered (to estimate the metal concentration in solution) and 4 whole samples i.e. non-filtered (to estimate the total load of metal (Raspor, 1980)). For the third objective, on 18 November 1987 additional sets of 8 samples were taken from the R. Ecclesbourne at 3 positions (1 regular and 2 other) in separate riffles about 50 metres from each other. Samples were treated immediately after they were taken to avoid changes in the distribution of metal between the different phases as rates of adsorption and desorption of metals on particles is rapid (Gardiner, 1974b). Samples were filtered under pressure, with nitrogen, to give a flow rate of the water of about 50 ml min^{-1}. Then all samples, whole and filtrates, were acidified with concentrated HCl to prevent loss of metal from solution onto the walls of the bottles. They were stored at 4 °C and analyzed for cadmium and lead on an atomic absorption spectrophotometer (electrolyte furnace with background noise correction) within one week of sampling.

Aspects of water quality were measured with a minimum of two observations per period (table 2). To allow for greater variability for some variables, provision was made for an appropriate increase in number of water samples. Flow rate in the river was estimated with an orange peel, as this floating object was both conspicuous and mostly submerged, hence not prone to interference from the wind during measurements (Hynes, 1972).

Given the information on the magnitude of changes in pH, phosphate (mean values and standard errors were for the River Ecclesbourne, Brailsford Brook South and Suttonbrook 0.92 ± 0.42, 0.14 ± 0.03 and 0.16 ± 0.04 respectively) and hardness of the water and consequent effects on metal concentration, analysis of the data was based on the premise that (changes in) flow rate and sampling period were important factors that affect metal concentrations. For statistical analysis, described later, the results of metal analyses were transformed onto a logarithmic scale with base 10 so that the residual variation in the data conformed more nearly to a normal distribution. Logarithms of flow rates on pairs of consecutive occasions were then compared to establish whether the flow rate had changed appreciably i.e. if the difference was greater than ± 0.041 (10%). Then consecutive series of sampling occasions were formed with a falling, stable or rising trend in flow rate. These three types of result were analysed separately, to avoid any confusion from a hysteresis effect: that the concentration of metal at a given flow rate is influenced by whether the flow rate is increasing or decreasing.

Sets of data on concentration of metal were analysed by a two-factor analysis of variance with flow rate of river water and filtration as the two, fixed, factors. The appropriate analysis for an unbalanced analysis of variance was then applied (Sokal & Rohlf, 1981). Some samples, particularly the filtered ones for lead from Brailsford Brook South and Suttonbrook (see table 1), contained metal levels below the limit of detection, which may result in a biased interpretation. Therefore, when no metal was detected, the concentration was taken to be the limit of detection, which ensures that no spurious differences will be detected although, of course, real differences (for any given degree of probability) could be overlooked.

Table 2 Mean values, standard deviations (sd) and number of measurements (n) of aspects of water quality at three sites during sampling of river water for heavy metals taken on 14 occasions: cation and anion concentrations in mg l^{-1}.

Variable	Site								
	River Ecclesbourne			Brailsford Brook South			Suttonbrook		
	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n
pH	7.4	0.1	8	7.5	0.1	8	7.7	0.2	8
Conductivity (µS)	361	57	8	433	36	8	445	50	8
Ca^{2+}	46.5	2.7	22	61.7	4.7	22	62.0	9.6	22
Mg^{2+}	9.75	1.90	22	20.3	2.4	22	20.8	4.2	22
K^{+}	6.33	1.95	22	9.40	1.07	22	9.00	1.14	22
Na^{+}	21.9	7.3	22	8.99	1.86	22	9.77	3.66	22
total - P	0.57	0.17	6[a]	0.24	0.08	10	0.30	0.13	10
PO_4^{3-} - P (dissolved)	0.45	0.02	6[a]	0.19	0.06	10	0.27	0.13	10
Org. N + NH_4^{+} -N	0.58	0.38	10	0.53	0.15	10	0.66	0.28	10
$NO3^{-}$ - N (dissolved)	4.6	0.7	10	5.6	1.8	10	5.57	1.60	10
NH_4^{+} - N (dissolved)	0.13	0.04	4[b]	0.46	0.24	4[a]	0.78	0.18	2[a]
Cl^{-}	34.9	3.6	10	22.2	0.9	10	20.6	3.0	10
SO_4^{2-} - S	18.8	2.4	10	19.7	2.5	10	18.4	5.5	10

[a] on 2 occasions in 2 samples each levels were anomalously high (3.1 and 1.5 mg l^{-1}) which were more than 3 standard deviations from the mean and were therefore discarded;
[b] difference from 10 indicates number of samples with levels below the limit of detection of 0.10 mg l^{-1}.

The results for the metal concentrations in filtrate and whole samples were assumed to be statistically independent from each other as they were obtained from different observations. The
proportion of total metal in solution (PMS) and its variance (var PMS) can be estimated for any flow rate by:

$$\mathrm{PMS} = \frac{\overline{mf}}{\overline{mw}} \qquad (1)$$

$$\mathrm{var\ PMS} = \frac{\overline{mf}^{2}}{\overline{mw}^{2}} * \left(\frac{\sigma^{2}_{\overline{mf}}}{\overline{mf}^{2}} + \frac{\sigma^{2}_{\overline{mw}}}{\overline{mw}^{2}} \right) \qquad (2)$$

in which: PMS : proportion of total metal in solution;

$\overline{mf}$: mean metal concentration in filtrate ($\mu g\ l^{-1}$);

$\overline{mw}$: mean metal concentration in whole sample ($\mu g\ l^{-1}$);

$\sigma^{2}_{\overline{mf}}$: variance of the mean metal concentration in filtrate;

$\sigma^{2}_{\overline{mw}}$: variance of the mean metal concentration in whole sample;

(see also Finney, 1964). Interaction between flow rate and filtration indicated that the proportion of total metal present in solution changed significantly with flow rate. Lack of this type of interaction implies that the proportion of metal in solution is not significantly different for the different sampling occasions with different within each of the periods.

RESULTS

Effect of flow rate and filtration

The results indicate that most of the cadmium is usually in solution at all sites, while most of the lead is associated with particles (figs. 1-3). The pH is relatively constant and well above 7 thus affecting speciation of cadmium and lead to only a minor extent (see also Davies, 1976).

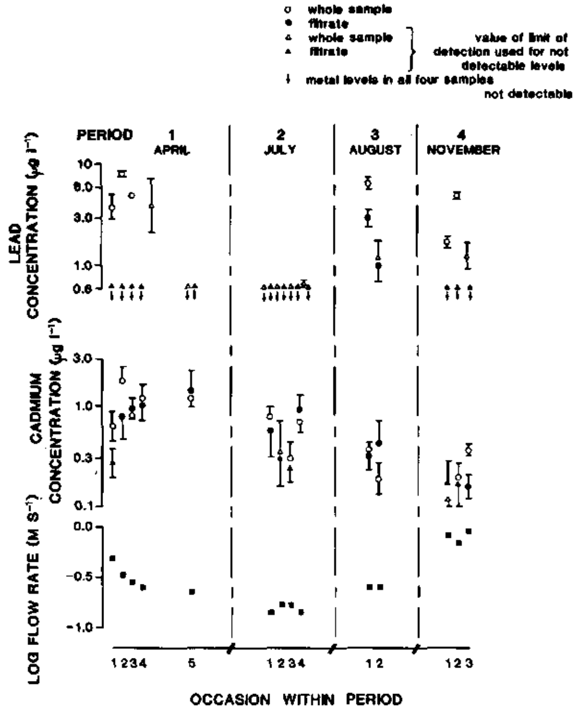

Fig 1 Mean concentrations and standard errors of lead and cadmium ($\mu g\ l^{-1}$) in whole samples and filtrates (passed through a filter of 0.45 µm pore size) in river water from Brailsford Brook South (Derbyshire). Flow rate was estimated at the times of sampling, in 4 periods during 1987.

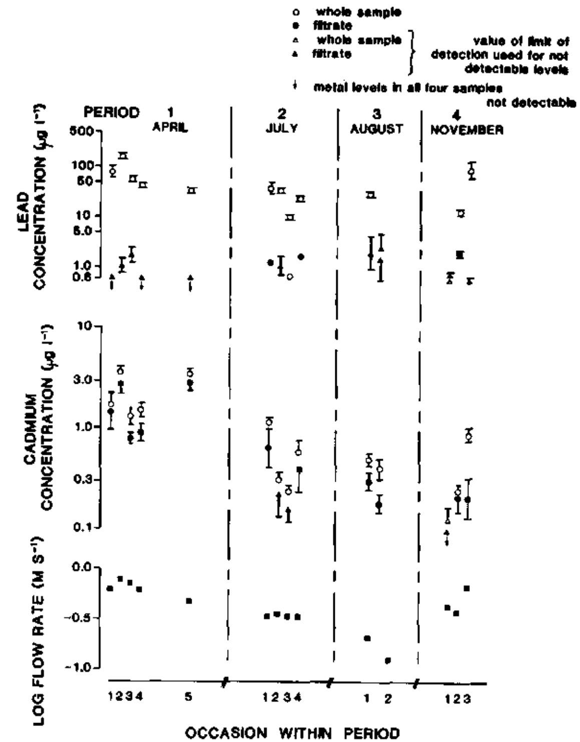

Fig 2 Mean concentrations and standard errors of lead and cadmium ($\mu g\ l^{-1}$) in whole samples and filtrates (passed through a filter of 0.45 µm pore size) in river water from the River Ecclesbourne (Derbyshire). Flow rate was estimated at the times of sampling, in 4 periods during 1987.

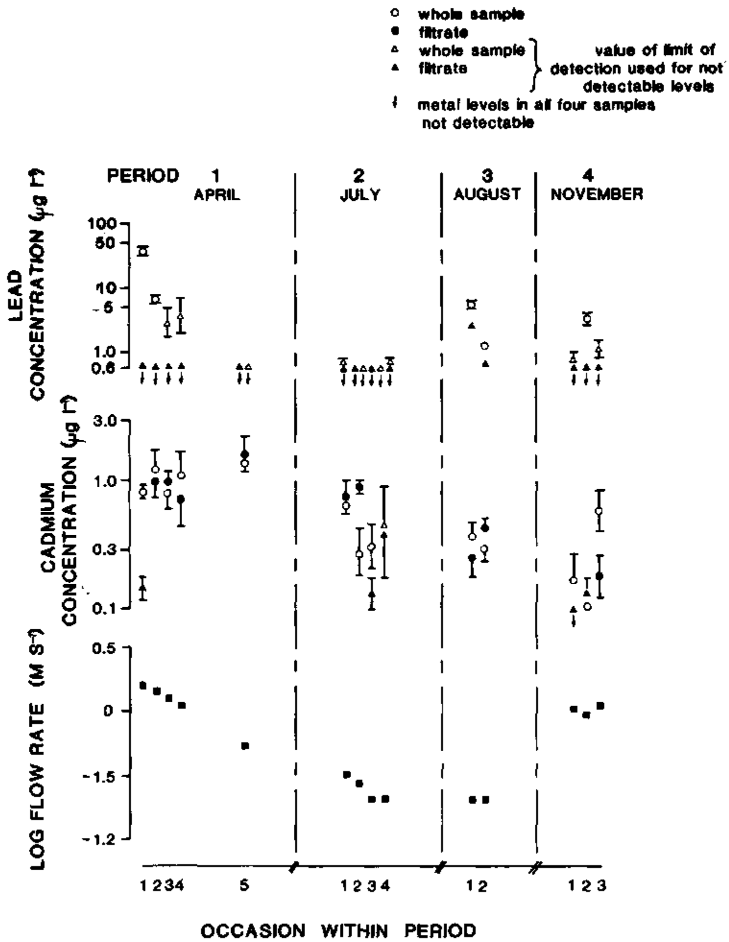

Fig 3 Mean concentrations and standard errors of lead and cadmium ($\mu g\ l^{-1}$) in whole samples and filtrates (passed through a filter of 0.45 µm pore size) in river water from Suttonbrook (Derbyshire). Flow rate was estimated at the times of sampling, in 4 periods during 1987.

Despite differences in ranges of flow rates between the sites, some generalisations can still be made. A wide range of concentrations of cadmium occurs and period overrides the effect of flow rate (fig. 4). The effect of flow rate on cadmium levels is inconsistent although flow rate is an important factor that affects cadmium levels in water samples at all sites.

Although for all trends of flow rate most of the cadmium is in solution at Brailsford Brook South ($F_{11,36}$=2.11, P<0.10) (mean proportion equals 1.10 ± 0.08), at Suttonbrook there is a minor effect of flow rate on the proportion of cadmium in solution ($F_{9,30}$=4.23, P<0.01): at the lowest flow rates (second and third period combined) the proportion equals 1.40 ± 0.42 while at higher flow rates (first and fourth period combined) this proportion equals 0.79 ± 0.17. This separation into two groups is based on flow rate and on interaction between the effect of flow rate and filtration. Neither proportion is significantly different from unity. In samples from the R. Ecclesbourne, for some trends of flow rate, concentration of cadmium was significantly lower up to the 0.01 probability level in the filtrate than in whole samples, in spite of slightly lower hardness of this water. Additionally, there is no substantial difference between the occasions with different flow rates ($F_{12,39}$=1.73; P<0.10); the overall proportion of cadmium in solution equals 0.74 ± 0.03. However, cadmium levels in the R. Ecclesbourne are generally higher than at the other sites (figs. 1-3).

In contrast to cadmium, the detailed results for lead are more difficult to interpret because in many samples, both whole and filtrates, levels were below the limit of detection of 0.6 $\mu g\ l^{-1}$. Only at higher flow rates at Brailsford Brook South and Suttonbrook was lead more readily detectable in whole samples. The same trend was found in whole samples from the R. Ecclesbourne while the concentration of lead in filtrates decreased with increasing flow rate (fig. 2). At all sites this resulted in a significant interaction between flow rate and filtration (figs. 1-3). Consequently, the proportion

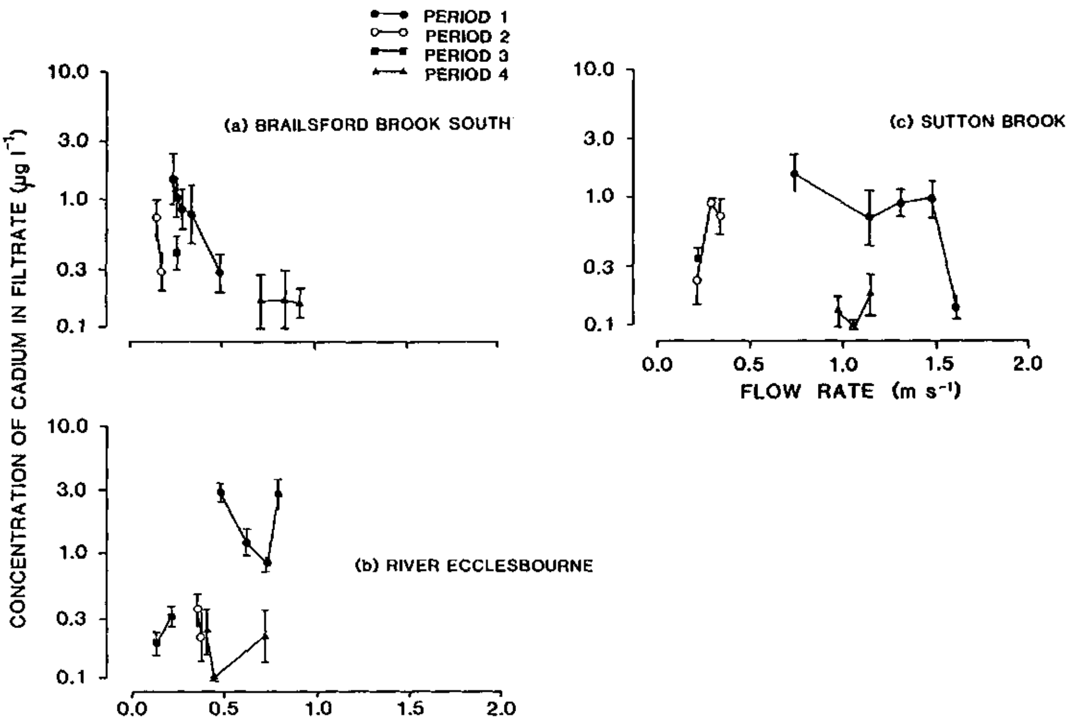

Fig 4 Mean concentrations and standard errors of cadmium ($\mu g\ l^{-1}$) in filtrates (passed through a filter of 0.45 µm pore size) of river water with estimated flow rate taken during four periods in 1987 at three sites in Derbyshire: (a) Brailsford Brook South, (b) River Ecclesbourne and (c) Suttonbrook. Value of limit of detection ($0.1\ \mu g\ l^{-1}$) used for not detectable levels.

of lead in solution decreased with increasing flow rate (table 3 and fig. 2). There appears to be some hysteresis effect on the proportion of lead in solution (table 3 and figs. 1-3).

Table 3 Mean values and standard errors of the proportion of the total amount of lead in solution in river water from three sites in Derbyshire taken at 4 different times; non-detectable levels were replaced by the limit of detection of 0.6 μg l^{-1}; see table 1 for details.

Site	Trend of flow rate	Period	Range of flow rates ($m\ s^{-1}$)	mean ± s.e.	Number of observations
Brailsford Brook South	decreasing	1	0.49	0.14 ± 0.04	4
			0.33	0.08 ± 0.00	4
			0.28	0.12 ± 0.00[b]	4
			0.25	0.11 ± 0.03	4
			0.23	1.00 ± 0.00[a]	4
		2	0.14 - 0.17	0.94 ± 0.19[b]	12
	stable	3	0.25	0.55 ± 0.19	8
	increasing	2	0.14	1.00 ± 0.00[b]	4
			0.17	1.00 ± 0.00[b]	8
	variable	4	0.71	0.12 ± 0.01	4
			0.84	0.34 ± 0.04	4
			0.92	0.43 ± 0.12	4
River Ecclesbourne	decreasing	1	0.79	0.01 ± 0.00	4
			0.73	0.04 ± 0.01	4
			0.62	0.01 ± 0.00	4
			0.48	0.02 ± 0.00	4
		2	0.35 - 0.37	0.05 ± 0.01	4
			0.35 - 0.37[c]	0.04 ± 0.01	16
		3	0.21	0.14 ± 0.07	4
			0.13	0.59 ± 0.59	4
		4	0.44	1.13 ± 0.35	4
			0.40	0.16 ± 0.02	4
	increasing	1	0.62 - 0.79	0.01 ± 0.00	8
		2	0.35 - 0.37	0.04 ± 0.01	4
		4	0.40	0.16 ± 0.02	4
			0.72	0.00 ± 0.00	4
			0.40 - 0.44[c]	0.16 ± 0.02	8
			0.72	0.00 ± 0.00	4
Suttonbrook	decreasing	1	1.61	0.02 ± 0.00	4
			1.47	0.09 ± 0.01	4
			1.30	0.15 ± 0.04	4
			1.13	0.11 ± 0.03	4
			0.73	1.00 ± 0.00[a]	4
		2	0.33	1.20 ± 0.14[b]	4
			0.28	1.00 ± 0.00[a]	4
			0.21	0.89 ± 0.06[b]	8
	stable	3	0.21	0.61 ± 0.27	8
	variable	4	0.97	0.17 ± 0.04	4
			1.05	0.73 ± 0.17	4
			1.14	0.56 ± 0.10	4

[a] all samples had lead levels below the limit of detection;
[b] almost all samples had lead levels below the limit of detection;
[c] variation in discharge of 10% was ignored.

For those sampling occasions in which lead was detected in all of the whole samples and filtrates, the estimates of the proportion of lead in solution confirm that usually little of total lead is present in solution and most of it is in suspension (table 4). Thus, lead is mainly associated with particulate matter.

Table 4 Proportion of lead in solution in water samples which contained detectable levels of lead in all 4 whole and 4 filtered samples taken on the same occasion.

Site	Period	Occasion	Flow rate ($m\ s^{-1}$)	mean ± s.e.
Brailsford Brook South	3	1	0.25	0.47 ± 0.10
River Ecclesbourne	2	1	0.35	0.03 ± 0.01
		3 + 4 *	0.35	0.06 ± 0.02
	4	2	0.40	0.16 ± 0.02
Suttonbrook		not applicable		

* data for the third and fourth occasion combined as they had the same flow rate; 8 observations used.

Variation between sampling times

Comparison of results for occasions from different periods that have similar flow rates are consistent in the sense that cadmium levels in all samples are significantly higher during the spring than during the summer and autumn (fig. 4). Filtration does not reduce the concentration of cadmium significantly at low flow rates and only slightly ($P<0.10$) at higher flow rates (figs. 1-3). Additionally, this is supported by the most complete results from the R. Ecclesbourne where more than one comparison of occasions include the first period: for flow rates 0.48/0.44 and 0.73/0.72 m s^{-1} the results are $F_{1,12}=395.19$ $P<0.001$ and $F_{1,12}=9.27$ $P<0.01$ respectively.

The results for lead are comparable with those for cadmium: higher levels in whole samples taken during the first and second period than in those from the fourth period for both the R. Ecclesbourne and the Suttonbrook (figs. 1 and 2). The relatively low flow rate for Brailsford Brook South may explain the lack of significance. Filtration reduces the concentration of lead in all samples significantly with a greater effect during the first period (table 3).

Change in metal concentration with decline in flow rate at one time

Flow rate did not affect cadmium and lead levels (fig. 5). The proportion of cadmium in solution was estimated to be 0.65 ± 0.04. However, this is presumably a biased result as in 10 of the samples amounts of cadmium were below the limit of detection of 0.1 µg l^{-1}. The proportion of lead in solution did not change with decreasing flow rate ($F_{2,9}=2.67$, $P>0.10$); estimated mean value 0.18 ± 0.01.

DISCUSSION

There is little detailed information on the effect of flow rate on, and fluctuations of, concentration of heavy metals in water that is relevant for the exposure-dose relationship in organisms.

Most published work deals with the complexation of trace metals with ligands and particulate matter, but detailed chemical aspects of adsorption/desorption processes are outside the scope of this paper. Suffice to say that the

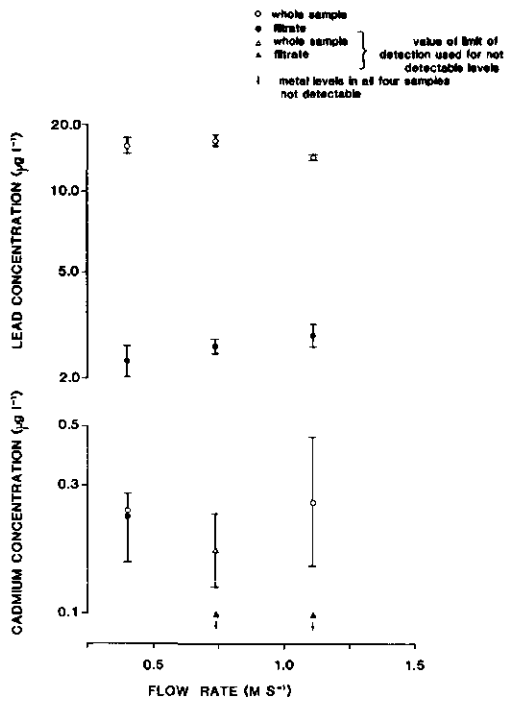

Fig.5.

Fig. 5 Mean concentrations and standard errors of lead and cadmium ($\mu g\ l^{-1}$) in whole samples and filtrates (passed through a filter of 0.45 μm pore size) in river water from the River Ecclesbourne taken on 18 November 1987 at three positions with different flow rate.

effect of flow rate on metal levels depends on several variables including the type of metal, the ratio of dissolved to particulate metal, metal content of the sediment and adsorption of dissolved metal by the sediment (Gardiner, 1974b; Reynolds, 1981). Cadmium and lead can be absorped differently onto humic material and onto clay and mineral particles (Stumm & Morgan, 1981). Förstner (1983a) concluded that the different dispersal behaviour of water constituents, especially of sediment fractions must be considered.

Concentrations of cadmium and lead in solution from Brailsford Brook South and the Suttonbrook are low and comparable with the results of Thorne *et al.* (1979) for their control sites thus confirming that they provide information on normal background levels. The River Ecclesbourne, a 'flashy' river (Moriarty *et al.*, 1982), with mineral veins at the head of the valley, had the highest concentrations of both cadmium and lead. Aston and Thornton (1977) also found higher levels in water from mineralised catchments. Nevertheless, this does not imply that the mechanism of transport is through the water; Moriarty and Hanson (1988) found for the R. Eccclesbourne that characteristics, such as size and density, of particles, with which most of the heavy metals are associated, change with distance from the mouth of the river. The characteristics of a particular stream determine to a large degree the distribution of metals in sediments (Bradley and Cox, 1986). Because of the interaction between trace metals and sediments, the extent of adsorption of metals onto particulate matter may vary between streams. The results of this study show that differences in concentration between streams were greater for lead than for cadmium. Concentrations of both metals correspond with those found by Reynolds (1981) for the R. Ecclesbourne.

Most of the cadmium is in solution at all flow rates at all three sites. For some periods, at relatively low flow rates increasing flow rate increases the concentration of dissolved cadmium (fig. 4). Similar results have been found for phosphate and silicate, which were presumably captured in interstitial water in the top layer of the sediment (Casey and Farr, 1982). Even so, the concentration of cadmium does decrease with increasing flow rate at relatively high flow rates. Förstner (1983b) got similar results for cadmium in water from the R. Rhine, and Gardiner (1974a) found that in unpolluted river water about 90% of the cadmium is present as free ions. Förstner (1983a) has reported for upper part of the R. Rhine that all cadmium is in solution when the concentration is low (about 0.2 $\mu g\ l^{-1}$).

In contrast with cadmium, the concentration of lead in whole samples increases with flow rate because most of the lead is associated with particles (see also Thorne *et al.*, 1979). Similar results have been found for zinc (Grimshaw *et al.*, 1976; Wilson, 1976). The concentration of that lead which is in solution decreases with increasing flow rate, presumably because of dilution. The difference in proportion of total metal between cadmium and lead that is in solution is presumably caused by difference in adsorption to particles with hydrous oxides surfaces (Sigg, 1987).

Particles from the river bed can be resuspended thus increasing the metal levels with increasing discharge. The results suggest that once particles are brought into suspension, they do not settle rapidly. Particles are carriers for adsorbed metal ions and thus contribute to the regulation of the chemical composition of aquatic systems (Sigg, 1987). Their transport is governed by many factors of which the motion of the water and, to some extent, inter-particle contacts are important (O'Meila, 1987). The data indicate that gravity had no significant effect on settlement of particulates in these particular circumstances, although size and density of particles in sediment show differences (Moriarty and Hanson, 1988). Therefore, flow rate can have a different effect on the concentration of metals in whole samples and in solution depending on the type of metal, which is confirmed by Hellmann (1987). The hysteresis effect on only lead is presumably caused by the same phenomenon.

The relatively high solubility of cadmium, and the difference between sites in the proportion of cadmium and lead that is in solution, may be explained, at least in part, by the difference in concentration of total phosphate. The concentration of total and orthophosphate, which to a first approximation are proportional to the amount of organic material, is higher in the R. Ecclesbourne than at the other two sites (table 2). Ortho-phosphate reduces the solubility of lead and the extent to which this can occur is greater in hard water than in soft water (Sheiham & Jackson, 1981). Humic substances also influence the speciation of trace metals in water (Mantoura *et al.*, 1978) and can reduce the solubility of lead. Ligands such as chloride and sulphates can form reversible complexes and result in lowered concentrations of dissolved metal (Schindler & Stumm, 1987). However, competition from Ca and Mg ions for adsorption sites on humic material may result in a higher proportion of cadmium than lead in solution (Laxen, 1985; Mantoura *et al.*, 1978).

There appears to be some temporal variation in the concentration of metals in samples taken at occasions from different periods with the same flow rate, which confirms Reynolds (1981). Similar results for a different area have been found by Aston and Thornton (1977). However, temporal differences cannot simply be attributed to seasonal variation because other variables can have an appreciable effect (Förstner, 1983b). Long-term studies can overcome these drawbacks.

This work indicates the degree of exposure of fish to lead and cadmium in water. A simple statement of total metal concentration in water may be misleading for understanding the exposure-dose relationship. Flow rate affects the metal concentrations in whole samples and filtrates. There is some temporal variation in metal concentrations.

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PART IV MODELLING

Chapter 7: A mathematical model for cadmium in the stone loach (*Noemacheilus barbatulus* L.) from the River Ecclesbourne Derbyshire.

Ecotoxicology and Environmental Safety, submitted.

A MATHEMATICAL MODEL FOR CADMIUM IN THE STONE LOACH (*NOEMACHEILUS BARBATULUS* L.) FROM THE RIVER ECCLESBOURNE DERBYSHIRE

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ABSTRACT

A mathematical model was developed using information from laboratory experiments and field studies, which linked metabolism of stone loach with cadmium dynamics. Three possible sources of cadmium were distinguished: water, food and sediment. Predicted results over one year, were compared with field observations from three sites in Derbyshire. The model adequately predicted growth of fish for all three sites. Predicted cadmium levels in loach were in good agreement with measured levels in fish from all three sites despite the fact that concentrations of cadmium in the environment were kept constant during each simulation. The weight of fish affected the relative importance of different pathways of cadmium intake under similar conditions. Uptake from water contributed substantially to body burden even though the concentration in water was lower than in food or sediment. However, uptake from both food and sediment could not be ignored given the measured levels of cadmium in the field. The relative importance of uptake from the three sources also differed with site. The model showed that metabolism, affected by temperature, is important to the dynamics of cadmium in the stone loach.

INTRODUCTION

The nature of the link between exposure to a contaminant in the environment and the dose received within an organism is a central problem in studies of the biological effects of pollutants in the environment: exposure determines the dose, which may then cause an effect on the organism (Doull *et al.*, 1980). There has been a long debate about the relative importance of different parts of the physical environment (e.g. food, water, sediment) as sources of persistent pollutants, such as heavy metals, for aquatic animals. Knowledge of the routes and rates of uptake of a pollutant from different sources is important for understanding and predicting effects of pollutants on wildlife.

This paper develops a mathematical model to describe results on rates of uptake and loss of cadmium from water (Douben 1989a), food (Douben 1989c) and sediment (Douben & Koeman, 1989) by the stone loach (*Noemacheilus barbatulus* L.). Data from laboratory experiments have been used together with field data on cadmium burden in loach from three sites in Derbyshire: River Ecclesbourne, Brailsford Brook South and Suttonbrook (Douben, 1989b). (For a detailed description of the study sites see Douben (1989d)).

The objectives of the mathematical model have been:

- to asses the effect of body size on cadmium burden;
- to quantify the intake of cadmium from food, water and sediment;
- to explain the observed fluctuations in cadmium body burdens under field conditions;

- to evaluate reliability of application in other habitats.

The stone loach is a common fish in British rivers. It is a bottom-feeder (Maitland, 1965), buries itself in the mud (Hyslop, 1982), and is active during the first few hours of darkness (Welton *et al.*, 1983).

DEVELOPMENT OF THE MODEL

First approach

The mathematical model is based on the premise that metabolism (metabolic rate) of stone loach is an important factor that affects metal burden, an approach first developed by DeFreitas and Hart (1975). Metabolism of fish (including growth) depends on body weight and the temperature of the water, and affects rates of uptake and loss of pollutant. The model can be divided into two major parts: one part describes metabolism of the loach, the other describes the flux of cadmium between fish and its surroundings. Values of parameters and variables for the model were estimated from data for the River Ecclesbourne, and validated with observations from the two other sites: the Brailsford Brook South and Suttonbrook.

Norstrom *et al.* (1976) developed a model which considered two routes of entry for mercury and PCB in yellow perch (*Perca flavescens*): food and water. A comparison between their model and the one described here is made where appropriate. Their model has been used as a basis for the mathematical model described in this paper. Data for the stone loach has been used where available.

Metabolism

Two rates of metabolism can be distinguished: low routine metabolism when fish show occasionally spontaneous movements, and maximum metabolism when fish are active and feeding at such quantities that growth occurs. The relationship between weight of fish and metabolic rate can be expressed as (Paloheimo and Dickie, 1966):

$$Q_{lr} = \alpha_{lr} W^{y} \qquad (1)$$

in which : Q_{lr} : low routine metabolism (cal day^{-1});
α_{lr} : coefficient for low routine metabolism (cal day^{-1} mg^{-1});
W : body weight (dry weight mg);
y : body weight exponent.

Numerous publications deal with the relationship between metabolism and size of the fish (e.g. Niimi and Beamish, 1974) or temperature (Beamish, 1974; Brett *et al.*, 1969). Data on oxygen consumption rate for the stone loach over a wide range of temperatures have been published (Douben, 1989c), and energy expenditure can be calculated by converting oxygen consumption into calories (see below). It was assumed that high activity was absent at 6 °C and below, because growth of loach had ceased under those conditions (Douben, 1989b). If any growth occurs, a component for change in weight with time, dW/dt, is introduced in equation 1 and total metabolic rate increases. Conversely, loss of weight makes energy available thus lowering the required intake of food.

Three age groups of loach have been distinguished: fish between 0 and 1 year old (0+), between 1 and 2 years old (I+) and 2 years and older (II+) (for details see Douben (1989b)). For each age group, a regression analysis of measured weights of fish on the time of each sampling occasion throughout one year was carried out and differentiation of the regression equation yielded growth rate as a function of time. Given that temperature affects growth rate and temperature of river water changed with time (Douben, 1989b), a subsequent regression analysis was carried out of growth rate on temperature, after allowing for the fact that estimates were based on growth rate over a period. This approach proved to be adequate for predicting weight of loach from the River Ecclesbourne.

The model for metabolism and ration (R) is then:

$$R = \frac{1}{E_f} \left[\alpha_{1r} W^{\gamma} + (\beta+1) \frac{dW}{dt} \right] \qquad (2)$$

The coefficient E_f allows for food passing through the gut unassimilated and another one, ß, takes the energetic cost of growth into account (Norstrom *et al.*, 1976).

Uptake of pollutant

Uptake of pollutant can occur from three sources: food, water and sediment. The model is based on the assumption that rates of uptake from these sources are independent and that, therefore, uptake of cadmium via different pathways is additive.

Uptake of cadmium from food depends on the ingested ration (R) and the concentration of pollutant in the food (C_{pr}). However, not all of the ingested cadmium is taken up by the stone loach. Results on rates of uptake of cadmium during dietary exposure, dCd/dt, have been published (Douben, 1989c), and are implemented in equation 3 to estimate the efficiency of uptake of cadmium from the food (E_{pr}).

$$\frac{dCd}{dt} = E_{pf} * C_{pf} * R \qquad (3)$$

Similarly, not all of the cadmium is taken up from the water flowing over the gills. Rate of uptake of cadmium from the water is related to the volume of water passing over the gills (V) and the concentration of cadmium in the water (C_{pw}). Water passes over the gills to supply the fish with oxygen. The volume of water is therefore a function of the metabolic requirement, the concentration of oxygen in the water (C_{ox}) and the efficiency with which the oxygen is taken up (E_{ox}). Oxygen can be converted into calories by Q_{ox}=3.42 cal mg^{-1} oxygen (which is equal to $3.42*10^{-3}$ cal μg^{-1} oxygen) (Warren and Davis, 1967). The model is given in equation 4.

$$\frac{dCd}{dt} = \left[\frac{E_{pw} * C_{pw}}{E_{ox} * C_{ox} * Q_{ox}}\right] * \left[\alpha W^{\gamma_w} + \beta \frac{dW}{dt}\right] \qquad (4)$$

Exposure to sediment contaminated with cadmium increased cadmium levels in stone loach under laboratory conditions (Douben & Koeman, 1989). Field observations also suggested that uptake of cadmium from the sediment could occur (Douben, 1989b). Rate of uptake will depend on concentration of cadmium in the sediment (C_{ps}). For practical purposes an efficiency coefficient (E_{ps}) of less than unity would imply that not all the cadmium is taken up. It was assumed that the connection between metabolic requirements and uptake of cadmium under these circumstances could be described in a similar way as for water-borne exposure, although the pathway of uptake may be different. Uptake of cadmium from sediment is then as described in equation 5:

$$\frac{dCd}{dt} = \left[\frac{E_{ps} * C_{ps}}{E_{ox} * C_{ox} * Q_{ox}}\right] * \left[\alpha W^{\gamma_s} + \beta \frac{dW}{dt}\right] \qquad (5)$$

Loss of pollutant

Loss of cadmium from loach was determined after both water-borne and dietary exposure (Douben, 1989a and 1989c). The rate constants for loss found in the laboratory during and after dietary exposure were higher than for other types of exposure. Concentrations used in food items during dietary exposure were close to levels in invertebrates (on which loach feeds) as found under field conditions (see below). Body size had no effect on the rate of loss per unit burden of cadmium, in contrast to the model used by Norstrom *et al.* (1976). Loss can be described by equation 6:

$$\frac{dCd}{dt} = k * Cd \qquad (6)$$

<u>Final model</u>

Sub-models for cadmium burden in the loach which included just one source of cadmium, were used to test the working of that model and compared with results from laboratory experiments. These sub-models were then amalgamated into one major model as given in equation 7:

$$\frac{dCd}{dt} = \left[\frac{E_{pf} * C_{pf}}{E_f}\right] * \left[\alpha W^{Y_f} + (\beta+1)\frac{dW}{dt}\right] + \left[\frac{E_{pw} * C_{pw}}{E_{ox} * C_{ox} * Q_{ox}}\right] * \left[\alpha W^{Y_w} + \beta\frac{dW}{dt}\right]$$

$$+ \left[\frac{E_{ps} * C_{ps}}{E_{ox} * C_{ox} * Q_{ox}}\right] * \left[\alpha W^{Y_s} + \beta\frac{dW}{dt}\right] - k * Cd \qquad (7)$$

ESTIMATION OF VARIABLES AND PARAMETERS

<u>Metabolism</u>

Oxygen consumption rate per day was calculated from low routine metabolism for 19 hours and maximum metabolism for 5 hours. Stone loach were fed different rations of *Tubifex*, from which maintenance rations were calculated (Douben, 1989c). The energetic value of the worms was taken to be 5.49 kcal g^{-1} dry weight *Tubifex* (Warren and Davis, 1967). The ratio of the amount of energy required, from oxygen consumption rate, to the amount of energy consumed at maintenance ration is taken as the estimate for efficiency of energy uptake from the diet, and yields a value of 0.70.

The value of α_{lr} (equation 2) was estimated from the results from oxygen consumption rate at different temperatures (Douben, 1989c). The coefficient relating uptake of energy to the amount of tissue deposited (ß) was estimated by using the results from feeding *ad libitum* (Douben, 1989c). A linear regression of growth rate on food consumption was carried out according to equation 8a to obtain b and then was estimated according to equation 8b (Norstrom *et al.*, 1976):

$$\frac{dW}{dt} = a + b * R \qquad (8a)$$

$$b = \frac{E_f}{1 + \beta} \qquad (8b)$$

where E_f equals 0.70.

Results for exponents of body weight, y_w, y_f and y_s (according to the route of entry: water, food and sediment respectively), relating weight to cadmium burden, from laboratory experiments yielded values of 0.34 ± 0.08, 0.70 ± 0.06 and 0.88 ± 0.13 respectively (Douben, 1989a and c; Douben and Koeman, 1989). A test was carried out to use a common exponent of 0.78 (the value of intake of food).

It was assumed water was always saturated with oxygen, when oxygen concentration (mg l^{-1}) can be described by:

$$C_{ox} = 14.45 - 0.413 * T + 0.00556 * T^2 \qquad (9)$$

in which T represents the temperature (°C) (Norstrom *et al.*, 1976).

The efficiency of oxygen uptake from the water was assumed to be 0.75 (Lloyd, 1961).

Efficiency of cadmium uptake

Rates of uptake of cadmium from water, food and sediment have been published (Douben, 1989a and c; Douben and Koeman, 1989). These data should be the same as the outcome of the appropriate parts of equation 7, which describe uptake from these sources. For exposure to water-borne cadmium and cadmium associated with sediment particles loss of weight, caused by starvation, was taken into account. Then, the efficiencies of cadmium uptake were estimated as 4.05, 6.29 and 0.05 % from water, food and sediment respectively.

Loss of cadmium

Laboratory experiments indicated that the rate constant of loss (k) is dependent on temperature. Loss was higher after than during dietary exposure and therefore the values of 0.22 at 8°C and 9.76 at 18°C have been used (Douben, 1989c). For intermediate temperatures k was obtained by interpolation, and below and above these k was kept constant at 0.22 and 9.76 (day^{-1}) respectively.

Concentration of Pollutant in the Environment

Water

Results from field observations on the concentration of cadmium in water (Douben, 1989c) were used to estimate maximum and minimum levels that can be expected in the River Ecclesbourne (table 1).

Food

Samples of invertebrates were taken on four occasions (29 May, 22 June, 21 September 1987 and 18 January 1988) for metal determination. Invertebrates were sorted into taxa; samples were weighed, dried at 85°C for 3 days and then reweighed. Also, the gut contents of some loach were analysed to determine the taxa actually eaten. The contents found in gut of stone loach were broadly similar to the taxa found in the invertebrate samples. For all three sites the concentration of lead and cadmium varied between times of sampling and taxa (fig. 1 and table 2) ($P<0.001$). Also there is probably a significant interaction between sampling time and taxa (P ranging from 0.10 to 0.05). The results (fig. 1) suggest that the concentration of both metals is higher in samples taken later in the year.

Sediment

Sediment samples were taken over a one year's period at the same time as fish (see Douben, 1989b). For details of sampling and chemical analysis see Douben and Koeman (1989). Table 1 lists the results of maximum and minimum concentrations found.

Temperature

To obtain data for temperature for each day, mean values of minimum and maximum temperature for each of the sampling occasions (Douben, 1989b) were interpolated between sampling occasions.

Initial values of the model

Mean values of dry weight and cadmium burden measured in loach per agegroup in the first sample of fish, taken during the one year sampling programme, have been used as initial values.

Time constant

Metabolism was simulated on a daily basis and predicted growth rate adequately. Values of rate constants for loss of cadmium required that change of cadmium burden was calculated for each hour.

Table 1 Minimum and maximum concentrations of cadmium in water, invertebrates and sediment from three sites in Derbyshire (for details of water see Douben, 1989e).

Site	Concentration of cadmium					
	water $\mu g\ l^{-1}$		invertebrates $mg\ kg^{-1}$		sediment $mg\ kg^{-1}$	
	min	max	min	max	min	max
River Ecclesbourne	0.1	2.8	0.01	100	40	75
Brailsford Brook South	0.16	1.4	0.01	10	1	2
Suttonbrook	0.1	1.6	0.01	10	1	2

Criterion for fit

Given that earlier work had shown that body weight was an important factor that influenced cadmium burden, comparison between observed data (Douben, 1989b) and predicted results were made for three different age-groups. The differences between the logarithm of predicted and observed mean values per sample for all times were regressed on sampling time to evaluate whether the difference changed with time. The regression coefficient was not different from zero on any occasion thus a bias in this difference with time had not occurred. The deviation of the difference about the regression line was then used as a measure for adequate fit. The mean difference indicated the overall closeness of the predicted to the observed values.

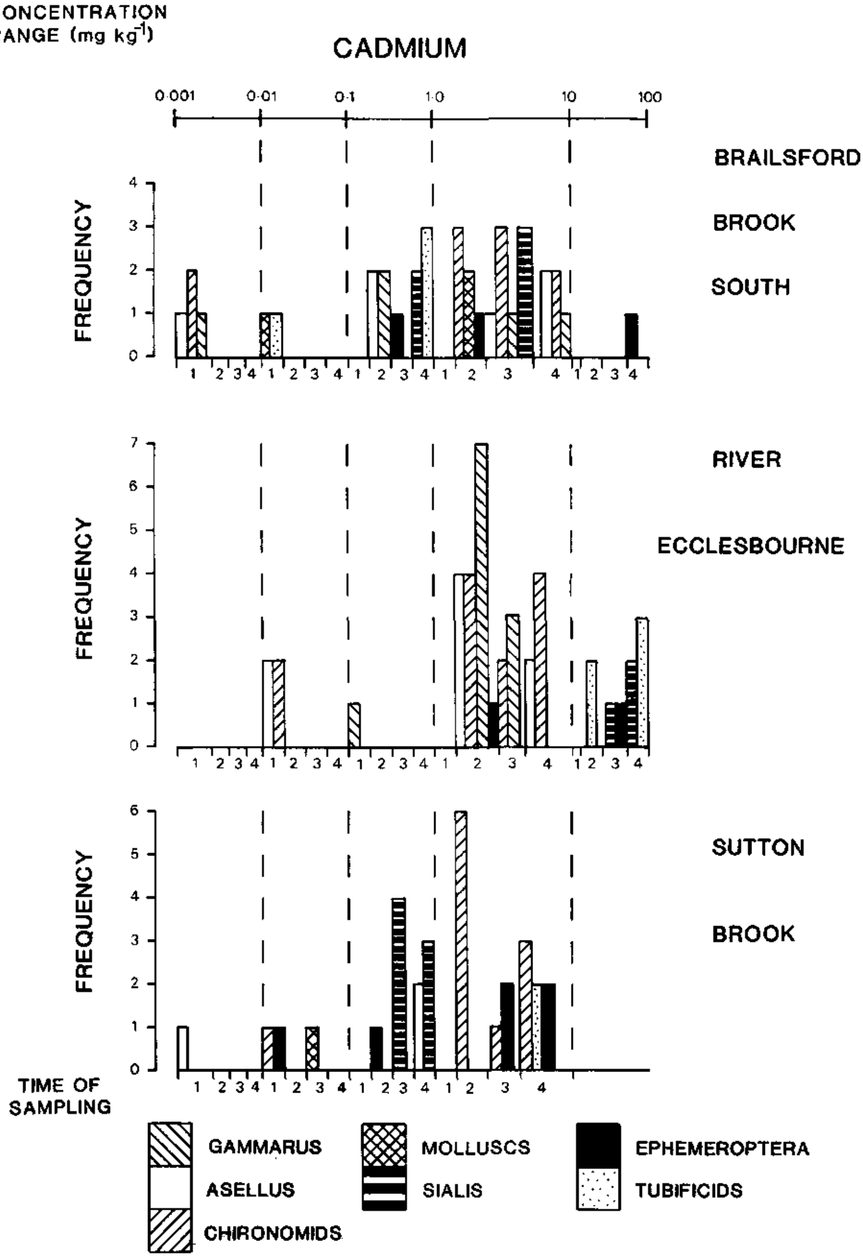


Fig. 1 Frequency distribution of concentration of cadmium in invertebrates from three sites in Derbyshire, sampled on four occasions. See text for sampling dates.

Table 2 Details of stone loach (*Noemacheilus barbatulus* L.) and their gut contents with estimated total number of animals.

Site	Fish no.	Length (mm)	Sex	Taxon								Total number
				Chironomidae	Crustacea	Erpobdellidae	Lumbriculidae	Oligochaeta	Prodiamesa	Simuliidae	Tubificidae	
R. Eccles-	1	77		+	-	-	-	-	-	+	-	> 100
bourne	2	82		+	-	-	-	-	-	+	-	> 50
Brailsford	3	105		+	+	-	+	-	+	+	+	> 100
Brook South	4	92		-	-	-	-	-	-	+	-	12
Sutton-	5	93		+	-	-	-	+	-	+	-	> 150
brook	6	67		+	-	+	-	-	-	+	-	> 100

RESULTS

Prediction of dry weight and effect on cadmium burden

Prediction of weight of I+ and II+ fish correlated significantly ($P<0.001$), with the observed data (see also table 3). Weight of 0+ fish was not adequately predicted, presumably because only few field observations were available. Food accounted for more than 50 % of the total intake of cadmium (table 4). Percentage uptake from water was of similar magnitude as from sediment for 0+ and I+ fish, but lower for II+ fish. Standard deviations of the regression of the difference between observed and predicted values were similar for I+ fish as for II+ fish but the predicted values matched the observed ones better for the first group of fish.

Table 3 Comparison of the regression of the difference between predicted and observed logarithmic dry weight for stone loach of different ages on time of sampling: standard deviation about the regression line (S_y), the mean difference between predicted and observed mean dry weight (mg), and the correlation coefficient.

Agegroup	Standard deviation about the regression S_y	Mean difference (mg)	Correlation coefficient between predicted and observed dry weight	Number of observations
II+	0.0453	0.0268	0.833***	13
I+	0.0855	0.1679	0.916***	12
0+	0.2129	-0.4321	0.369	5

*** indicates significant correlation at the 0.001 probability level.

Table 4 Effect of age of fish on the relative contribution of water, food and sediment to the intake of cadmium: minimum (min) and maximum (max) on one day, and overall covering the entire simulation period, and on the regression of the difference between predicted and observed logarithmic burden of cadmium in stone loach on time of sampling, the standard deviation about the regression and mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 mg kg^{-1} dry weight and 10 mg kg^{-1} dry weight respectively.

Age-group	Percentage intake from									Standard deviation about regression S_y (ng)	Mean difference between predicted and observed mean	Number of observations
	water			food			sediment					
	min	max	over-all	min	max	over-all	min	max	over-all			
II+	2.0	16.3	11.3	57.4	70.8	61.1	24.3	30.6	27.7	0.3787	-0.5219	13
I+	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079	12
0+	18.4	27.7	22.6	55.7	67.5	62.6	12.9	18.5	14.8	0.2880	-0.7877	5

Range of minimum and maximum cadmium burden

Standard conditions refers to the values of parameters as listed in table 5. Two levels of concentrations of cadmium in the environment (water, food and sediment) have been distinguished: minimum and maximum (table 1). Using these concentrations throughout different simulations yields the widest range

Table 5 Values of variables for standard conditions in the mathematical model which predicts cadmium burden of I+ stone loach.

Variable	Value
Dry weight (mg)[a]	300
Cadmium burden (ng)[a]	15
y_w	0.34
y_f	0.70
y_s	0.88
C_{pw} ($\mu g\ l^{-1}$)	1.0
C_{pf} (mg kg^{-1} dry weight)	10
C_{ps} (mg kg^{-1} dry weight)	10
E_{pw}	$4.05 * 10^{-2}$
E_{pf}	$6.29 * 10^{-2}$
E_{ps}	$5.211 * 10^{-4}$
E_{ox}	0.75
Q_{ox}	$3.42 * 10^{-3}$
E_f	0.70
ß	0.83

[a] refers to initial situation for fish from the River Ecclesbourne.

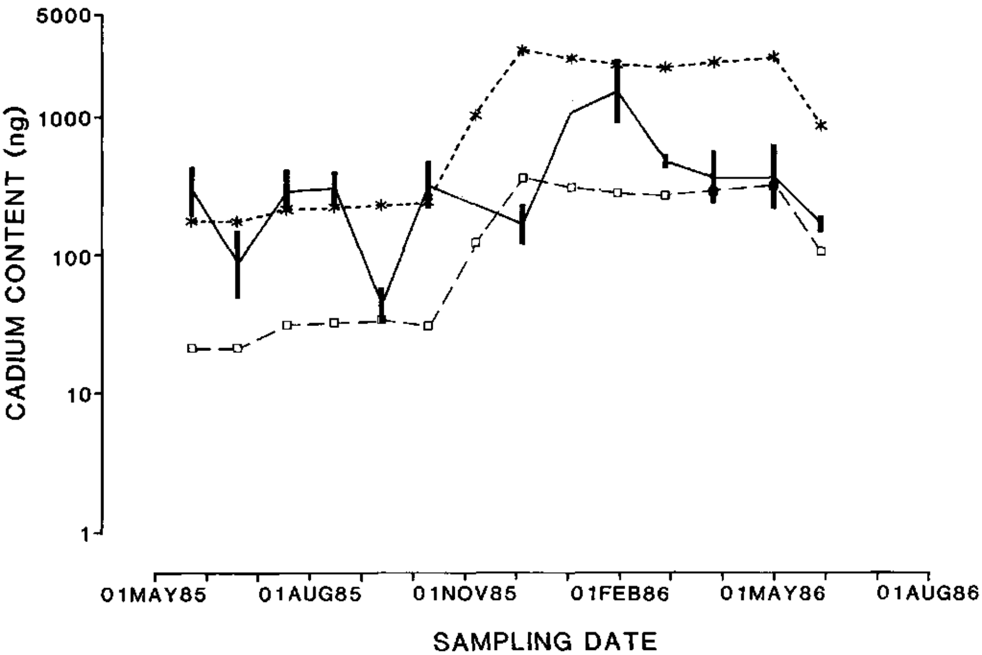
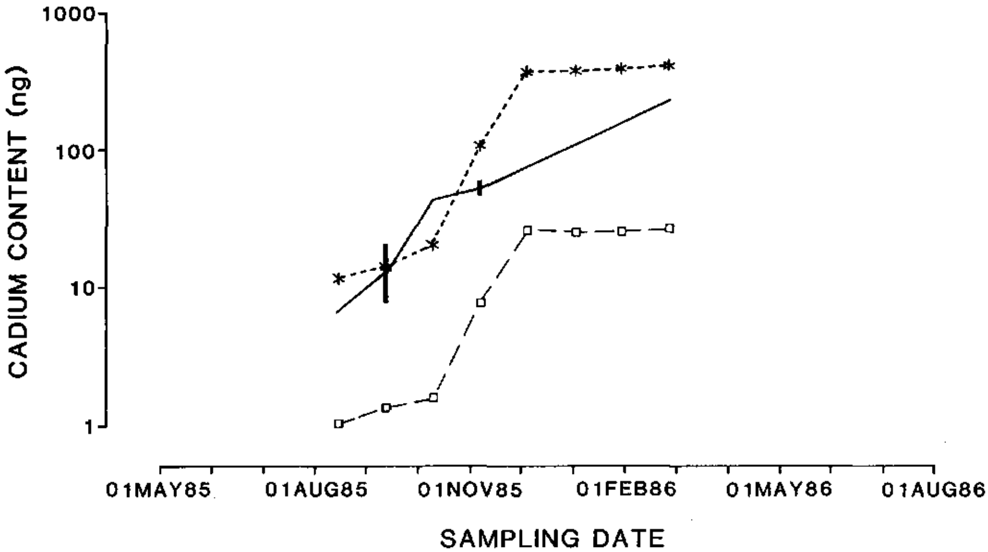
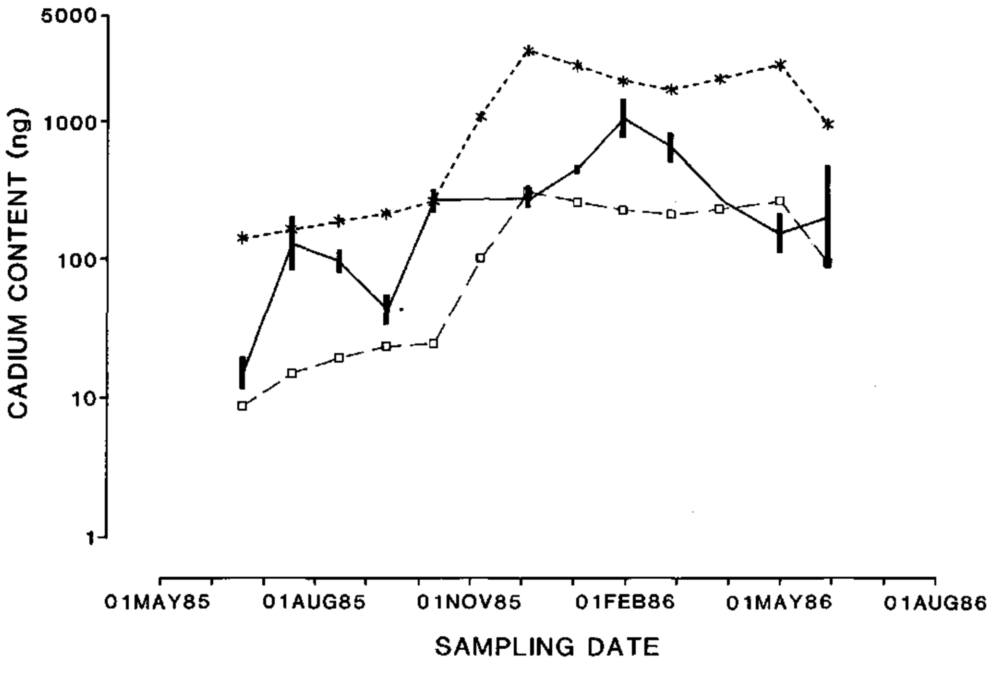


Fig. 2 Mean cadmium content (●), with standard errors, of stone loach sampled at about 4-weekly intervals from the River Ecclesbourne, Derbyshire. Values predicted with highest (✱) and with lowest (□) measured concentrations of cadmium in water, food and sediment. Three different agegroups were distinguished: (a) 0+ fish, (b) I+ fish and (c) II+ fish.

of body burden in loach that can be expected. The observed values for the three age groups fell within the respective ranges (figs. 2a, b and c): 8 out of 13, 11 out of 12 and 4 out of 5 sampling occasions for II+, I+ and 0+ fish respectively. The model predicted a plateau in cadmium burden for each agegroup of fish during the winter, which for the maximum concentrations


1000
100
10
1
CADIUM CONTENT (ng)
01MAY85
01AUG85
01NOV85
01FEB86
01MAY86
01AUG86
SAMPLING DATE


5000
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1
CADIUM CONTENT (ng)
01MAY85
01AUG85
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01FEB86
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01AUG86
SAMPLING DATE

was higher than the observed peaks for both I+ and II+ fish. The rise in cadmium content is predicted to occur later than observed. Because I+ fish approach the maximum weight by the start of the second winter, there is hardly any difference in predicted values between I+ and II+ fish from that time onwards (figs. 2b and c).

Given the availability of data for I+ fish, subsequent comparisons are based on results for I+ fish only with standard conditions.

Effect of change in cadmium in water, food and sediment

All predictions of minimum intake per day of cadmium from water are zero (table 6). This is due to the loss of weight during the winter period. The energy derived from this loss of weight is calculated to be greater than the requirements for Q_{1r}; provisions were made that no negative intake could take place through the water i.e. additional loss via the uptake component, which was then set to zero.

An increase in the cadmium concentration in water from 0.1 to 1.0 $\mu g\ l^{-1}$, with concentrations remaining constant in food and sediment, obviously increased the percentage intake of cadmium from this source whilst the overall percentage intake from water tends towards the higher part of the range (table 6a). Maximum predicted intake from sediment remains unaffected but its overall contribution drops from 24.2% to 6.6%. Deviation between observed and predicted values increases.

When the concentration of cadmium in food increases from 0.01 to 1.0 mg kg^{-1} then its contribution to the total intake increases most rapidly towards the top end of the concentration range used. The increase in concentration from

Table 6 Comparison of the regression of the difference between predicted and observed logarithmic burden of cadmium in stone loach on time of sampling for different concentrations of cadmium in water, food and sediment; the relative contribution of these sources to the intake of cadmium: minimum (min) and maximum (max) per day and overall covering the entire simulation period, the standard deviation about the regression and mean difference between predicted and observed logarithmic cadmium burden. Concentrations in food and sediment based on dry weight. Results for fish in agegroup I+; see text and table 5 for details of other conditions.

(a) change in water

Concentration of cadmium*			Percentage intake from									Standard deviation about regression S_y	Mean difference between predicted and observed mean cadmium burden (ng)
			water			food			sediment				
water $\mu g\ l^{-1}$	food $mg\ kg^{-1}$	sediment $mg\ kg^{-1}$	min	max	overall	min	max	overall	min	max	overall		
0.1	10	10	0.0	5.2	2.7	70.2	79.7	73.1	15.2	29.4	24.2	0.3812	-0.3843
1.0	10	10	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079
10.0	10	10	0.0	84.5	73.5	13.0	71.5	19.9	2.5	29.4	6.6	0.4603	0.0653

(b) change in food

1	**0.01**	10	0.0	77.1	52.7	0.1	0.3	0.1	22.7	99.8	47.2	0.4018	-0.7319
1	**0.1**	10	0.0	76.3	52.0	1.2	2.5	1.4	22.5	97.7	46.6	0.4015	-0.7254
1	**1.0**	10	0.0	69.0	46.1	10.4	20.1	12.5	20.3	80.7	41.3	0.3985	-0.6649
1	**10**	10	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079
1	**100**	10	0.0	6.0	3.4	92.1	96.2	93.5	1.8	4.0	3.1	0.3889	0.5080

- continued -

- continuation -

(c) change in sediment

1	10	**0.1**	0.0	39.3	26.9	60.6	99.6	72.9	0.1	0.4	0.2	0.4015	-0.4087
1	10	**1.0**	0.0	38.9	26.3	60.0	96.2	71.3	1.1	4.0	2.4	0.4003	-0.3984
1	10	**10**	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079
1	10	**100**	0.0	18.2	7.9	19.1	28.2	21.4	53.7	80.7	70.7	0.3662	0.1449
1	1	**0.1**	0.0	86.4	78.1	13.3	96.2	21.2	0.3	4.0	0.7	0.4805	-0.9795
1	1	**1.0**	0.0	84.5	73.5	13.0	71.5	19.9	2.5	29.4	6.6	0.4604	-0.9346
1	1	**10**	0.0	69.0	46.1	10.4	20.1	12.5	20.3	80.7	41.3	0.3985	-0.6649
1	1	**100**	0.0	24.4	9.8	2.3	3.8	2.7	71.9	97.7	87.6	0.3606	0.0499

* figures in **bold** emphasize the source in which change has occurred.

10 to 100 mg kg^{-1} has a considerably less effect. The deviation between the observed and predicted value drops (table 6b).

An increase in the concentration in the sediment also reduces the difference between observed and predicted values (table 6c). When the concentrations of cadmium in water, food and sediment are 1.0 $\mu g\ l^{-1}$, 1 mg kg^{-1} and 100 mg kg^{-1} respectively, the least deviation for all comparisons listed in table 6a, b and c is obtained and the mean of the difference is lowest: this indicates that the observed and predicted values are reasonably close.

Effect of exponent of body weight

The relatively low contribution from the water component may be, at least to some extent, due to the numerically lower value of the exponent relating body weight to cadmium uptake from water. When the exponents were given the value of 0.78, the value for uptake of food, entry of cadmium through the water increases to nearly 2.5 times, the relative contribution from food has dropped to about 69% of its original contribution and from sediment to about 35% (table 7). The deviation about the regression of the difference between observed and predicted values on time is reduced.

Relationship between metabolic rate and cadmium uptake from sediment

It was assumed that the connection between metabolic rate and uptake of cadmium from sediment is similar to that for water-borne exposure. Replacing the part of metabolic rate by the equation used for dietary exposure increases the proportions taken up from water and food at the cost of intake from sediment (table 8). The predicted cadmium burdens do not match the observed ones as well as the standard model does.

Table 7 Effect of the exponent of body weight on the percentage uptake of cadmium from water, food and sediment, on the standard deviation about the regression of the difference between predicted and observed cadmium burden on time of sampling and the mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 mg kg^{-1} dry weight and 10 mg kg^{-1} dry weight respectively.

Body weight exponent for			Percentage intake from									Standard deviation about regression S_r	Mean difference between predicted and observed mean cadmium burden (ng)
			water			food			sediment				
water	food	sediment	min	max	over-all	min	max	over-all	min	max	over-all		
0.34	0.70	0.88	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079
0.78	0.78	0.78	48.0	55.8	52.7	37.0	45.8	40.5	6.2	7.2	6.8	0.3753	-0.1355

Table 8 Effect of change in connection between metabolic rate, from water-borne equivalent to dietary equivalent, and uptake of cadmium from sediment, on the percentage uptake of cadmium from water, food and sediment, on the standard deviation about the regression of the difference between predicted and observed cadmium burden on time of sampling and the mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 $mg\ kg^{-1}$ dry weight and 10 $mg\ kg^{-1}$ dry weight respectively.

Type of metabolic rate	Percentage intake from water			food			sediment			Standard deviation about regression S_y	Mean difference between predicted and observed mean cadmium burden (ng)
	min	max	over-all	min	max	over-all	min	max	over-all		
as during water-borne exposure	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079
as during dietary exposure	0.0	39.1	26.7	60.3	98.0	72.3	0.6	2.1	1.1	0.4011	-0.4043

Effect of rate constant of loss

At lower temperatures, body burden increases because rate of intake is reduced more than rate of loss. Given that cadmium burden in both I+ and II+ fish peaked in January, it seems likely that below 8°C loss is further reduced and not constant as assumed in the standard model. The same relationship between k and temperature extrapolated below 8°C with a minimum value of 0.0 results in a greater difference between the observed and predicted values (table 9).

Effect of temperature

Hitherto temperatures used were as observed in water from the River Ecclesbourne (Douben, 1989b). Predictions were carried out with one and two degrees difference for all temperatures without adjusting for a possible change in growth rate. Entry of cadmium from water is lowest at the standard set of temperatures but the predicted minimum percentage intake from water increases with temperature (table 10). Minimum, maximum and overall percentage uptake from food increases with drop in temperature, while the overall relative contribution from sediment decreases with decreasing temperature.

Given that the model is based on metabolism of stone loach, it is not surprising that predicted weight of fish is higher for higher temperatures at the end of the simulation period. Also, the maximum predicted cadmium burden is increased.

There was little difference between the predicted burden of cadmium up to the middle of October for the different sets of temperatures (fig. 3). Thereafter, the predicted increase is delayed for higher temperatures.

Table 9 Effect of reduction of the value for rate constant of loss k below 8°C on the relative contribution of water, food and sediment, to the cadmium burden of the loach, on the standard deviation about the regression of the difference between predicted and observed cadmium burden on time of sampling and the mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 $mg\ kg^{-1}$ dry weight and 10 $mg\ kg^{-1}$ dry weight respectively.

Value of k below 8°C	Percentage intake from water			food			sediment			Standard deviation about regression S_y	Mean difference between predicted and observed mean cadmium burden (ng)	Maximum predicted cadmium burden (ng)
	min	max	over-all	min	max	over-all	min	max	over-all			
0.22	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.3911	-0.3079	425.2
temperature dependent[a]	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	0.6667	0.3798	11416.1

[a] value of k below 8°C calculated as: k=-7.412+0.954*temperature (°C) with a minimum of 0.0; see text for details.

Table 10 Effect of change in temperature on weight and on the relative contribution of cadmium in water, food and sediment to the body burden of loach, on the standard deviation about the regression of the difference between predicted and observed cadmium burden on time of sampling and the mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 $mg\ kg^{-1}$ dry weight and 10 $mg\ kg^{-1}$ dry weight respectively.

Change of temperature in comparison with observed data[a]	Percentage intake from water			food			sediment			Predicted final dry weight (mg)	Maximum predicted cadmium burden (ng)
	min	max	overall	min	max	overall	min	max	overall		
+ 2°	5.6	35.2	22.3	52.4	68.8	57.8	11.3	25.6	19.9	2153.2	464.6
+ 1°	2.0	35.3	22.0	53.1	70.6	58.3	10.7	27.5	19.7	1960.5	439.9
0°	0.0	35.2	21.7	53.8	71.5	58.9	10.4	29.4	19.4	1767.9	425.2
- 1°	0.0	35.6	21.9	54.1	71.7	59.2	10.3	31.6	18.9	1575.3	409.9
- 2°	0.0	34.0	22.3	54.3	71.8	59.4	10.3	35.5	18.3	1382.7	348.1

[a] observed data from the River Ecclesbourne (Douben, 1988c).

Consequently, the maximum predicted burden is higher and occurs later. During the mid-winter period there is clearly an effect of temperature on the value of the plateau: higher temperatures cause higher cadmium burden. In late spring, the cadmium load drops for temperatures at and above standard. There was little difference between these three predicted levels of cadmium at the end of the simulation period. Cadmium burden increases for temperatures below standard level at the end of the simulation period. However, given the overall tendency, it is likely that cadmium burden would drop with rise in temperature during the second summer.

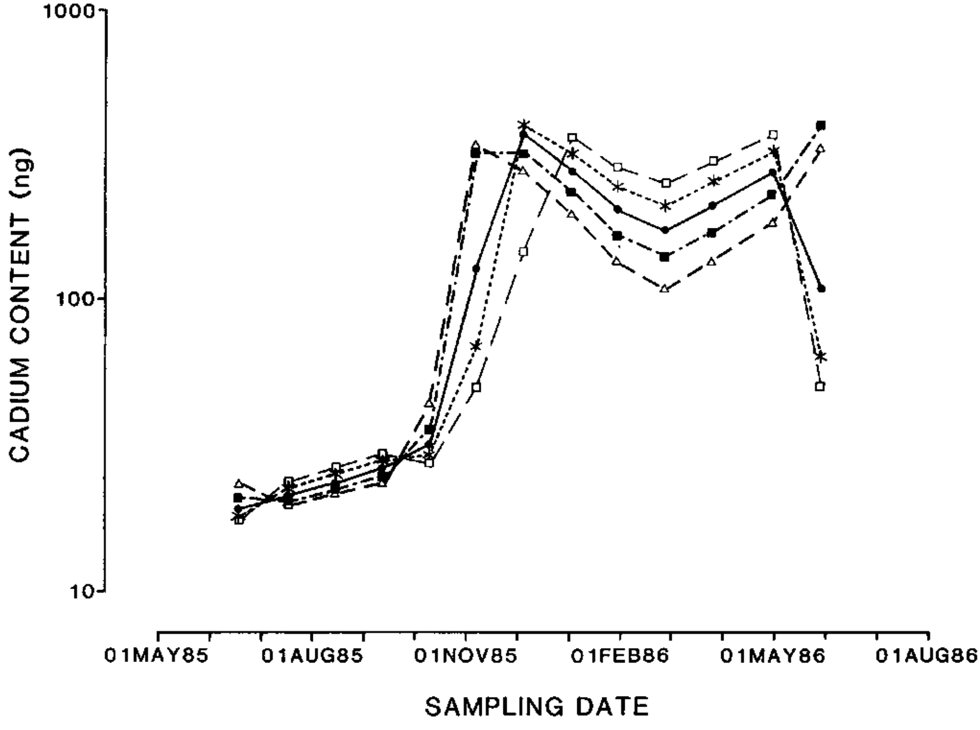

Fig. 3 Effect of change in temperature on predicted cadmium content (ng) of stone loach for standard conditions in the environment (see text for details): (●) observed set of temperatures in the River Ecclesbourne, (∗) all temperatures increased by 1°C, (□) all temperatures increased by 2°C, (■) all temperatures decreased by 1°C, (△) all temperatures decreased by 2°C.

Predictions from the model

Period of simulation

Analyses of the model was performed on the results for I+ fish apart from the comparison for the effect of body weight. Assuming that environmental conditions (e.g. temperature, concentration of cadmium in water, food and sediment) remain the same for another year, the simulation period covered two years continuously, using initial values of cadmium burden and dry weight for I+ fish only. Predicted results for dry weight were highly correlated ($P<0.001$) with observed results for I+ and II+ fish caught in the River Ecclesbourne (Douben, 1989b); 89% of the variation in predicted dry weight was explained by observed values. Predicted cadmium burden follows the trend as observed (fig. 4). The relative contribution of water, food and sediment are, as expected, between the results obtained for I+ and II+ fish separately (compare table 4 with table 11).

Predictions for different sites

Maximum and minimum measured concentrations of cadmiun in water, food and sediment from Brailsford Brook South and Suttonbrook (table 1) were used together with data for temperature at those sites (Douben, 1989b and c). Only I+ fish were considered. The predicted minima were always lower than the observed cadmium burden (fig. 5 a and b), and, in Suttonbrook, were usually lower than the predicted maxima. The observed cadmium burden in fish from Brailsford Brook South was higher than the predicted burden up to September 1985, but within the range of predicted minima and maxima. Thereafter, the rise in cadmium at the end of the autumn was predicted for both sites. The drop at the end of the simulation period occurs too late in the model for Brailsford Brook South and too early for Suttonbrook (fig. 5 a and b).

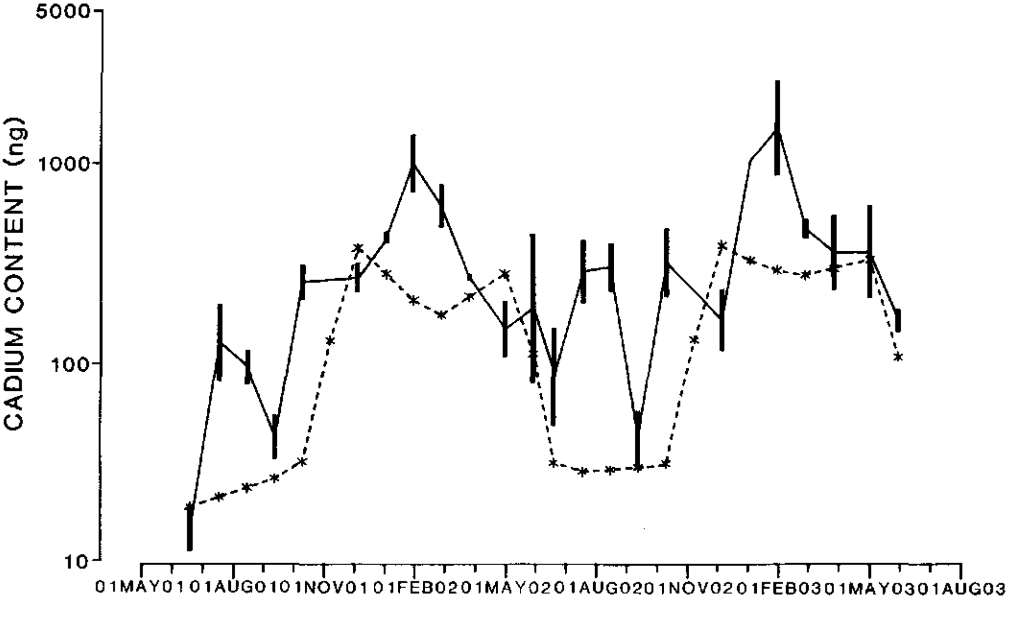

Fig. 4 Cadmium content (*) in stone loach, predicted continuously for two years, with concentration of cadmium in water, food and sediment of 1.0 µg l^{-1}, 10 mg kg^{-1} dry weight and 10 mg kg^{-1} dry weight respectively, and mean cadmium content (●) with standard errors of loach sampled at about 4-weekly intervals from the River Ecclesbourne, Derbyshire, for I+ and II+ fish in increasing order of age. For details see text.

Table 11 Predicted percentage intake of cadmium from water, food and sediment by stone loach during two years, starting with initial values for fish of one year old, and comparison of the regression of the difference between predicted and observed logarithmic burden of cadmium, the standard deviation about the regression (S_y), the mean difference between predicted and observed logarithmic cadmium burden. Concentrations of cadmium in water, food and sediment were 1 µg l^{-1}, 10 mg kg^{-1} dry weight and 10 mg kg^{-1} dry weight respectively.

Percentage intake from									Standard deviation about regression S_y	Mean difference between predicted and observed mean cadmium burden (ng)
water			food			sediment				
min	max	over-all	min	max	over-all	min	max	over-all		
0.0	35.2	15.5	53.8	71.5	60.1	10.4	31.7	24.4	0.4197	-0.3615

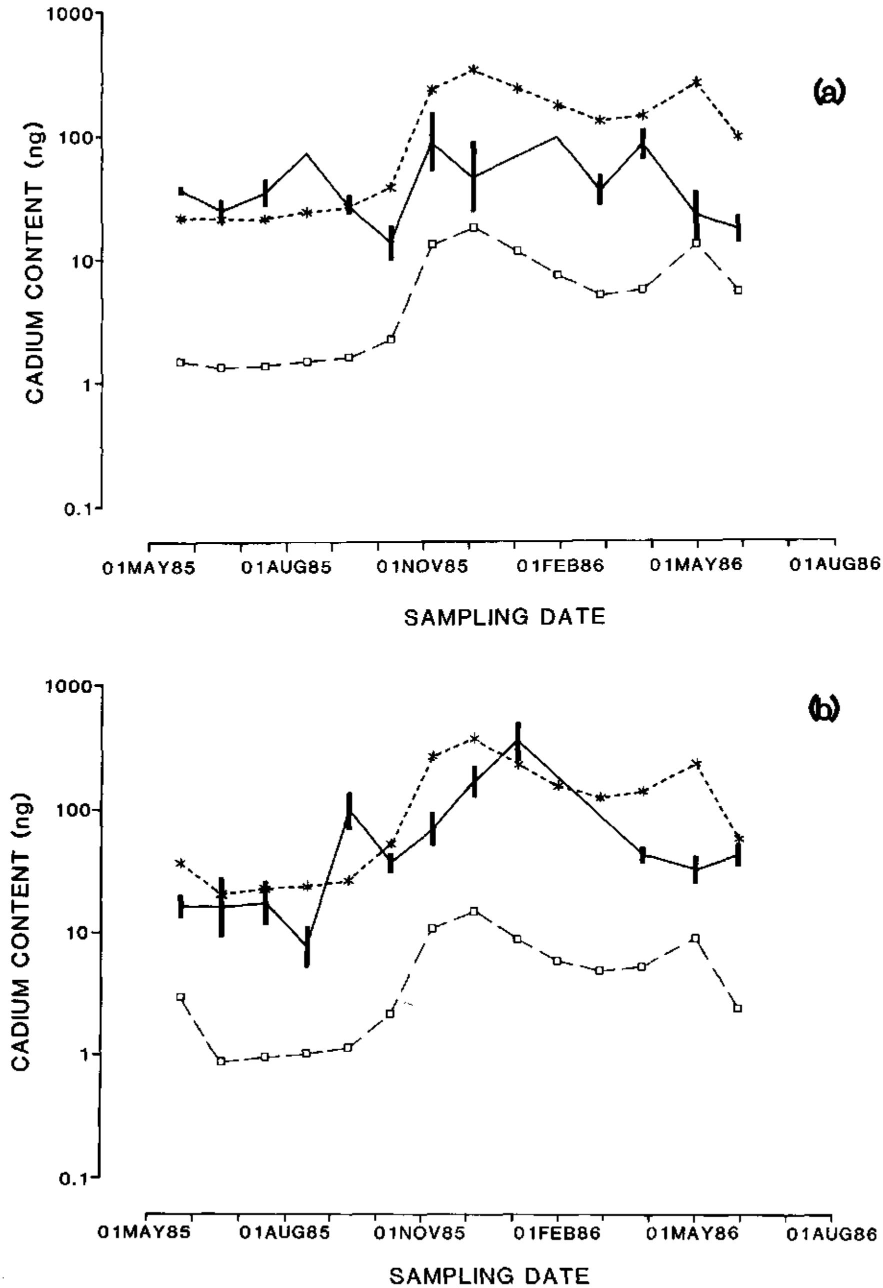

Fig. 5 Mean cadmium content (●), with standard errors, of stone loach in the agegroup I+, sampled at about 4-weekly intervals from two streams in Derbyshire. Values predicted with highest (*) and with lowest (□) measured concentrations of cadmium in water, food and sediment. (a) Brailsford Brook South and (b) Suttonbrook.

Prediction of weight of loach with the equation for growth rate derived from observations from the River Ecclesbourne and data for temperature from the respective sites, show that they are in good agreement with the observed weights (table 12a). The standard deviation about the regression for Brailsford Brook South fish is smallest. Using the same concentrations of cadmium in water, food and sediment (1 $\mu g\ l^{-1}$, 10 $mg\ kg^{-1}$ and 1 $mg\ kg^{-1}$ respectively) for all sites, then the results show that the relative contribution of these sources are comparable both as far as the range and as far as the overall percentages are concerned (table 12b). Predictions for Brailsford Brook South were closer to the observed data.

DISCUSSION

The approach, to construct the mathematical model to predict cadmium burden in stone loach, depends heavily on adequate information on both metabolism and pollutant dynamics and on the link between these two components. The predicted levels of cadmium indicated that these conditions were reasonably met: reproduction of trends follows cadmium burden of stone loach under field circumstances. Coupling of metabolism of the stone loach with pollutant dynamics helps to explain the relative importance of the different sources of cadmium.

The results of the predictions of weight of loach indicate that they are in good agreement with the observed weights in I+ fish from the three rivers, and in II+ fish from the River Ecclesbourne whilst too few data are available for 0+ fish (tables 3 and 12a).

Table 12 Comparison, for stone loach in the agegroup I+, of the regression of the difference between predicted and observed logarithmic (a) dry weight and (b) cadmium content on time of sampling from three sites in Derbyshire (for details see Douben, 1989b). Standard deviation about the regression line (S_y), the mean difference between predicted and observed mean dry weight (mg)/cadmium content (ng), the correlation coefficient between predicted and observed dry weight/cadmium content, and the relative contribution of water, food and sediment to intake of cadmium: minimum (min) and maximum (max) per day and overall covering the entire simulation period. Concentrations of cadmium in water, food and sediment were 1 $\mu g\ l^{-1}$, 10 $mg\ kg^{-1}$ and 1 $mg\ kg^{-1}$ dry weight respectively.

(a) dry weight

Site	Standard deviation about the regression S_y	Mean difference (mg)	Correlation coefficient	Number of observations
River Eccles-bourne	0.0855	0.1679	0.916***	12
Brailsford Brook South	0.0831	0.1689	0.871***	13
Sutton-brook	0.1094	0.2639	0.864***	12

(b) cadmium content

Site	Percentage intake from water min	water max	water over-all	food min	food max	food over-all	sediment min	sediment max	sediment over-all	Standard deviation about regression S_y	Mean difference between predicted and observed mean cadmium burden (ng)
River Eccles-bourne	0.0	38.9	26.3	60.0	96.2	71.3	1.1	4.0	2.4	0.4003	-0.3984
Brailsford Brook South	0.0	39.3	27.0	59.7	96.2	70.9	1.0	4.0	2.1	0.2999	0.2298
Sutton-brook	0.0	38.9	27.1	60.0	96.2	70.8	1.0	4.0	2.1	0.3817	0.1739

*** indicates significant correlation at the 0.001 probability level.

The premise that metabolism of the loach is an important factor in explaining its metal burden, seems to be justified by the similarities of predicted and observed cadmium burden. For example, the higher levels observed during the winter period were reasonably well predicted. Cadmium levels tended to be higher in bullhead (*Cottus gobio* L.) from the River Ecclesbourne in November than in those sampled in August even after allowing for differences in weight (Moriarty *et al.*, 1984). Similar observations were found in mussels (Amiard *et al.*, 1986). This phenomenon can be explained by the effect of temperature on rates of intake and loss: rates of intake are more affected than rates of loss. The rate constant for loss k was a function of temperature in this model in contrast to the model used by Norstrom *et al.* (1976), but in accordance with their suggestion. These results correspond with those found by Jimenez *et al.* (1987) for the bluegill sunfish (*Lepomis macrochirus*). Although the value of k appeared to be different during and after dietary exposure (Douben, 1989c), no provision was made to allow for this phenomenon in the model because no adequate information was available for the effect of the concentration of cadmium in the environment on k.

In field conditions, even in the unlikely event of a constant exposure, the intake of cadmium by the stone loach will be influenced by factors associated with metabolism such as change in temperature (fig. 3). This was demonstrated by all simulations particularly by the one run for two years. However, the high rate of loss of cadmium in May/June of the second year was observed later than predicted (fig. 4). Norstrom *et al.* (1976) concluded that estimates of pollutant concentrations in the environment were most prone to errors due to inadequate definition of seasonal variation. Despite the fact that the concentration of cadmium in samples of invertebrates tended to be higher during the autumn (fig. 1) and that the concentration of cadmium in water fluctuated due to flow rate and, perhaps, season (Douben, 1989d), predictions of cadmium in loach were in reasonable accordance with measured levels.

There were three possible routes of entry of cadmium: water, food and sediment. Water is more important for 0+ and I+ fish than for II+ fish. Food contributes substantially to the body burden of loach in all agegroups. There is still some controversy over the importance of food as a source of metal for fish (Kay, 1985; McCracken, 1987). Sediment as a source of metal is often overlooked; the results of other studies indicated that cadmium associated with sediment particles was taken up by aquatic organisms, including the stone loach (Douben and Koeman, 1989; Gillespie, 1972; Luoma, 1983), possibly through ingestion of sediment (Tessier and Campbell, 1987). The results of the predictions in this paper demonstrate that cadmium in loach originates partly from sediment; its importance increases with age (weight) of loach (table 4). The problem of uptake from sediment particles directly or from interstitial water is outside the scope of this paper. There is increasing evidence that differences in behaviour affect routes of entry of metals in general into fish (Ney and Van Hassel, 1983).

The link between metabolism and cadmium uptake from sediment was the same as between metabolism and uptake from water. From equation 5 it can be deduced that changing the link into the dietary equivalent, reduces the intake of cadmium from sediment, despite the increase of the coefficient for growth rate into +1, because $1/E_r$ (which is equal to 1/0.70) is replaced by $1/(E_{ox}*C_{ox}*Q_{ox})$ $(1/0.0026*C_{ox})$ while C_{ox} ranges from 14.45 to 8.71 mg l^{-1}. The results in table 8 quantify the end result.

Comparison of the cadmium burden in loach in the agegroup I+ from different sites indicates that there is great similarity between the predicted cadmium burdens in loach with maximum and minimum concentration of cadmium in water, food and sediment as far as trend is concerned as well as levels in fish from Suttonbrook and Brailsford Brook South. For the same concentrations of cadmium in the environment, the relative contribution of cadmium intake from the sediment is slightly higher for the predictions for the River Ecclesbourne in comparison with other streams due to higher temperatures during the summer which increased growth rate; predicted final weights were 1767.9, 1709.5 and 1654.2 mg dry weight for loach from the River Ecclesbourne, Brailsford Brook South and Suttonbrook respectively. DeFreitas *et al.* (1974) concluded that the major route of entry of methylmercury in pike (*Esox lucius*) differed with location. Given that the concentration of cadmium in sediment is substantially higher in samples from River Ecclesbourne than in those from Brailsford Brook South and Suttonbrook, this results in a higher percentage uptake of cadmium from the sediment. To some extent the same argument applies to food. This clearly demonstrates that conclusions about the origin of cadmium in fish depend on environmental conditions.

CONCLUSIONS

The mathematical model described in this paper couples metabolism of the stone loach with uptake and loss of cadmium in the fish. It predicts levels of cadmium in the fish with a high degree of realism. Metabolism affects cadmium burden. Cadmium is taken up from water, food and sediment at different rates. The relative importance of these sources is affected by body size. Temperature has a major influence on the cadmium burden.

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PART V SUMMARY AND CONCLUSIONS

Chapter 8 : Summary and concluding remarks

SUMMARY AND CONCLUDING REMARKS

This thesis is concerned with exposure of stone loach (*Noemacheilus barbatulus* L.), a fairly common fish in British rivers, to cadmium and lead. The work described in this thesis was performed to assess the relative importance of different routes of entry (water, food and sediment) on the body burden of cadmium and lead in stone loach under field conditions. These studies were based on the premise that metabolic rate of stone loach is an important factor affecting metal burden. Metabolic rate in fish is dependant on factors which include body weight, temperature, and physiological condition. Therefore, a wide weight range of fish was deliberately used in this study, to obtain information on the effect of body weight, in contrast to common practice in laboratory studies where uniformity of body weight is used to reduce the variation in effect on uptake of pollutants.

This thesis is divided into the following parts:

- introduction (part I);
- laboratory studies (part II);
- field studies (part III);
- mathematical modelling (part IV);

Part I of this thesis describes where lead and cadmium are present in the environment and the effect of human activities on distribution of the metals. This distribution was studied in areas in Derbyshire (UK), where one site (the River Ecclesbourne with mineral veins at the head of the valley), had higher metal levels in sediment and water than two other sites (both with 'background' levels of metals). In general, uptake of cadmium and lead by fish may occur via three pathways, but there is no consensus over their relative importance, not even within one species used in different studies. The literature is reviewed on environmental fate and effects on organisms of cadmium and lead. (chapter 1).

Part II (laboratory experiments) describes the laboratory experiments which were aimed at obtaining quantitative information on rates of uptake and loss of cadmium from three possible sources. Chapter 2 reports exposure to water-borne cadmium by the stone loach at different temperatures and over a range of cadmium concentrations. Rates of uptake and loss of cadmium were found to be affected by size of the fish and to increase with temperature. There was some evidence that cadmium burden of the loach reached a maximum after exposure around 16 °C. Fed fish appeared to have a higher rate of cadmium uptake from the water than had starved fish. Published rates of loss after exposure to cadmium cannot be readily compared because duration of exposure may affect rates at which a fish gets rid of cadmium.

Metabolic rate was estimated by the oxygen consumption rate. Two levels were distinguished: a low 'routine' level, when the fish were at rest during light, and a higher 'active' level, when fish were swimming, which was observed for a few hours after the onset of darkness. The difference between these two levels narrowed towards the upper end of the temperature range used (6 - 18 °C), suggesting that loach became stressed at high temperatures. A similar effect of temperature on rate of cadmium uptake from *Tubifex*, used as food items, into fish was found. Absorption efficiency of cadmium from food by the loach decreased with increasing cadmium concentration in the food; also, in spite of increased food consumption at higher temperatures, cadmium burden of stone loach did not rise in proportion because of reduced absorption efficiency of the metal. (chapter 3).

Exposure of stone loach to sediment with levels of cadmium and lead higher than 'background' increased body burdens of both metals (chapter 4). High rates of loss resulted in the rapid development of a steady state. The biological half-life for cadmium was longer than for lead in these experiments.

The field studies (part III) were carried out to provide information on the growth rate of stone loach, fluctuations in the fishes' body burden and in concentrations of cadmium and lead in the environment (water, food and sediment) in relation to fluctuations in temperature with season. These field studies included a one-year sampling programme of stone loach in Derbyshire (UK) (chapter 5). The selected streams were the River Ecclesbourne, Brailsford Brook South and Suttonbrook. Growth rate of fish, which depended on temperature and age, was greatest during the spring and summer and was highest in young loach. Fish from all streams had reached their maximum length (about 110 mm) after two years and also their maximum cadmium burden. Differences in body size accounted for most of the variation in cadmium levels between loach of different age groups, but was less important for lead levels; cadmium burden fluctuated more than lead burden. Loach caught in the River Ecclesbourne had higher body burdens of both cadmium and lead; no significant differences were measured between the Brailsford Brook South and Suttonbrook.

Water samples were taken from all three streams at four periods and on several occasions during each period (chapter 6). Most of the cadmium, in contrast to lead, was in solution. Flow rate of the water appeared to have a greater effect on the cadmium concentration than on the lead concentration.

There was some evidence for seasonal fluctuations: metal concentrations were higher in the early spring than in the autumn.

Kick-samples from the streams were examined for the range of invertebrate species. Remains of most of these species were found in the gut contents of loach, which suggests that loach are not very selective feeders on invertebrates. Cadmium levels in invertebrates showed seasonal variation: lowest levels were measured in samples taken during the spring and highest levels in those sampled during the autumn. (chapter 7).

Sediment samples were taken at about 4-weekly intervals from the same sites, at the same time, as the fish. Concentrations of lead and cadmium were higher in samples from the River Ecclesbourne. Although concentrations of both metals fluctuated with sampling time, there was no consistent trend at any of the sites. (chapter 7).

Part IV describes the development of a mathematical model which incorporates both laboratory and field studies (chapter 7). This model predicted adequately the range of cadmium levels found in stone loach in the field. Stone loach take up cadmium from water, food and sediment. The relative importance of these sources differed between the three sites because of differences in measured concentrations. For the R. Ecclesbourne, younger fish took up relatively more cadmium from water than did older fish; the latter received relatively more cadmium from sediment. The model predicted that consistent lower temperatures caused the metal burden to be lower during the winter period and consistent higher temperatures had the opposite effect.

The results of the work presented clearly show the effect of body size on metabolic rate. The model that relates body weight (W) of fish to a dependant variable (M), e.g. cadmium burden, includes an exponent (y) as follows:

$$M = \alpha * W^{y}$$

The values of the exponent y were comparable for respiration rate, food consumption on different rations and for cadmium burden during and after dietary exposure, during exposure to sediment and in field data. However, the value obtained from water-borne studies was lower. This suggested that, for similar values of α, water was less important as a source of cadmium than food and sediment; comparison of observed cadmium burdens during exposure in the laboratory with field data confirmed this. The values of the exponent for the lead burden obtained from field data and exposure to sediment, were lower compared to the value derived from respiration studies. This suggests that uptake and loss of lead is less affected by metabolic processes than is cadmium. Body burden of lead on exposure to sediment was comparable with that in loach from the River Ecclesbourne, suggesting that most of the lead is taken up from the sediment.

Cadmium burden is higher during the winter than during the summer. This is caused by the greater effect of temperature on rate of intake than on rate of loss. The relative contribution of cadmium in water, food and sediment to cadmium burden of loach depends on environmental conditions, such as cadmium concentration in these sources, temperature and body weight. There is virtually no uptake of cadmium from water during the winter; most cadmium originates at this time from food. Particles with which most of the lead is associated and which are a prime source of lead for stone loach, are brought into suspension at higher flow rates and, therefore, exposure increased.

The data in this study, compared with the published literature, suggest that the fish's handling of cadmium depends on the duration of exposure; after short-term exposure, the fish eliminate cadmium rapidly while, after long-term exposure, cadmium is not easily lost. This phenomenon may be explained by the time of exposure required for the induction of cadmium-binding proteins. There is also some evidence that the type of exposure affects the rates of loss, presumably in parallel with the fate of cadmium within the fish. Based on a one-compartment model, the cadmium derived from food seems to remain longer in the body than cadmium originating from water and sediment. There is evidence that stone loach get rid of cadmium rapidly after short term exposure to both dietary and water-borne cadmium. This corroborates the observed fluctuations of cadmium burden in stone loach from the field.

The importance of different routes of uptake of both lead and cadmium differs between fish species. Water is often regarded as the sole source of metal although there is evidence that diet can contribute significantly to the body burden of fish. Bottom feeders, in particular, seem able to acquire some metal from sediment. Moreover, concentrations in the different environmental compartments determine their relative contributions to the body burden.

Chapter 9: Samenvatting en slotbeschouwingen

SAMENVATTING EN SLOTBESCHOUWINGEN

Dit proefschrift behandelt de blootstelling aan cadmium en lood door het bermpje (*Noemacheilus barbatulus* L.), een algemeen voorkomende vis in Britse rivieren. Het werk dat hierin beschreven is had tot doel de relatieve bijdrage te bepalen van diverse opname routes (water, voedsel en sediment) aan de belasting van het bermpje onder veldomstandigheden. Het studie was gebaseerd op de veronderstelling dat het metabolisme van het bermpje een belangrijke factor is die de belasting aan zware metalen beinvloedt. Het metabolisme van vissen is ondermeer afhankelijk van het gewicht van de vis, temperatuur en fysiologische conditie. Derhalve werden vissen met verschillende gewichten gebruikt om inzicht te krijgen over het effect van het lichaamsgewicht, dit in tegenstelling tot hetgeen over het algemeen toegepast wordt in laboratorium experimenten, waar gestreefd wordt naar uniformiteit van gewicht, zulks om variatie in het effekt van opname van contaminanten te minimaliseren.

Dit proefschrift is opgesplitst in de volgende delen:
- introduktie (deel I);
- laboratorium proeven (deel II);
- veld onderzoek (deel III);
- modelbouw (deel IV);

Het eerste deel van dit proefschrift behandelt waar lood en cadmium aanwezig zijn in het milieu en de gevolgen van humane aktiviteiten op de verspreiding van deze metalen. Deze verspreiding is bestudeerd in Derbyshire (Groot Brittannië), waar een rivier (de Ecclesbourne welke ontspringt waar hoge concentraties mineralen in de bodem aanwezig zijn) hogere concentraties van de metalen in sediment en water had, in vergelijking met twee andere rivieren (beide met 'achtergronds' concentraties). In principe kunnen vissen lood en cadmium via drie routes opnemen, maar er bestaat geen éénluidende conclusie over de relatieve belangrijkheid van deze routes, zelfs niet voor dezelfde vissoort. Een overzicht van de relevante literatuur over het lotgeval van lood en cadmium in het milieu, beschikbaarheid voor aquatische organismen, alsmede mogelijke effekten is aangegeven. (hoofdstuk 1).

Deel II beschrijft de laboratorium proeven die gericht waren op het verschaffen van kwantitatieve gegevens over opname- en eliminatie snelheid van cadmium via genoemde routes. Hoofdstuk 2 beschrijft de blootstelling van het bermpje aan cadmium in het water bij verschillende temperaturen en concentraties. Opname en eliminatie snelheden werden beinvloed door gewicht van de vis; verhoging van temperature vergrootte deze snelheden. Er waren aanwijzigingen dat de grootste cadmium belasting in het bermpje plaatsvond na blootstelling bij 16 °C. Ook bleek dat vissen die gevoerd waren tijdens de proef een hogere opname snelheid hadden dan niet gehonderde vissen. Vergelijking van deze resultaten met die in de literatuur is niet zondermeer mogelijk omdat tijdsduur van blootstelling de eliminatie van cadmium kan beinvloeden.

Metabolisme van de vis was gemeten door middel van zuurstof consumptie. Twee niveaus werden onderscheiden: een laag 'routine' niveau wanneer de vissen merendeels rustten, en een hoger 'aktief' niveau, wanneer de vissen zwommen, hetgeen aan het begin van een donker periode werd waargenomen. Het verschil tussen deze niveaus werd kleiner naarmate de temperature toenam (6 - 18 °C bestudeerd), hetgeen suggereerd dat de vissen onderhevig waren aan stress. Temperatuur had een soortgelijk effekt op de consumptie van *Tubifex* (gebruikt als voedsel) door de vissen. Opname efficiëntie van cadmium vanuit het voedsel nam af met toename van de cadmium concentratie in het voedsel. Ondanks een hogere voedsel consumptie bij hogere temperatuur nam de hoeveelheid cadmium in de vis niet in dezelfde mate toe vanwege lagere absorptie efficiëntie. (hoodstuk 3).

Blootstelling van het bermpje aan sediment met hogere dan 'achtergronds' concentraties cadmium en lood leidde tot een hogere belasting van de vis (hoofdstuk 4). Een evenwichtstoestand werd snel bereikt vanwege hoge eliminatiesnelheden. De biologische halfwaarde tijd van cadmium onder de gebruikte omstandigheden leek langer.

Deel III beschrijft het veldonderzoek dat uitgevoerd werd om gegevens te verzamelen over groeisnelheid van het bermpje, fluctuaties in het gehalte lood en cadmium in de vis, water, voedsel en sediment in relatie tot temperatuursschommelingen met de jaargetijden. Dit veldonderzoek omvatte ondermeer een één-jaar monster programma van bermpjes in Derbyshire (Groot Brittannië)) (hoofdstuk 5). De bemonsterde rivieren waren de Ecclesbourne, Brailsford Brook South en de Suttonbrook. Groeisnelheid van de vis (afhankelijk van temperatuur en leeftijd) was het grootste gedurende de lente en zomer en het hoogste in jonge bermpjes. Vissen van twee jaar oud hadden hun maximale lengte bereikt (ongeveer 11 cm) alsmede het maximale cadmium gehalte (zulks afhankelijk van de rivier). Het grootste deel van de variatie in cadmium belasting was te wijten aan verschillen in gewicht; dit gold niet voor lood. Belasting van cadmium in de vis schommelde meer die van lood. Bermpjes gevangen in de Ecclesbourne hadden een hogere cadmium en lood belasting; geen verschillen konden worden aangetoond voor de Brailsford Brook South en de Suttonbrook.

Gedurende vier periodes en op verschillende dagen binnen elke periode werden water monsters genomen (hoofdstuk 6). Bijna al het cadmium was in oplossing in tegenstelling tot lood. Stroomsnelheid bleek een groter effekt te hebben op de concentratie cadmium dan op de lood concentratie. Er bleken ook seizoensverschillen op te treden: hoge concentraties werden gevonden in het voorjaar in vergelijking met de herfst.

Macrofauna van de rivieren werd onderzocht. Restanten van het merendeel van deze soorten werden aangetroffen in het maag-darmkanaal van het bermpje. Cadmium concentraties in de macrofauna waren lager in het voorjaar dan in de herfst. (hoofdstuk 7).

Monsters van het sediment werden op dezelfde tijdstippen (onm de vier weken) genomen als de vissen. Concentraties lood en cadmium waren hoger in monsters van de Ecclesbourne. Ofschoon er fluctuaties optraden, was er geen consistente trend voor alle monsterpunten (hoofdstuk 7).

In deel IV wordt het mathematisch model beschreven dat gebaseerd was op de resultaten van de laboratorium en veldstudies (hoofdstuk 7). Het model voorspelde de range in cadmium in het bermpje bevredigend. Het bermpje neemt cadmium op vanuit het water, voedsel en sediment. De relatieve belangrijkheid van deze bronnen was afhankelijk van de rivier vanwege de gemeten concentraties aldaar. Voor de Ecclesbourne werd aangetoond dat jonge vissen relatief meer cadmium opnemen van het water dan oudere vissen; deze laatste namen relatief gezien meer op vanuit het sediment. Het model voorspelde dat een constante lagere temperatuur resulteerde in een lager cadmium gehalte in de vis gedurende de winter; een constant hogere temperatuur had het omgekeerde effekt.

De studies die hierin beschreven zijn geven het effekt van lichaamsgewicht duidelijk aan. Het model dat de relatie legt tussen lichaamsgewicht van de vis (W) en een afhankelijke variabele (M), bijvoorbeeld het cadmium gehalte in de vis, omvat een exponent (y) en wel als volgt:

$$M = \alpha * W^{y}$$

De waarde van de exponent y waren vergelijkbaar voor zuurstof verbruik, voedsel consumptie (zelfs bij verschillende rantsoenen) en cadmium belasting gedurende en na blootstelling via het voedsel, gedurende blootstelling aan sediment en in de resultaten van het veldonderzoek. Echter een lagere waarde werd gevonden voor blootstelling aan cadmium in het water. Dit suggereert dat, voor vergelijkbare waarden van α, het water minder belangrijk was dan het voedsel en het sediment als een bron van cadmium. Dit werd bevestigd door vergelijking van de waargenomen cadmium belasting gedurende laboratorium proeven en veld gegevens. De waarde van de exponent voor lood gehalte zoals bleek uit blootstelling aan sediment in het laboratorium en veldgegevens, waren lager dan de waarde voor zuurstof verbruik. Dit suggereert dat opname en eliminatie van lood in mindere mate van metabolisme afhankelijk is dan cadmium. Loodgehalte in het bermpje na blootstelling aan het sediment was vergelijkbaar met dat in bermpjes van de Ecclesbourne, hetgeen duidt op het feit dat het merendeel van het lood afkomstig is van het sediment.

Cadmium belasting is hoger in de winter dan in de zomer. Dit wordt veroorzaakt door het grotere effekt van temperatuur op de opname snelheid dan het effekt op de eliminatie snelheid. De relatieve bijdrage van cadmium in water, voedsel en sediment in het bermpje wordt beinvloedt door milieu omstandigheden zoals de cadmium concentratie in deze bronnen, temperatuur en lichaamsgewicht. De studie geeft aan dat er vrijwel geen cadmium wordt opgenomen vanuit het water gedurende de winter; het merendeel van de opgenomen cadmium komt dan uit het voedsel. Deeltjes waaraan het lood geassocieerd is en welke de belangrijkste bron van lood zijn voor het bermpje, worden in suspensie gebracht bij hogere stroomsnelheden: de blootstelling is dan groter.

De gegevens in deze studie, vergeleken met de literatuur, suggereren dat de wijze waarop de vis omgaat met cadmium afhangt van de periode van blootstelling; na acute expositie raakt de vis cadmium snel kwijt, terwijl na chronische blootstelling cadmium langer in de vis blijft. Dit kan mogelijk verklaard worden door de tijd die nodig is voor de induktie van eiwitten die cadmium binden. Er zijn ook aanwijzigingen dat het type blootstelling de eliminatie snelheid beinvloedt, waarschijnlijk zoals het lotgeval van cadmium in de vis daardoor beinvloed wordt. Cadmium opgenomen vanuit het voedsel schijnt langer in de vis te blijven dan cadmium opgenomen vanuit het water en sediment hetgeen gebaseerd is op het gebruik van een één-compartiment model. Het bermpje elimineert cadmium snel na acute blootstelling aan cadmium in voedsel en water. Dit bevestigt de waargenomen fluctuaties in het bermpje van het veldonderzoek.

De belangrijkheid van de verschillende opname routes van cadmium en lood hangt van de vissoort af. Het water wordt vaak als de enige bron van deze metalen beschouwt, terwijl er aanwijzingen zijn, ook in de literatuur, dat het voedsel een belangrijke bijdrage kan leveren aan de metal belasting van vissen. Met name vissen die op de bodem van de rivier leven, zijn in staat metalen op te nemen vanuit het sediment. Bovendien bepalen de concentraties van zware metalen in de diverse milieu componenten hun relatieve bijdrage aan het gehalte in vissen.

CURRICULUM VITAE

Peter E.T. Douben werd geboren te Belfeld op 1 november 1956. In 1975 behaalde hij het diploma Gymnasium ß aan het St. Thomascollege te Venlo. Dat zelfde jaar startte hij met zijn studie biologie aan de toenmalige Landbouwhogeschool (LH) (nu Landbouwuniversiteit) te Wageningen. In 1983 behaalde hij het ingenieursdiploma met als hoofdvakken Toxicologie (prof. dr. J.H. Koeman) en Hydrobiologie (prof. dr. C. den Hartog).

Gedurende enige tijd was hij verbonden aan de Middelbare Land- en Tuinbouwschool te Breda en aan de afdeling Onderwijs, Wetenschap en Planning van de LH. In 1984 was hij werkzaam bij de vakgroep Toxicologie in een projekt van de Wereld Gezondheidsorganisatie (WHO) in Togo (West-Afrika).

Ondersteund door een fellowship van de EEG was hij sinds 1985 verbonden aan Monks Wood Experimental Station (NERC's Institute of Terrestrial Ecology) te Huntingdon, Groot Brittannië. Het onderzoek voor dit proefschrift is aldaar uitgevoerd.