Mercury accumulation by the eelpout (*Zoarces viviparus* L.) in the Forth Estuary, Scotland

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To Mum and Dad,
for your continuing love and support

Declaration

I hereby declare that the work presented in this dissertation is my own original work, excepting where acknowledgement has been made of assistance received.

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Abstract

The Forth Estuary, in eastern Scotland, has received inputs of the toxic metal, mercury, from an industrial discharge for many decades. This has led previously to accumulation of relatively high levels of mercury in the environment and biota of the estuary, although both inputs and levels in biota have fallen sharply since the early 1980s. The general literature on mercury in the marine environment is reviewed, including the human health implications of mercury in aquatic food chains. A review is also presented of current knowledge of mercury in the Forth Estuary environment and biota, as carried out by the statutory water quality authority, the Forth River Purification Board (FRPB) and others.

Studies by the FRPB have indicated that a resident teleost fish species, the eelpout (*Zoarces viviparus* L.) may be a suitable candidate for use as a bioaccumulation monitoring species for mercury in the Forth. Its potential and demonstrated use in other pollution studies in North Sea and Baltic waters are reviewed. In order to investigate the use of this species for bioaccumulation monitoring of mercury, the variability of mercury concentrations in a number of tissues was investigated, in relation to biological, temporal (both seasonal and annual) and spatial factors, during the period November 1989 to March 1992. Although skeletal muscle and liver were investigated in most detail, kidney, testes, ovary, and tissues of the viviparous brood were also studied. Mean mercury concentrations in all tissues were relatively low, although skeletal muscle, liver and kidney tended to have higher values than the reproductive tissues.

The log of mercury concentration in skeletal muscle increased in a statistically significant linear fashion with length, weight and age of the fish, in almost all samples. There was no statistically significant difference in the rate of increase (i.e. the slope of the linear regression of log concentration on length) between the sexes. There was a clear seasonal pattern in the rate of increase of mercury concentration with length in both years, with highest values in the Spring period, March to May, followed by a sharp decrease in the rate in Summer, June to August. Differences between seasons were not, however, statistically significant in either year. The rate was negatively correlated with the allometric condition factor. The total mass of mercury in the skeletal muscle of a standard fish showed little significant variation between seasons, although there was considerable seasonal difference in total muscle mass. It is suggested that seasonal variability of mercury levels in eelpout skeletal muscle in the Forth Estuary is related principally to seasonal changes in skeletal muscle mass, rather than to changes in mercury burden.

Both liver and testes showed a seasonal peak in relative weight (somatic index) in Summer months, with a seasonal minimum mercury concentration in the same period. There was little difference between seasons in the total mercury burdens of these organs, with respect to fish size. Thus, the seasonal variability of mercury concentrations in the liver and testes appears to be due to seasonal growth and regression patterns. Mercury concentrations and burdens of the

female reproductive tissues were studied in relation to the viviparous reproduction of the eelpout. The mercury concentration of the intra-ovarian brood is significantly correlated with that of the maternal skeletal muscle. The increase of brood mercury burden through the brood development period was slow initially, followed by a rapid increase which was closely related to a period of rapid increase in brood weight. The distribution of mercury in the ovary was described, and a high concentration of mercury was found in a cellular fraction of the ovarian fluid, relative to the supernatant fluid. Larval eelpout are known to consume this cellular fraction, including red blood cells, which is released from the inner wall of the ovary. It is possible that this matrotrophic form of nutrition may provide the main route for accumulation of mercury, at least in older larvae.

Uptake, internal dynamics and elimination of methylmercury by eelpout was investigated under laboratory conditions by the administration of ^{203}Hg-labelled methylmercuric chloride, by oral dosing or intra-peritoneal (i.p.) injection. Patterns of tissue redistribution of mercury with time suggest that the dynamics of i.p. administered methylmercury are related closely to the structure and biological functions of the tissues. The transfer of a portion of an i.p. administered dose of methylmercury, from the peritoneum to the intra-ovarian tissues of the eelpout (including brood and ovarian fluid), was demonstrated for the first time in a limited investigation of mercury transfer between generations.

Elimination of orally administered methylmercury, measured by whole body counting of live fish, was initially rapid, with 20% of the dose lost over two days, with a slower loss of a further 20% over the following two weeks. The initial period of rapid loss coincides with the loss of mercury from the intestine of i.p. dosed fish, suggesting a two compartment loss. More detailed analysis of biological half-times of elimination was not permitted by the restricted duration of the experiment.

The literature and concepts of trend monitoring and Environmental Capacity for mercury in the marine environment were reviewed. Linking of archive data, collected by the FRPB between 1978 and 1988, with results from the present study, allowed an analysis of temporal trends of mercury in Forth Estuary eelpout. There has been a clear trend of decreasing mercury concentrations in eelpout from the Forth Estuary since the early 1980's, although levels in fish from the Firth of Forth, where environmental mercury levels are lower, have remained relatively unchanged. In the early 1980's, mean concentrations in fish from the site closest to the main mercury discharge were in excess of 0.3 mg.kg^{-1}, the acceptable limit established by the European Community Environmental Quality Standard (EQS) for mercury in fish muscle. Mercury concentrations in eelpout from the Firth were lower in than those from the Estuary in all years. Using this data, an *a posteriori* estimation was made, of the Environmental Capacity of two Forth Estuary sites to receive mercury inputs. The meeting of the EQS was taken as the acceptable 'endpoint' of the calculation. The estimated values of Environmental Capacity, of 1858 kg per year at the site closest to the discharge would now be in excess of the currently permitted inputs of mercury to the Forth Estuary.

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"We had good treatment there, met charming people, did good and bad things,and left with reluctance". John Steinbeck and Ed Ricketts (1941), from *Log from the Sea of Cortez*

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Chapter 2 Variability of mercury in tissues of the eelpout, *Zoarces viviparus* (L.), from the Forth Estuary and Firth of Forth, Scotland

" Generally held to be coarse and eaten only by the poor, but Lowe says it tastes much better and fatter than other Blennies. Neill states it is often brought to the Edinburgh market. At St. Andrew's, a most intelligent mechanic informed me that no better fish could be obtained. "

F. Day (1884). *The Fishes of Great Britain and Ireland*, Vol. 1., on *Zoarces viviparus*

Chapter 1 Mercury in the Marine Environment: A review

1.1 Properties and Uses of Mercury

1.1.1 Introduction

Marine pollution has been defined by the Food and Agriculture Organisation of the United Nations as: "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities" (FAO, 1984).

Mercury, as such a substance, has attracted much attention in recent years. The toxicity of mercury to humans has been known for centuries, the first recorded case of poisoning being of a mercury miner in the 15th Century (Takizawa, 1979). Mercury is probably the only contaminant introduced by man into the marine environment which has certainly been responsible for human fatalities (Clark, 1986). The most significant and well-known incidents occurred in Japan at Minimata (1953 - 1960), and Niigata in 1965 (Takizawa, 1979).

The first incident involved the onset of a strange disease with peculiar neurological symptoms in the population of a fishing village in Minimata Bay. On investigation, the condition proved to be a classical case of methylmercury poisoning. Methylmercury is more toxic to humans than the inorganic form because it cannot be excreted easily. Methylmercury passes into the brain causing progressive and irreversible brain damage (Clark, 1986).

The disease was linked with the occurrence of methylmercury in the discharges from an acetaldehyde plant, manifesting itself via a food-chain of grossly contaminated fish and shellfish consumed in large quantities by the fishing community of Minimata (Takizawa, 1979). A total of around 2200 cases were recorded. In the long term, there were 750 fatalities, with many survivors suffering severe permanent disabilities. The second incident, resulting in 55 fatalities, at Niigata, near the mouth of the Agamo river, was also caused by the gross contamination of fish by mercury from an industrial effluent, in this case from a factory 60 km upstream (Clark, 1986).

One outcome of the Minimata disaster has been a greater appreciation of the risk of mercury poisoning from eating contaminated seafood. This led initially to the introduction of legal standards setting maximum levels for mercury in food offered for sale. For example, in the USA and Canada, the maximum permitted mercury level in seafood is 0.5 $\mu g\ g^{-1}$ fresh weight , while in Japan the standard is 1.0 $\mu g\ g^{-1}$ f.w. (Nauen, 1983). The risk to human health of dietary exposure to mercury is discussed more fully in section 1.4.

Mercury and its compounds are included in the "Black List" of the Paris Convention on Dumping of Wastes from land-based sources, and by the "Dangerous Substances" Directive of the European Community. This is a list of substances which should be banned or substantially reduced from discharges (McLusky, 1989). More recently, mercury was included in a 'Red List' of substances whose control, and subsequent elimination from discharges, is seen as a priority, adopted by the UK and other North Sea nations in 1987 (Anon., 1990).

1.1.2 Natural Mercury Inputs to the Environment

A 'soft-acid' transition metal, mercury (Atomic Number 80; Atomic Weight, 200) is a silver-white liquid at ambient temperatures (Andren and Nriagu, 1979). It occurs naturally in the Earth's crust in three major forms: sulphides (primarily cinnibar, HgS), oxides, and elemental mercury. Sulphide minerals at the Earth's surface are subjected to weathering reactions, mainly promoted by hydrogen ion activities in precipitation, surface and ground waters. These weathering processes all lead to mobilisation of bivalent mercuric (Hg^{2+}) ions into surface and ground water, representing the beginning of one of the two main routes of mercury transport to the oceans, the other being atmospheric transport (FAO, 1984).

It is estimated that about 3500 tonnes of mercury per year are derived from weathering of mercury-containing rocks, while between 25,000 and 150,000 tonnes per year are released to the atmosphere as gases from volcanic areas, geothermal vents, and other sources of degassing of Earth's crust. No accurate estimates are available regarding the input of mercury from the atmosphere to the oceans, although it is may be significant as water concentrations in open oceans vary between 0.5 and 5 $\mu g.l^{-1}$ (Bruland, 1983).

1.1.3 Human Uses of Mercury

The widespread industrial and agricultural applications of mercury and its compounds result from the unusual physico-chemical properties of these materials. For example, mercury's liquidity at normal temperatures, along with its high surface tension, uniform volume expansion and inability to wet and cling to glass have combined to make mercury useful for barometers, thermometers, manometers and other measuring devices. Mercury's use in the industrial recovery of metals exploits its ability to form amalgams with many metals. The toxicity of mercury and its compounds has led to their widespread use as bactericides, fungicides and insecticides (especially in agriculture), and in a wide variety of pharmaceuticals (Nriagu, 1979).

Since the 16th Century, when mercury was principally used in medicine and paints, the

number of applications has grown dramatically and is currently believed to be in excess of 3000. In recent years, the two principal uses of mercury, accounting for over 50% of the total consumed, have been for electrical apparatus and in the production of caustic soda and chlorine by the chlor–alkali process (Nriagu, 1979).

The chlor–alkali process involves the simultaneous production of chlorine and caustic soda by the electrolysis of brine solutions using a flowing cathode of metallic mercury. The sodium which amalgamates with mercury at the cathode is converted to NaOH with water and the mercury which is liberated is recycled in the cell (Nriagu, 1979). In actual fact, it is common with this process for 150–200g. of mercury to be lost to the atmosphere and in waste waters for each tonne of product (Clark, 1986). Because of pressure from environmental groups, many countries have now passed 'Chloralkali Mercury Regulations' aimed at minimising the release of mercury from chloralkali plants (Nriagu, 1979). Many older plants, however, have difficulty in reducing the mercury content in their effluent below 8 $\mu g.ml^{-1}$ (Clark, 1986).

The growth in use of mercury in the electrical industries has remained steady with these industries being the leading consumers of mercury in the USA and Canada in 1976 (Nriagu, 1979). The use of inorganic mercurials as toxicants in anti–fouling marine paints has virtually ceased (at least in Canada and the USA). Considerable quantities of organo-mercurial compounds, phenylmercuric compounds and mercury dodecenylsuccinate are still used as bactericide and fungicide products in the paint industry. Mildew proofing was the third largest consumer of mercury in the USA in 1976, accounting for 12% of total consumption (Nriagu, 1979).

The use of organomercurials as slimicides in the pulp and paper industries was widespread in the 1960's but was outlawed in Sweden in 1967 following a rise in concern about contamination of both the waterways, and the resultant paper products (Swedish Expert Group, 1971; Nriagu, 1979; Clark, 1986). Many countries were prompted to follow Sweden's example shortly thereafter. Legislative control measures implemented by the European Community and the United Kingdom are discussed in more detail later, in Chapter 2.

1.1.4 Anthropogenic Sources of Mercury in the Environment

Many human activities lead to the unintentional mobilisation of mercury at the Earth's surface. Anthropogenic inputs are estimated to contribute 10 500 tonnes of mercury per year, as compared to a natural input of some 3000 tonnes per year (Phillips, 1980). Of a total mercury load of 90 tonnes mercury entering the North Sea each year, however, some 48% is estimated to be from anthropogenic sources (Wilson, 1988).

Many mercury-containing effluent discharges are made directly into coastal waters or estuaries, as well as to river systems (Clark, 1986). Important sources in terms of the quantity available for transport to the oceans, either directly through surface waters, or via the atmosphere, are the chlor-alkali industry, and the application of organomercurial agrochemicals as fungicides and pesticides (FAO, 1984). Other activities which contribute significantly to mercury mobilisation include quarrying, mining, fossil fuel burning and the incineration of domestic and industrial wastes (FAO, 1984), including crematoria, where mercury from dental amalgam is released (Mills, 1990). Marine dumping of sewage sludge also contributes mercury directly to the marine environment. The United Kingdom dumps approximately 11 million tonnes of sewage sludge per year to coastal waters, which contributes about 0.5 tonnes of mercury per year (George *et al.*, 1983).

1.1.5 Mercury Speciation

As mentioned above, natural and anthropogenic processes lead to the mobilisation of bivalent mercuric (Hg^{2+}) ions into surface and ground waters, and directly to the marine environment. Once in solution, Hg^{2+} ions are readily complexed by inorganic and organic ligands, the nature of the complexes varying with the composition of the surface waters. Computer models based on thermodynamic principles predict that soluble inorganic mercury species are present in seawater exclusively as chloro- complexes with $HgCl_4^{2-}$ as the dominant species (Bernhard and George, 1986). Such complexation of mercury (by both organic and inorganic ligands) plays an important role in the behaviour and migration of mercury in natural waters (Benes and Havlik, 1979).

In addition to complex-formation, bivalent mercury forms an important group of organomercuric compounds where one or two organic radicals (R or R') are directly linked via their carbon atom to the mercury atom as: R-Hg-X or R-Hg-R', where X is an inorganic ligand. The commonest organomercurials encountered are methyl and phenyl groups, with chloride, hydroxide, nitrate and sulphate anions as the most frequently encountered inorganic ligands (Benes and Havlik, 1979).

The complexity of the physicochemical state and behaviour of mercury in natural waters is made greater by the tendency of mercury to become adsorbed onto solid particles present in waters, by the low solubility of certain mercury compounds, and by biological accumulation of mercury in aquatic organisms (Benes and Havlik, 1979; Nelson and Donkin, 1985). In fact, Fitzgerald and Lyons (1973) found that as much as 50-60% of the mercury in rivers and coastal seawater may exist either as organic compounds or in association with organic matter.

Mercuric ions are adsorbed by natural organic matter principally through Hg^{2+} reactions

with:

1) Organic sulphur sites in proteinaceous materials
2) Negative surface charge sites on silicate materials, especially clay minerals.
3) The Fe/Mn oxy-hydroxide and sulphide phases common to all soils and sediments (FAO, 1984).

Thus, in addition to dissolved (soluble) forms of mercury, there can be a number of larger, solid particles containing mercury, representing particulate, suspended or insoluble forms (Benes and Havlik, 1979; Balls, 1989).

1.1.6 Transformations of Mercury in the Aquatic Environment

Discharges of mercury to the marine environment from natural and anthropogenic sources are primarily in the form of inorganic mercury (Hg^0, Hg^{2+}) rather than organic mercury (Windom and Kendall, 1979). A very large percentage, however, of the total mercury observed in coastal and marine organisms, especially vertebrates, is methylmercury (Bishop and Neary, 1974, Gardner *et al.* 1978).

This paradox was found to be the result of biological and chemical transformations of inorganic mercury to methylmercury. Methylmercury is the most hazardous mercury species, due to its high stability (George, 1991), in combination with its lipid solubility (Lakowitz and Anderson, 1980), and covalent properties (Simkiss, 1983). This leads to a high ability to penetrate membranes in living organisms. Other organic mercurial compounds like phenylmercury and the alkoxy-alkyl mercurials decompose rapidly in the environment and thus behave more like inorganic mercury (Beijer and Jernelov, 1979).

As stated above, mercury in the aquatic environment may be methylated both biologically and chemically, and possibly also through a mixed process. Bacterial synthesis of methylmercury compounds from inorganic mercury was first demonstrated by Jensen and Jernelov (1967, cited in Beijer and Jernelov, 1979) in natural lake sediments, and in aquaria sludge (Jensen and Jernelov, 1969), while Wood *et al.* (1968) found a mercury methylation by cell-free extracts from methanogenic bacteria.

Subsequent to these initial discoveries, the importance of biological cycles in sediments in synthesis and degradation of organomercurial compounds has been well-established in studies of bacteria by several authors (Imura *et al.*, 1971; Spangler *et al.*, 1973; Langley, 1973; Wood, 1974). Wood *et al.* (1968) proposed two mechanisms of mercury methylation

1. Enzymatic microbial methylation, and
2. Non-enzymatic transfers of methyl groups by methylcobalamine-type

compounds (vitamin B_{12}) to Hg^{2+} in biological systems.

Methylation, presumed to be enzymatic, has been reported in sediments and rotting fish (Jensen and Jernelov, 1969) and in pure cultures of the bacterium *Clostridium cochlearium* (Yamada and Tonomura, 1972). A number of workers have suggested that non-enzymatic methylation may be possible in all micro-organisms and higher animals capable of synthesising methylcobalamine (Vitamin B_{12})-type compounds (Wood, 1971, cited in Windom and Kendall, 1979; Imura *et al.*, 1971).

Matsumura *et al.* (1973) studied the factors involved in the process of methylmercury formation from Hg^{2+} ions in the livers of several marine fish species. They concluded that a methylcobalamine-independent methylation process was taking place. These findings clearly indicate that much work remains to be done to identify those micro-organisms and biochemical systems responsible for methylmercury synthesis.

Hamdy *et al.* (1977) isolated mercury resistant strains of the bacterium *Enterobacter aerogenes* which were capable of surviving 1200 μg $Hg^{2+}ml^{-1}$ medium. These workers noted a cyclic pattern to bacterially-mediated methylmercury synthesis and suggested that methylmercury synthesis may possibly be a mechanism of detoxification by bacteria exposed to inorganic mercury in sediments.

Chemical methylation is also possible in the aquatic environment in the presence of suitable methyl donors. Transalkylation reactions in aqueous solutions have been demonstrated for several metals (Jewett and Brinkmann, 1974, cited in Beijer and Jernelov, 1979), such that mercury will be alkylated by tin and lead alkyls, with trimethyl lead being as effective a methylator as methylcobalamine for mercury.

A mixture of biotic and abiotic processes may be responsible for another methylation pathway for mercury. A species of the bacterium *Pseudomonas* capable of producing a methyltin species from tin (IV) was also found to be quite active in forming elemental mercury from phenylmercuric acetate or mercuric ions. It was found (Huey *et al.*, 1974, cited in Beijer and Jernelov, 1979) that in the presence of both mercury (II) and tin (IV), the net result was methylmercury, probably formed through a chemical alkylation by methyltin.

1.1.7 Degradation of methylmercury

Methylmercury is not readily decomposed by most organisms. It is, in fact, persistent to all but specific biochemical processes (Beijer and Jernelov, 1979). Methylmercury synthesised in sediments may, however, be rapidly decomposed by micro-organisms (Spranglet *et al.* 1973; Sommers and Floyd, 1974) into elemental mercury (Hg^0) and methane.

$$CH_3Hg^+ \text{ -----micro-organisms-----> } CH_4 + Hg^0$$

Biological decomposition of methylmercury has been demonstrated to occur in mercury–polluted sediments from lakes and rivers, while methylmercury decomposition activity has also been detected in heavily–polluted sediments from the Northern Baltic (Beijer and Jernelov, 1979).

It has been shown (Komura *et al.*, 1971, cited in Windom and Kendall, 1979) that the mercury–resistant bacterium *Escherichia coli* is capable of transforming inorganic mercury ($HgCl_2$) to a volatile inorganic mercury in the presence of NADPH. Bacteria in sediments are thus capable of remobilising mercury by either methylmercury synthesis and/or degradation and volatilisation, or by directly reducing inorganic mercury to a more volatile form (Windom and Kendall, 1979). The major transformations involving mercury in the aquatic environment are summarised in Figure 1.1.

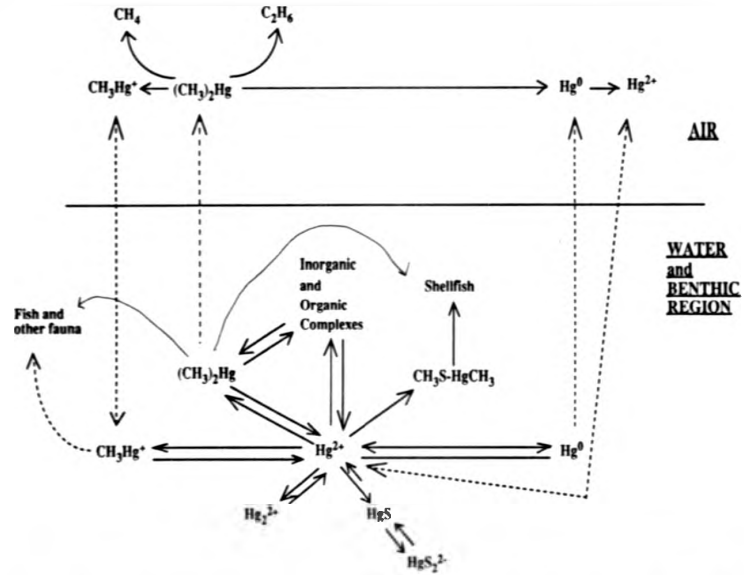


Fig. 1.1 Principal transformations of mercury related to the aquatic environment (from Beijer and Jernelov, 1979)

1.2 Mercury Concentrations in Components of the Marine Ecosystem

1.2.1 Mercury Concentrations in Seawater

The analysis of metal levels in seawater can be relatively problematical as concentrations are generally very low and there is a strong possibility of contamination of a sample during collection or analysis (Bryan *et al.*, 1985). A survey of the studies reporting mercury concentrations in seawater reveals a clear trend of decreasing mean concentrations with time. This is probably the result of improvements in quality control of both sample handling and analytical methods (GESAMP, 1986a).

In open ocean waters, most mercury is in the dissolved form, and particulate levels are very low. Sample filtration is not, therefore, usually employed prior to analysis (Bryan *et al.*, 1985). A summary of determinations of mercury in Atlantic oceanic waters is given in Table 1.1. Bruland (1983) indicated that, although oceanic distribution of mercury is incompletely understood, open ocean mercury concentrations appear to lie in the range 0.5-5 ng.l^{-1}. Similarly, Aston and Fowler (1985) reported that the open-ocean waters of the Mediterranean Sea, away from coastal influences, have mercury concentrations in the range 0.5-2.5 ng.l^{-1}.

Mercury concentrations in coastal waters tend to be higher than in open-oceanic waters (GESAMP, 1986a; Cossa and Fileman, 1991), reflecting the higher levels of suspended particulate material and greater proximity to mercury input sources, whether natural or anthropogenic. As particulate metals may comprise the largest fraction in coastal waters, a filtration step (usually through a 0.45 µm filter) is usually necessary prior to analysis. Ferrara *et al.* (1990) reported total mercury concentrations in the coastal waters of the Ionian and Aegean Seas in the range 3.5-10.5 ng.l^{-1} (means 4.8 and 6.9 ng.l^{-1} respectively), with dissolved mercury concentrations in the range 0.30-0.69 ng.l^{-1}. The same workers (Ferrara and Maserti, 1988) reported similar total mercury concentrations, of 2-6 ng.l^{-1} in coastal waters of the Straits of Gibraltar. These values are much lower than the total mercury concentrations of <20-204 ng.l^{-1} reported in unfiltered Tyrrhenian coastal water by Renzoni *et al.* (1973).

Total mercury levels in English coastal waters of the English Channel, were reported in the range 14-21 ng.l^{-1} by Burton and Leatherland (1971). A more recent study, by Cossa and Fileman (1991) reported much lower total mercury concentrations, of 0.21-4.1 ng.l^{-1}. This decrease in observed mercury concentrations in an area with time may be the result of the previously-mentioned improvements in sampling and analytical methodology, as suggested by GESAMP (1986a).

Coastal waters have been shown to have elevated mercury concentrations as a result of

Table 1.1 Summary of some measurements of total mercury concentrations in filtered Atlantic Ocean waters[1]

Region	Depth (metres)	Sample Size	Hg Conc. (ng.l^{-1}) Mean	Range	Reference
NE Atlantic Ocean	0-4660	9	13	<3-20	Leatherland *et al.*, 1971
NE Atlantic Ocean	0-4030	11	54	17-142	Leatherland *et al.*, 1973
Sub-tropical Atlantic	0	10	35	10-54	Gardner, 1975
SE Atlantic Ocean	0 (upwelling)	5	14	6-25	Gardner, 1975
NE Atlantic Ocean	0	7	15	trace-34	Gardner, 1975
Atlantic Ocean	not given	not given	5	not given	Buffoni *et al.*, 1982

Note: [1]. except Leatherland *et al.* (1971), who measured mercury concentration in unfiltered seawater

Table 1.2 Comparison of typical total and dissolved mercury concentrations (ng.l^{-1}) in waters of several UK estuaries.

Estuary	Total Hg Mean	Range	Dissolved Hg Mean	Range	Source
Thames	29	1.4-310	1.2	0.5-2.8	1
Humber	28	3.4-100	0.9	0.43-1.71	1
Tyne	7.9	3.2-15	1.3	0.47-3.5	1
Tees	3.1	2.1-6.0	0.68	0.44-1.4	1
Inner Forth	not given	16-119	2.9	1.5-3.6	2, 3
Outer Forth	not given	<10	4.0	1.14.0	2, 4

Sources: 1. from MAFF (1991); 2. from Davies and Paric (1978); 3. from FRPB (1985); 4. from FRPB (1991)

anthropogenic inputs of mercury. Total mercury concentrations in coastal waters near Kurwar in western India appear to be heavily influenced by the discharge of mercury with effluents of a caustic soda factory (Sanzgiry *et al.*, 1988; Krishnakumar and Pilai, 1990). Mercury concentrations were measured in the range 260-2680 ng.l^{-1} in the vicinity of the discharge, compared to 30-130 ng.l^{-1} offshore, with a mean of 61 ng.l^{-1} (Sanzgiry *et al.*, 1988). In general, for coastal areas not directly influenced by anthropogenic mercury inputs, total mercury concentrations may be of the order of 20 ng.l^{-1} (Fitzgerald, 1979).

Mercury concentrations in estuarine waters are highly variable due to the greater number of mercury sources, and a higher but variable suspended particulate loading. Adsorption to and absorption by particulate material rapidly removes dissolved mercury from solution in estuarine waters (Campbell *et al.*, 1986; MAFF, 1991). Dissolved mercury concentrations tend to be much lower than total concentrations in estuarine waters due to the scavenging of dissolved mercury by particulate material. This is illustrated in Table 1.2, which compares the level of dissolved and total mercury in waters of a number of UK estuaries. An environmental quality standard (EQS) has been established by the European Community (EC) for protection of marine and other life in coastal waters. As a consequence of its implementation, dissolved mercury levels must be less than 300 ng.l^{-1} in coastal and estuarine waters, or 500 ng.l^{-1} in waters affected by mercury discharges from the chloralkali industry (European Communities, 1982). Similar values are laid down by a sister Directive covering discharges from industries other than the chloralkakli industry (European Communities, 1984). Mercury concentrations in UK coastal and estuarine waters, as shown in Table 1.2, are often orders of magnitude lower than these values (FRPB, 1991; MAFF, 1991). The great variation of concentrations in each case is probably the result of the highly variable suspended particulate concentrations common to most estuaries (Salomons *et al.*, 1988).

1.2.2 Mercury concentrations in marine, coastal and estuarine sediments

Sediments are the principal sites of mercury accumulation in the marine environment (GESAMP, 1986a). Table 1.3 gives a summary of studies of total mercury concentrations in marine and coastal sediments outwith the United Kingdom, from both unpolluted and polluted sediments. Background mercury concentrations in clean sediments, distant from anthropogenic influence, are usually less than 0.1 μg.g^{-1} (e.g. Baffin Bay, Canada: Campbell and Loring, 1980; Izmir, Turkey: Balci and Turkoglu, 1993). The highest mercury concentrations ever recorded in marine sediments (2010 μg.g^{-1} wet weight) were collected near the chlor-alkali plant discharge in Minimata Bay in Japan (Takizawa, 1979).

Studies of total mercury concentrations in some United Kingdom estuarine and Irish Sea sediments are summarised in Table 1.4. Mercury inputs from a variety of sources have led

Table 1.3 Summary of total mercury levels in marine, coastal, and inshore sediments outwith UK waters

Location	Sediment Fraction	Total Mercury (μg.g^{-1}) Mean	Range	Comments	Reference
Californian coast (La Jolla), USA	Whole	not given	0.2-1.0	higher values close to sewer outfall	Klein and Goldberg (1970)
Tyrrhenian coast, Italy	Whole -sand	not given	up to 1.3	-	Renzoni *et al.* (1973)
Halifax Bay, Queensland, Australia	Whole	0.0039	not given	sub-surface, pre-industrial	Knauer (1976)
Halifax Bay, Queensland, Australia	Whole	0.0117	not given	surficial, post-industrial	Knauer (1976)
Arctic nearshore, Baffin Bay, Canada	Whole	0.05	0.02-0.08	-	Campbell and Loring (1980)
Arctic offshore, Baffin Bay, Canada	Whole	0.07	0.04-0.11	-	Campbell and Loring (1980)
Bellingham Bay, Washington, USA	whole	not given	0.98-1.2	surface sediment	Bothner *et al.* (1980)
Bellingham Bay, Washington, USA	whole	not given	4.1-5.4	sediment 10-15 cm. depth	Bothner *et al.* (1980)
Nissum Broad, Denmark	whole	not given	1.77-21.9	adj. to former Hg discharge	Kuorboe *et al.* (1983)
Nissum Broad, Denmark	whole	not given	0.25-3.4	500m. offshore from above	Kuorboe *et al.* (1983)
Kanwar coast, India	Whole -sand	not given	0.002-0.1	close to chlor-alkali outfall	Krishnakumar and Pillai (1988)
Kanwar coast, India	Whole -mud	not given	0.007-1.3	close to chlor-alkali outfall	Krishnakumar and Pillai (1988)
Haifa Bay, Israel	Whole	not given	0.03-0.78	Coastal sites	Hornung *et al.* (1989)
Haifa Bay, Israel	Whole	not given	1.0-1.5	adjacent to chlor-alkali factory outfall	Krom *et al.* (1990)
Port Phillip Bay, VA, Australia	Whole	not given	0.01-0.06	wet weight basis	Phillips *et al.* (1992)
Nissum Broad, Denmark	Whole	not given	>1.22->2.27	adjacent to former chlor-alkali discharge 0-5 cm. depth	Andersen (1992)
Nissum Broad, Denmark	Whole	not given	0.051-0.138	1500 m.from above former discharge 0-5 cm. depth	Andersen (1992)
Saguenay Fjord, Canada	Whole	3.63	not given	0-0.5 cm depth, highly industrialised	Gagnon *et al.* (1993)
Saguenay Fjord, Canada	Whole	0.43	not given	13-15 cm. depth	Gagnon *et al.* (1993)
Izmir Bay, Turkey	Whole	0.55	0.31-1.30	<3 km. from industrial Hg input	Balci and Turkoglu (1993)
Izmir Bay, Turkey	Whole	0.06	0.04-0.09	>30 km. from ind. Hg inputs	Balci and Turkoglu (1993)

Table 1.4 Summary of total mercury concentrations in UK estuarine (and Irish Sea) sediments

Location	Sediment Fraction	Total Mercury ($\mu g.g^{-1}$ dw) Mean	Range	Comments	Reference
Mersey estuary	whole	not given	<0.05- 4.83	-	Craig and Moreton (1976)
Hale, Mersey estuary	whole	not given	4.3- 8.6	-	Bartlett *et al.* (1978)
Fiddlers Ferry, Mersey estuary	whole	not given	1.2- 11.3	-	Bartlett *et al.* (1978)
Stanlow Marshes, Mersey estuary	whole	not given	4.5- 6.4	-	Bartlett *et al.* (1978)
Inner Clyde estuary	whole	not given	0.4- 4.4	-	Bartlett *et al.* (1978)
Outer Clyde estuary/ Firth of Clyde	whole	not given	B.D.L.- 0.3	-	Bartlett *et al.* (1978)
Irish Sea sediments	whole	not given	B.D.L.- 0.2	-	Bartlett *et al.* (1978)
Lune estuary	whole	0.21	0.11- 0.36	-	Langston (1982)
Wyre estuary	whole	1.53	0.21- 3.71	-	Langston (1982)
Humber estuary	whole	0.56	0.28- 0.74	-	Langston (1982)
Mersey estuary	whole	2.76	1.31- 3.96	-	Langston (1982)
Clyde estuary	whole	0.48	0.08- 1.31	-	Langston (1982)
Carron estuary (Firth of Forth)	whole	not given	<0.05- 3.95	top 3-5 cm.	Craig and Moreton (1983)
Lower Forth estuary	<63 µm	1.04	not given	adjacent to mercury discharge	FRPB (1992)
Lower Forth estuary	<63 µm	0.496	0.153- 0.915	18 km. downstream from above	FRPB (1992)
Firth of Forth, 1990	<63 µm	0.250	0.108- 0.302	36 km. downstream from above	Dr. S. Hull, FRPB pers. comm. (1992)

Note. B.D.L. = concentration below detection limit

to a considerable accumulation of mercury in the sediments of Liverpool Bay and the Mersey Estuary, with mercury concentrations up to 11.3 $\mu g.g^{-1}$ dry wt. Major contributors to these inputs were chlor-alkali plants (whose discharges entered the Mersey and Wyre estuaries), especially prior to 1974 when proper effluent treatment began (Campbell *et al.*, 1986). Although the inputs have been greatly reduced over the last decade, there remains a reservoir of mercury in the sediments of the estuaries and inshore areas (Rowlatt, 1988; Camacho-Ibar *et al.*, 1992). Despite this, mercury concentrations in fish tissue have fallen (MAFF/ Franklin, 1987), although tissue levels do reflect the geographical distribution of mercury in the sediments. Mercury levels in three fish species (plaice, *Pleuronectes platessa*, dab, *Limanda limanda*, and lesser spotted dogfish, *Scyliorhinus caniculus*) were higher in fish from inshore sites, in the Mersey and Wyre estuaries (Leah *et al.*, 1991).

Notably high mercury concentrations have also been recorded in riverine sediments influenced by discharges containing mercury. The mean mercury concentration in unaffected sediments upstream of a mercury discharge from a cellulose factory on the River Gota in Sweden was 0.164 $\mu g.g^{-1}$ dry weight (Hasselrot, 1968). Immediately downstream of the factory (550 m), mean mercury concentrations were almost two orders of magnitude higher, at 11.6 $\mu g.g^{-1}$ d.w. (maximum value was 26.5 $\mu g.g^{-1}$ dry weight). Levels fell rapidly downstream from from this point (at 750 metres downstream, 1.2 $\mu g.g^{-1}$ dry weight; at 10 km, 0.140 $\mu g.g^{-1}$ dry weight).

1.2.3 Behaviour of mercury in the estuarine environment

The estuary has been defined by Pritchard (1967) as "a semi-enclosed coastal body of water, which has a free connection with the sea, and within which sea water is measurably diluted with fresh water derived from land drainage". Fine sedimentary deposits are an especially characteristic feature of estuaries, with sedimentary material introduced into the estuary by rivers and sea, and by shore erosion (Schubel, 1977; McLusky, 1989).

Historically, man has long used estuaries as a resource, initially to provide food, then for transport and communication, with more recent use as sites for industry and development. Many estuaries are, however, under pressure through their use as repositories for the effluent of industrial processes and domestic waste (Wilson, 1988; McLusky, 1989).

The use of estuaries as receiving waters for effluent discharges means that many are subject to considerable metal loadings (e.g. Ems, Netherlands (Essink, 1980, 1985); Mersey, UK (Bartlett and Craig, 1981; Campbell *et al.*, 1986); Forth, UK (Davies, 1987); Kishon, Israel (Hornung *et al.*, 1989). Sediments deposited in an estuary will contain a record of the pollutant status of the estuary at the time of deposition (French, 1993). Comparison of copper, lead and zinc concentrations in modern intertidal sediments from the Severn estuary with those of

saltmarsh deposits, known to have been deposited before any large-scale industrial development, revealed a systematic enrichment of these metals in present-day sediments, of the order of 2-4 times the levels in pre-industrial sediments (French, 1993).

The rationale for discharging of effluents to estuaries is based on the assumption that waste will be diluted effectively and carried, by regular tidal flushing, to the open sea for disposal and dispersal. Compared with the behaviour of metals in the oceans, however, the estuarine system is more complex due to variable suspended matter concentrations, strong gradients in the chemical composition of the water, and complex hydrodynamic conditions (Salomons *et al.*, 1988).

It has been well-established that, on entering an estuary, mercury rapidly becomes adsorbed onto particulate materials, with preference for the finer fractions (Andren and Harris, 1973; Nelson, 1981; Campbell *et al.*, 1986). As was described for several UK estuaries in section 1.2.1, the proportion of total mercury in the dissolved phase of estuarine waters is very low, although highly variable. The majority of both inorganic and organic mercury appears to be associated with particulate material, colloids and high-molecular weight organic matter, where it is probably co-ordinated with sulphur ligands (Reimers and Krenkel, 1974; Mantoura *et al.*, 1978; Wallace *et al.*, 1982, GESAMP, 1986a).

The processes which affect metal-to-particulate adsorption and desorption phenomena under estuarine conditions were summarised by Salomons *et al.* (1988):

1. Oxidation either of organic particles containing trace metals, or oxidation of metal sulphides,
2. Chlorinity increase causing competition between chloride ions and particulates for complexation of the dissolved metals;
3. Turbidity changes which may offer additional sites for adsorption;
4. pH changes which affect adsorption-desorption processes significantly;
5. Formation of new particulate material, including release of dissolved organic material (DOM) from interstitial pore waters;
6. Flocculation of DOM and changes in surface properties of estuarine molecules, consistent with the formation of a macromolecular film.

With reference to the second point above, Lockwood and Chen (1973) demonstrated that the adsorption of mercury onto hydrous manganese oxides is prevented by the presence of chloride ions at the concentration found in seawater.

Although the suspended loading in any particular area may remain relatively constant, there is a continuous turnover of particles. Fresh particles are generated by fluvial and atmospheric inputs, primary production, and by resuspension. These sources are balanced by advection or sedimentation. The effect of this continual particle throughput is to strip metals from solution

(Balls, 1989).

Fine particulate material in suspension will tend to settle in suitable deposition sites in estuarine and coastal zones, regardless of source (Young *et al*, 1985). The deposition of sediments within the estuary is controlled by current speed and sediment particle size, with greater current speeds required to mobilise or transport larger particles (McLusky, 1989). In consequence, the strong tidal currents and river flows encountered at the lower and upper ends of estuaries result in the mobilisation and transport of all particle sizes. The slower currents of the mid to upper estuary, where the greatest mixing of fresh and sea waters occurs, create suitable conditions for the finer silt and clay particles to settle out, and muds are deposited. The rate of settling of the finest particles is very low, however, resulting in naturally-high turbidity in estuarine waters (Salomons *et al.*, 1988). The rate of deposition can be accelerated by flocculation processes, while disturbance factors (e.g. storms, sediment washing by tidal action, springtime ice breakup) all contribute to sedimentary resuspension (Burton, 1976). Since metals tend to associate most closely with the finest particulate material, and these tend to settle in upper estuarine areas, estuarine sediments may thus become 'reservoirs', or 'sinks' for metals discharged into the estuary (Langston, 1982; McLusky, 1989).

The most frequently-observed association regarding mercury in marine and estuarine sediments appears to be between total mercury and organic material, or organic carbon, rather than with sediment type (Bartlett and Craig, 1981; Langston, 1982). Methylmercury levels in estuarine sediments, however, appear to be controlled more by the sulphide content of sediments than by factors like total mercury levels or organic content (Craig and Moreton, 1983, 1984).

Chemical leaching experiments support the idea that redox potentials in sediments influence mercury partitioning (Langston, 1982). It was found that 64% of the mercury in predominantly-oxidized surface sediments was bound to organic material rather than sulphides. The amount bound to organic material in reduced, sulphide-rich sediments near a sewage outfall, however, was 0%. A small proportion of mercury could be associated with oxide and hydroxide phases, as indicated by a significant correlation between mercury and iron, although it was suggested that this was relatively unimportant in overall mercury partitioning processes. Humic and fulvic acids provide favourable binding sites for mercury, accounting for 4-32% of total mercury in British estuarine sediments (Langston, 1982).

A study of the loss of mercury from contaminated sediments in Bellingham Bay, USA (Bothner *et al.*, 1980) revealed a mercury concentration as high as 3.5 $\mu g.l^{-1}$ in the interstitial water of anoxic sediments, which was 126 times the level in the overlying water column, but a maximum concentration of only 0.06 $\mu g\,l^{-1}$ in the interstitial water of oxidised surface sediments. Thus, although mercury fluxes could be measured from anoxic sediments, losses could not be detected from the oxidised sediments. Mercury may be released from sediments mainly as organic or

polysulphide complexes (Lindberg and Harris, 1974). It has also been postulated, and demonstrated experimentally, that the formation of stable chloro-complexes in the presence of seawater may cause the release of mercury from estuarine sediments (Feick *et al.*, 1973).

For a fuller review of the current understanding of processes affecting metal concentrations in estuarine and coastal marine sediments, see Luoma (1990), who indicates that physical transport and sorting of sediments is the most important factor controlling the distribution of metals in a wide range of estuarine systems. The distribution of particulate material and its associated metal load will be determined by natural depositional processes in most estuarine and coastal marine systems (Luoma, 1990). The highest concentrations of metals in whole sediments occur in such environments where the finest particles accumulate in natural depositional areas, as shown for mercury by Figueras *et al.* (1985) in a number of west European estuaries.

The importance of the estuarine turbidity maximum has been stressed with regard to the mobilising of metals in the estuarine environment (Wilson, 1988). This area is the focus of the sedimentation/deposition regime of an estuary and has, additionally, strong salinity and dissolved oxygen gradients. Within the turbidity maximum zone, and within the course of its movement up and down the estuary with the tidal cycle, there is a considerable mobilization and flux of metals between the sediments and water column.

In conclusion, considering the close associations of mercury with sediment, especially fine particulate material, it seems likely that the movement, and perhaps availability to biota, of mercury in estuaries, is controlled principally by sediment fluxes.

1.3 Accumulation of mercury by aquatic biota

1.3.1 Introduction

Mercury has been shown to accumulate to high levels in the tissues of aquatic organisms exposed to elevated concentrations in the environment. The accumulation of mercury in its various forms in marine organisms will make it available for transfer to other organisms within food chains. This section reviews the observed concentrations and the accumulation of mercury by various groups of aquatic organisms, and then considers the food chain transfer of mercury in aquatic ecosystems.

1.3.2 Plants

Relatively little work appears to have been published on the mercury concentrations in marine phytoplankton, although the phytoplankton community plays a major role in the vertical transport of metals, amongst other materials, from the mixed surface layers of the oceans to bottom waters (Windom and Kendall, 1979).

In coastal waters, mercury levels in phytoplankton appear to reflect local inputs. Knauer and Martin (1972) found the average mercury concentration in phytoplankton samples from the north western Pacific coast of the USA, collected over a one-year period, to be about 0.2 μg Hg g^{-1} dry weight. As this area receives no significant anthropogenic mercury inputs, the low mercury concentrations are probably indicative of ambient levels. The same authors, however, found mercury concentrations in phytoplankton samples collected on a transect from the west coast of the USA (Monterey) to Hawaii to average 0.4 μg Hg g^{-1} dry weight. It was suggested that the additional presence of radiolarians and other small zooplankton forms in the samples contributed to the higher mercury concentrations.

Skei *et al.* (1976) reported mercury concentrations of an order of magnitude or more higher than those of Knauer and Martin in phytoplankton collected from a Norwegian fjord which received an industrial mercury discharge (3kg daily) at the head. Mercury concentrations in samples, principally composed of dinoflagellates of genus *Ceratium* , ranged from 25 μg Hg g^{-1} dry weight near the head of the fjord, to 0.52 μg Hg g^{-1} dry weight at the mouth.

The accumulation of mercury and other metals by phytoplankton may be primarily by adsorption of the elements to the cell wall rather than by true absorption and transport into the cytoplasm (Phillips, 1980). Similarly in macroalgae, it is suggested (Phillips, 1977) that the plant probably responds almost entirely to metals in solution, and binding appears to occur by adsorption to alginates in the cell wall as an ion-exchange process. Laboratory studies of trace metal uptake indicate that a linear relationship approaching direct

proportionality exists between metals in the algae and those in the water (Bryan, 1969), suggesting little regulation of metal uptake. Some successful attempts have also been made to correlate levels of trace metals other than mercury in algae with those in the ambient water (Morris and Bale, 1975; Seeliger and Edwards, 1977). Work by Gekeler *et al.* (1988) demonstrated unequivocally that algae can sequester heavy metals, including mercury, by an identical mechanism to that of higher plants, via complexation to phytochelatins, a series of cysteine-rich peptides.

In a comparison of field and experimental data, Seeliger and Corduzzo (1982) indicated that green algae of the genus *Enteromorpha* combined several essential criteria for the biological monitoring of dissolved copper and mercury. *Enteromorpha* spp. was also used successfully to monitor dissolved heavy metal concentrations (including mercury) in several UK estuaries (Say *et al.*, 1991). Jones *et al.* (1972) reported elevated levels of mercury in several species of macroalgae in the region of the Tay estuary, Scotland. Mercury levels were particularly high in *Ulva lactuca*, *Porphyra umbilicalis* and *Ceramium rubrum* (25.5, 2.3, and 3.0 μg $Hg.g^{-1}$ tissue dry weight respectively) collected within the Tay estuary, compared with levels in the same species from a fully marine site outwith the estuary (all 0.001 μg $Hg.g^{-1}$ dry weight. The authors gave no indication of anthropogenic inputs of mercury to the estuary, although they implied that mercury levels were elevated in the waters of the estuary.

In a study of mercury contamination in components of the Forth estuarine ecosystem in eastern Scotland, Elliott and Griffiths (1986) showed that mercury content of the brown algae, *Fucus vesiculosus* corresponded relatively closely with the overall distribution of total mercury concentration in the water column. They demonstrated a concentration gradient for mercury in this species taken from sites along the estuary. The highest mercury concentrations (up to 0.9 μg $Hg.g^{-1}$ dry weight) were recorded in plants collected near an industrial discharge of inorganic mercury compounds, with levels in the algae decreasing with distance from the site.

The uptake of mercury by marine higher plants has also been investigated. The rooted salt marsh plant *Spartina alterniflora*, an important primary producer in the extensive salt marsh ecosystems of the southeastern Atlantic and Gulf coasts of the USA, has been shown to accumulate mercury predominantly in the organic form, with a higher concentration factor than for a number of other metals (Dunstan and Windom, 1975 cited in Windom and Kendall 1979).

Windom *et al.* (1976) found no methylmercury in *Spartina* plants from a contaminated salt marsh, although primary consumers had considerable amounts of the methylated form. The uptake of mercury by *S. alterniflora* is mainly through the root system and, following

uptake, mercury is transferred to other parts of the plant (Rahn, 1973 cited in Windom and Kendall, 1979). Similarly, root system uptake of mercury was suggested as the cause of a correlation between sediment mercury levels and mercury concentrations in the marine plant, *Posidonia oceanica*, growing in sea water close to an industrial input of mercury (Maserati *et al.*, 1988). Mercury levels in leaves were high, up to 0.19 μg Hg.g^{-1} fresh weight, compared to a level of 0.02 μg Hg.g^{-1} fresh weight in plants from an area not contaminated by mercury.

1.3.3 Zooplankton

There is relatively little published literature concerning uptake or concentrations of mercury in the zooplankton. Both Knauer and Martin (1972), and Cocoros and Kahn (1973) noted the concentrations of mercury in zooplankton, the latter in estuarine zooplankton and the former in marine species. Both sets of workers recorded higher mercury levels in phytoplankton than in zooplankton. Parrish and Carr (1976) found minimal retention of inorganic mercury by the calanoid copepod, *Acartia tonsa* fed algae, *Croomonas salina* previously exposed to mercuric chloride. It is possible that uptake of mercury by zooplankton is similar to that observed in macro-invertebrates belonging to the same taxonomic phyla.

1.3.4 Molluscs

Due to the commercial importance of many mollusc species and their position in the food chain, often with a direct input into the human diet, this group has been the most widely-studied of the macro-invertebrates with regard to accumulation and effects of heavy metals. Particular attention has been focussed on mussels of the genus *Mytilus* e.g. *M. edulis*. Since mussels are sedentary, filter-feeding molluscs, of wide distribution in coastal waters, and are easily collected they appear to have favourable characteristics for use as indicators in studies of environmental quality (Davies and Pirie, 1977). As a result, many studies have been made of metals levels in wild mussel populations from areas thought to be at risk from pollution by metals (De Wolf, 1975; Goldberg, 1975; Eganhouse and Young, 1976; Phillips, 1976a, b; Davies and Pirie, 1978). Many of these studies have been co-ordinated under the "Mussel Watch" Programme for worldwide monitoring of the coastal marine environment (Goldberg, 1975).

The following arbitrary, purely descriptive guidelines for mercury concentrations in shellfish have been adopted for the purposes of the Joint Monitoring Programme (JMP) of the Oslo and Paris Commissions: lower band: ≤0.6 μg.g^{-1} dry wt. Medium Band: 0.6-1.0 μg.g^{-1} dry wt. Upper Band: >1.0 μg.g^{-1} dry wt. In co-ordinating the monitoring, by the River Purification Boards, of mercury contamination in mussels (*M. edulis*) in Scottish

estuaries, the Association of Directors and River Inspectors of Scotland established an uncontaminated background concentration of 0.3 $\mu g.g^{-1}$ dry wt (FRPB, 1991).

Bivalve molluscs can accumulate trace metals with concentration factors of 10^3 to more than 10^6, depending on the species and the metal involved (Phillips, 1977). Filter-feeding bivalves, such as the genera *Mytilus* and *Crassostrea*, will take up metals rapidly from solution or from food, but also from ingestion of inorganic particulate materials (Phillips, 1980). Relative proportions of the total body trace metal content derived from each of the three routes in bivalves are uncertain. Concentration factors recorded for mussels (*Mytilus edulis*) for the uptake of metals from solution alone are relatively low, however, when compared with those found in mussels from the environment (Pentreath, 1973; Phillips, 1976), suggesting uptake from food to be the most important route.

Similarly, in deposit-feeding bivalves such as *Scrobicularia plana* it has been shown that the digestive gland in animals from both contaminated and relatively clean estuaries contained the highest concentrations, with up to 96% of the body total (Bryan and Uysal, 1978; Bryan and Hummerstone, 1978). A high percentage of the total body metal load in the digestive gland suggests that uptake from ingested sediment is particularly important.

Wrench (1978) attempted to correlate the tissue distribution of mercury in the oyster (*Ostrea edulis*) with amounts of soluble protein in the tissues and found that inorganic mercury uptake by isolated gill tissues required a metabolisable substrate and was ATP dependent.. Active uptake of a substance is related to the metabolic activities of the cells and has a higher temperature dependency than simple passive diffusion (Pringle *et al.*, 1968, cited in Windom and Kendall, 1979). Temperature-dependent uptake, generally regarded as a positive indication that mercury uptake is closely linked with metabolic pathways in the organism, has been observed in the mussel, *Mytilus edulis* (Fowler *et al.*, 1978) and the American oyster, *Crassostrea virginica* (Cunningham and Tripp, 1975a). In a study of the uptake of mercury by three species of freshwater clams from the Family Unionidae, however, Smith *et al.* (1975) found that differences in temperature at which the experiment was run did not contribute significantly to differences in mercury uptake. This led them to suggest that uptake was by diffusion followed by the formation of stable complexes within the animal

1.3.5 Fish

1.3.5.1 Mercury concentrations in marine fish

The principal reason for concern about mercury in fish is the position of fish in food chains leading to man. The tendency for fish to accumulate mercury in their tissues can lead to a potential human hazard. Worldwide awareness of the previously-mentioned Japanese and

Swedish experiences, with deleterious ecological and public health effects caused by mercury contamination in fish, has resulted in a considerable volume of literature on the subject. The published research on mercury concentrations in tissues of wild fish, especially the edible skeletal muscle, is particularly extensive, and a comprehensive review is not possible here.

A useful summary of several large international, or Governmental agency, databases of monitoring programmes for mercury in muscle of commercial fish species, is provided by GESAMP (1986a). Of most relevance to the work to be presented in later chapters is the GESAMP summary of selected data from the International Council for the Exploration of the Sea (ICES) database of mercury concentrations from Northern Atlantic fish. The results indicate that mercury levels in plankton-feeding fish species do not exceed 0.24 $\mu g.g^{-1}$ fresh weight (FW), although mean values are typically less than 0.1 $\mu g.g^{-1}$ FW. Fish which feed on other marine organisms tend to have slightly higher mercury levels. The reported range for cod (*Gadus morhua*), for example, is 0.03 to 0.48 $\mu g.g^{-1}$ FW.

Preston and Portmann (1981) reported background mercury levels in muscle of flounders (*Platichthys flesus*) from uncontaminated UK coastal waters, of 0.2 $\mu g.g^{-1}$ FW. Summaries are also produced periodically by the UK Government, of monitoring programmes for mercury levels in muscle of fish from UK coastal waters (MAFF, 1990, 1991; Brown, 1992). From these summaries, it appears that mercury levels in muscle of UK fish vary between species, and between areas. Mean concentrations are, however, typically less than the 0.3 $\mu g.g^{-1}$ FW level established as a guideline to indicate elevated mercury concentrations, by the Joint Monitoring Group of the Oslo and Paris Commissions. This value has been adopted by the European Community as an Environmental Quality Standard for mercury in fish muscle (European Communities, 1982, 1984; see Chapter 2). The mercury levels in fish from Scottish waters, described in section 1.4 in relation to human exposure to mercury through seafood products, are frequently lower than the 0.2 $\mu g.g^{-1}$ FW background value described above (Brown, 1992).

Mercury in fish tissues appears to be predominantly in the methylated form (Westoo, 1973, Bloom, 1992). The source of this methylmercury has puzzled researchers, as the concentrations of methylmercury in seawater are extremely low (Bernhard, 1985). It appears, however, that the high percentages of methylmercury in fish tissues may result from a high efficiency of uptake of methylmercury, both from water and from food, in combination with extremely strong binding of methylmercury with components of tissues, particularly the sulphydryl groups of proteins (Pentreath, 1976b, c; Carty and Malone, 1979). This behaviour is in contrast to that observed for inorganic forms of mercury, which are accumulated with much lower efficiency, and excreted with much shorter biological half-lives, than methylmercury (Hannerz, 1968; Olson *et al.*, 1973; Pentreath,

1976a, c). The percentage of mercury as inorganic mercury is, however, frequently higher in tissues other than muscle, e.g. liver and kidney (Elliott *et al.*, 1988; Barghigiani *et al.*, 1989). It is possible that a certain amount of methylmercury demethylation may occur in these tissues, as a precursor to excretion via the kidney (Burrows and Krenkel, 1973).

Most studies of mercury concentrations in fish report a trend of increasing concentrations with size or age of fish. This phenomenon is so widely reported in the literature as to be almost ubiquitous, regardless of habitat or lifestyle. A summary of such studies is presented in Table 1.5. Increasing mercury levels with size or age means that very high mercury levels are frequently found in long-lived or large fish (see section 1.3.5.2). Possible reasons for this correlation between age or size of fish and mercury concentration are discussed more fully in Chapter 2.

To conclude, a short summary of the present understanding of accumulation and transformation of mercury in coastal and marine fish was presented by Windom and Kendall (1979): "Both inorganic and organic forms of mercury can be accumulated directly from sea water by fish. Methylmercury is generally accumulated more efficiently from food than inorganic forms. Mercury absorbed into the fish by either pathway is transferred through the animal via its blood until removed by the liver and spleen. Inorganic mercury is stored in these tissues until removed through the kidney by excretion. Methylmercury is apparently not excreted to a great extent but accumulates in muscle tissue. A portion of the methylmercury may be degraded to the inorganic form in the liver (Burrows and Krenkel, 1973). Mercury concentrations increase with fish age, and therefore individuals of species with longer life spans generally have higher levels of the metal."

1.3.5.2 Mercury accumulation by large and predatory fish

The highest mercury concentrations in marine biota have been recorded among high-ranking marine predators. Black Marlin (*Makaira indica*), from the apparently-unpolluted waters of north eastern Australia, have the highest concentrations yet reported on a wet weight basis for a teleost fish species (Mackay *et al.*, 1975), in both skeletal muscle (mean concentration = 7.3 $\mu g.g^{-1}$), and liver tissue (mean concentration = 63 $\mu g.g^{-1}$).

High mercury concentrations were recorded (Gardner *et al.*, 1975, cited in Windom and Kendall, 1979) on a dry weight basis, in several shark species from southeastern USA coastal waters. Most notably, a total mercury concentration of 10.4 $\mu g.g^{-1}$ dry weight (dw) was measured in the spleen of the Lemon Shark (*Negaprion brevirostris*). A similarly-high concentration (10.4 $\mu g.g^{-1}$ dw) was measured in the muscle of the bull shark, *Carcharhinus leucas*. Similar mercury concentrations were also presented by Walker (1976) for two species of Australian sharks, the School Shark, *Galeorhinus australis*, and the Gummy Shark, *Mustelus antarcticus*.

Table 1.5 Summary of types of relationship between biological variables and mercury concentration in fish muscle

Common Name	Species Name	Biological Variables: Length	Weight	Age	Model Fitted	Reference
Freshwater Fish						
Brown Trout	*Salmo trutta*	n	+	n	C	Brooks *et al.*, 1976
Pike	*Esox lucius*	n	+	+	L	Johnels *et al.*, 1967
Pike	*E. lucius*	n	+	+	L	Bull *et al.*, 1991
Perch	*Perca fluviatilis*	+	n	n	L	Luten *et al.*, 1987
Perch	*P. fluviatilis*	n	+	+	L	Bull *et al.*, 1991
Yellow Perch	*P. flavescens*	+	+	+	L	Grieb *et al.*, 1990
Roach	*Rutilis rutilis*	n	+	+	L	Bull *et al.*, 1991
(No common name)	*Catla catla*	+	+	n	L	Tariq *et al.*, 1992
(No common name)	*Chela chanius*	+	+	n	L	Tariq *et al.*, 1992
Pelagic Marine Fish and Sharks						
Yellowfin Tuna	*Neothunnus albacora*	n	+	n	L	Menasveta and Siriyong, 1977
Bigeye Tuna	*Parathunnus sibi*	n	+	n	L	Menasveta and Siriyong, 1977
Snapper	*Chrysophrys auratus*	+	n	n	Ex	Chvojka *et al.*, 1990
Yellowtail Kingfish	*Seriola laland*	+	n	n	Ex	Chvojka, 1988
Bluefish	*Pomatomus saltatrix*	n	+	n	L	Cross *et al.*, 1973
Saithe	*Pollachius virens*	+	n	n	L	Topping *et al.*, 1975
Saithe	*P. virens*	+	n	n	L	McKie and Topping, 1982
Herring	*Clupea harengus*	+	+	+	Log	Braune, 1987
Swordfish	*Xiphius gladius*	+	n	n	Ex	Monteiro and Lopes, 1990

(Table 1.5 contd. over)

Table 1.5 contd.

Common Name	Species Name	Biological Variables: Length	Weight	Age	Model Fitted	Reference
Black Marlin	*Makaira indica*	+	+	n	Ex	Mackay *et al.*, 1975
Black Marlin	*M. indica*	+	n	n	Ex	Barber and Whaling, 1983
Spiny Dogfish	*Squalus acanthias*	+	+	+	L	Topping and Graham, 1978
Lesser Spotted Dogfish	*Scyliorhinus caniculus*	+	n	n	L	Leah *et al.*, 1991a, b
Tope	*Galeorhinus galeus*	+	n	n	L	McKie and Topping, 1982
Gatuzo Shark	*Mustelus schmitti*	+	n	n	L	Marcovecchio et al., 1986
Marine and Estuarine Demersal Species						
Cod	*Gadus morhua*	+	n	n	L	Luten *et al.*, 1987
Ling	*Molva molva*	+	+	+	L	Topping and Graham, 1978
Blue Whiting	*Micromesistius poutassou*	+/ns	+/ns	+	L	Topping and Graham, 1978
Halibut	*Hippoglossoides hippoglossoides*	n	n	+	L	Topping and Graham, 1977
Plaice	*Pleuronectes platessa*	+	n	n	L	Leah *et al.*, 1991a
Dab	*Limanda limanda*	+	n	n	L	Leah *et al.*, 1991a
Megrim	*Lepidorhombus bosci*	+	n	+	L	Pellegrini and Barghigiani, 1989
Sole	*Solea vulgaris*	+	n	+	L	Pellegrini and Barghigiani, 1989
Flounder	*Platichthys flesus*	+	n	n	L	McKie, 1983
Flounder	*P. flesus*	+	n	n	L	Luten *et al.*, 1987
Flounder	*P. flesus*	+	n	n	L	Stronkhurst, 1992
Eelpout	*Zoarces viviparus*	+	n	n	L	Essink, 1980, 1985, 1988

Notes: + (positive significant regression); n (variable not considered in study); +/ns (positive, non-significant regression); L (linear regression); Ex (exponential regression); C (Correlation only calculated); Log (double-log linear regression)

A combination of factors is suggested by the above workers as possible causes of elevated mercury concentrations in these organisms. Factors such as longer exposure times with long lifespan, increasing methylmercury content in larger prey items, and lower tissue turnover and mercury excretion rates in older fish may all contribute to the accumulation of mercury to the high concentrations in large or predatory fish, as reported above (see also section 1.3.7).

1.3.6 Marine Mammals

Marine mammals have generally been found to have the highest tissue mercury concentrations of all aquatic organisms (GESAMP, 1986a). The position of most marine mammals at high trophic levels in the marine food web, in conjunction with their long life spans, apparently low excretion rates for mercury, and possible mercury detoxification mechanisms makes them particularly likely to accumulate high levels of mercury (Koeman *et al.*, 1975). The highest mercury concentration recorded in literature appears to be a maximum value of 1544 $\mu g.g^{-1}$ wet weight in liver tissue of a striped dolphin (*Stenella coeruleoalba*) from the French Mediterranean (Andre *et al.*, 1991). High levels (up to 860 $\mu g.g^{-1}$ wet wt) have also been reported in marine mammals in UK waters (Law *et al.*, 1992).

One notable feature of the tissue distribution of mercury is that both seals (pinnipeds) and whales (cetaceans) seem to show the same pattern, with highest levels in liver tissue, intermediate levels in kidney tissue and lower concentrations in muscle tissue. This has been illustrated for a number of pinniped species (Smith and Armstrong, 1975; Roberts *et al.*, 1976; Drescher *et al.*, 1978; Kari and Kauranen, 1978; McKie *et al.*, 1980, Yamamoto *et al.*, 1987; Pena *et al.*, 1988) and cetacean species (Honda *et al.*, 1983; Fujise *et al.*, 1988; Morris *et al.*, 1989; Marcovecchio *et al.*, 1990).

In reviews of mercury levels in marine mammals (Wagemann and Muir, 1984; Thompson, 1990), similar patterns of mercury accumulation are apparent in both pinniped and cetacean species. In both groups, there is extensive inter and intra-species variation of mercury levels, complicated by a tendency for levels to increase in an age-related manner. Mercury concentrations in liver samples of forty-two mammal of six species in UK waters ranged from 0.5 to 430 $\mu g.g^{-1}$ wet wt (Law *et al.*, 1992), a factor of 860 times.

A summary of the typical ranges of mercury concentrations in livers of a number of marine mammal species is shown in Table 1.6. Comparison of different studies of the same species from different areas (e.g. see *Phoca vitulina* or *Phocoena phocoena*) reveals considerable intra-species differences in mercury levels. These differences are likely to be the result of a combination of several factors, including different mercury content of prey species from different areas, differences in mobility and behaviour patterns, and different age composition of samples. These sources of variability, along with frequent failure to present age/size

Table 1.6 Mercury Concentrations in livers of marine mammals (μg.g^{-1} wet weight)

Species	Common Name	Location	Sample Size	Mean	Range	Reference
		Pinnipeds:				
Phoca vitulina	CS	German Wadden Sea	70	-	1.5-160	1
P. vitulina	CS	W. Scotland, UK	6	-	0.5-1.13	2
P. vitulina	CS	East Anglia, UK	7	-	1.5-106	2
P. vitulina	CS	Hokkaido, Japan	14	2.9	0.11-11.8	3
P. vitulina	CS	UK coastal waters	13	50.6	1.0-170	4
Phoca hispida	RS	NW Territory, Canada	80	27.5	30.1 (sd)	5
P. hispida	RS	Finland	12	91	14-300	6
Erignathus barbatus	BS	NW Territory, Canada	6	143	170 (sd)	5
Leptonychotes weddellii	WS	Syowa, Japan	2	5.8	3.1-8.5	7
Halichoerus grypus	GS	Eastern Scotland	23	28.2	2.6-89.3	8
H. grypus	GS	UK coastal waters	21	108	1.5-430	4
Otaria flavescens	SSL	Coastal Argentina	7	47	23.1-64.5	9
Odobenus rosmarus	PW	Alaska	62	1.5	3.18 (sd)	10
		Cetaceans:				
Phocoena phocoena	HP	Baltic Sea	2	1.6	0.7-2.5	1
P. phocoena	HP	Coastal NE Scotland	26		0.28-15.9	11
P. phocoena	HP	Cardigan Bay, Wales	4	1.25	0.6-2.8	12
P. phocoena	HP	County Down, Ireland	4	10.25	5.2-26	12
Phocoenoides dalli	DP	NW Pacific	1	6.38	-	13
Tursiops truncatus	BND	Coastal Wales	2	20.5	20.21	12
Tursiops gephyreus	BND	Coastal Argentina	1	86	7.3 (sd)	14
Stenella coeruleoalba	SD	Coastal Japan	20	205	1.7-485	15
S. coeruleoalba	SD	Coastal Wales	2	10.5	10-11	12
Hyperoodon ampullatus	BNW	North Sea	1	0.38	-	1
Delphinapterus leucas	BW	Baltic Sea	1	4.4	-	1
Kogia breviceps	PSW	Coastal Argentina	1	11.7	-	14
Monodon monoceros	NW	Baffin Island, Canada	1	7.28	-	16
Balaenoptera acutorostrata	MW	South Wales	1	1.8	-	4

Notes: **Species:** CS,Common Seal; RS, Ringed Seal; BS, Bearded Seal; WS, Weddell Seal; GS, Grey Seal; SSL, Southern Sea Lion; PW, Pacific Walrus; HP, Harbour Porpoise; DP, Dall's Porpoise; BND, Bottlenose Dolphin SD, Striped Dolphin; BNW, Bottlenose Whale; BW, Beluga Whale; PSW, Pigmy Sperm Whale; NW, Narwhal; MW, Minke Whale.

References: 1. Harms *et al.*, 1978; 2. Roberts *et al.*, 1976; 3. Tohyama *et al.*, 1986; 4. Law *et al.*, 1992; 5 Smith and Armstrong, 1975; 6. Kari and Kauranen, 1978; 7. Yamamoto *et al.*, 1987; 8. McKie *et al.*, 1980; 9. Pena *et al.*, 1988; 10. Taylor *et al.*, 1989; 11. Falconer *et al.*, 1980; 12. Law *et al.*, 1991; 13. Fujise *et al.*, 1988; 14. Marcovecchio *et al.*, 1984; 15. Honda *et al.*, 1983; 16. Wagemann *et al.*, 1984.

information with the concentration data, make sensible comparisons between studies very difficult (Thompson, 1990, Law *et al.*, 1992). Thompson (1990) indicates that tissue mercury levels in marine mammals are likely to be related to dietary mercury levels within different prey, moderated by age-related processes.

The chemical form of mercury in marine mammal tissues has been examined by several workers. The large majority of the mercury in liver tissue is in the inorganic form. In livers of common seal (*Phoca vitulina*) from UK waters, less than 15% of the mercury was in the organic, methylated form (Roberts *et al.*, 1976). Even lower percentages, of 5.6 and 0.38% organic mercury, were reported in livers of Canadian Ringed Seals, *Phoca hispida*, and Bearded Seals, *Erignathus barbatus*, respectively (Smith and Armstrong, 1975). Only 10% of the mercury measured in livers of *P. hispida* from Finland was in the organic form (Kari and Kauronen, 1978). By contrast, the majority of the mercury in seal muscle tissue appears to be in the methylated form (Smith and Armstrong, 1975; Kari and Kauronen, 1978).

It has been suggested that the relatively high mercury levels in some marine mammal species are natural, with some species having become adapted to dietary mercury exposure over the course of evolutionary time, possibly with different detoxification mechanisms (Thompson, 1990). Several features of mercury accumulation in the Common Seal (*Phoca vitulina*), namely high liver and kidney levels of mercury, low brain mercury concentrations, and poor mother-to-foetus transfer of mercury, are indicative of inorganic mercury absorption (Roberts *et al.*, 1976). The diet of this species is almost entirely fish, and the majority of mercury in fish is in the methylated form. The authors proposed, therefore, that mercury taken up by the seal as the more toxic methylmercury was being demethylated and stored in the liver as the less toxic inorganic form, with only a small amount redistributed to the other tissues as inorganic mercury.

The presence of a cytosolic metal-binding protein (metallothionein), capable of binding inorganic mercury, in the liver and kidney of the seal *Phoca vitulina*, suggests a possible cellular mechanism for the binding of mercuric ions from demethylated methylmercury (Tohyama *et al.*, 1986). Both mercury and metallothionein concentrations in the liver showed age-related increases. Metallothionein was also shown to be present in the liver and kidney of the Narwhal (*Monodon monoceros*), although in this case only a small fraction of the total mercury was shown to be bound to the thionein protein (Wagemann *et al.*, 1984).

1.3.7 Mercury transport in aquatic food chains

Food chain amplification, or biomagnification, is the concentration of persistent substances up the food chain. Thus, relatively low and innocuous levels at the foot of the chain may be progressively accumulated to harmful or lethal dose levels in organisms at the top (Wilson, 1988). Several workers have demonstrated evidence for biomagnification of mercury in aquatic

food chains.

Bernhard (1985) developed a mathematical model which explained the high levels of mercury in tuna as the result of amplification of mercury through a pelagic food chain:

seawater → plankton → sardine → tuna.

His model predicts that small differences in seawater mercury concentrations are sufficient to explain the large differences in mercury levels observed for tuna (*Thynnus thynnus*) from the Atlantic and the Mediterranean. He also showed that the fraction of total mercury present as methylmercury increases with the age and trophic level of an organism. Other specific food chain pathways have also demonstrated biomagnification of mercury. Pelletier and Laroque (1987) demonstrated experimentally the biomagnification of organic mercury along the simple food chain: "particles → mussel (*Mytilus edulis*) → starfish (*Lepasterias polaris*)". The starfish retained about 50% of the organic mercury ingested.

By ranking estuarine ecosystem components according to their mercury concentration factors over the water column soluble mercury fraction, Elliott and Griffiths (1986) were able to illustrate an increase in mercury levels along several direct consumer routes in the Forth Estuary: suspended material to suspension feeders; sediment and infauna to estuarine demersal fish and waders; macroalgae to grazers. The two most critical pathways of mercury biomagnification, leading to the highest biota concentration factors (in wading birds, mussels and estuarine fish) appear to be:

1. Suspended solids → mussels (*Mytilus edulis*) → oystercatchers (*Haematopus ostralegus*);
2. Sediment → infauna → estuarine demersal fish (principally flounder, *Platichthys flesus* and eelpout, *Zoarces viviparus*) and wading birds.

Other routes, however, demonstrated no increase in mercury levels, i.e. shrimps to marine demersal fish, and plankton to clupeids.

Elliott and Griffiths felt that, in general, their results agreed with those of other workers (Kiorboe *et al*., 1983) in suggesting that the concept of biomagnification across all trophic levels had not been demonstrated for mercury. These latter workers studied mercury levels in a wide range of marine organisms in the immediate vicinity of a redundant chemical factory, previously responsible for discharging large quantities of mercury. They reported no consistent pattern of increase of mercury concentration with trophic level, with highest Hg concentrations in deposit-feeding bivalves, lower in suspension feeders and lowest in predatory polychaetes and fish.

It appears that few other workers have studied a large number of species of different trophic status, within a relatively small area, to discover if any relationships exist between the mercury concentrations of species and their food chain /food web position. Such a study was carried out

by Ratkowsky *et al.* (1975) who reported mercury levels for 16 species of Tasmanian estuarine fish. The position of a species in the food chain appeared to be an important factor determining its mercury content. Approximately 51% of individual fish of species with a diet consisting principally of other fish species had mercury concentrations in excess of 0.5 $mg.kg^{-1}$. By contrast, 24% of invertebrate predators, and only 7% of herbivorous individuals had similar mercury levels. Similarly, Levitan *et al.* (1974) observed much higher mercury levels (>0.5 ppm) in several carnivorous fish species in Israel, compared to herbivorous species collected from the same area (all samples <0.1 ppm, with most <0.03 ppm). These works confirmed earlier suggestions by Jernelov and Lann (1971) that the efficiency of food web transfers of mercury to predators may be largely a function of of the chemical form of mercury in the prey. They found that fish preferentially assimilate methylmercury and excrete inorganic mercury (c.f. Farmanfarmaian, 1985). As the percentage of total mercury burden as methylmercury generally increases in prey from higher trophic levels, the efficiency of mercury transfers through the food web should increase towards the predators. Thus, it might be expected, as shown by Westoo (1973), Bishop and Neary (1974), and Bloom (1992), that the percentage of mercury as methylmercury is generally high in predatory aquatic species, in the range 70-100%.

1.4 Human health implications of mercury in aquatic environments

1.4.1 Human toxicology of methylmercury

The pharmacokinetics of methylmercury in the human system have been reviewed comprehensively by several workers (Swedish Expert Group, 1971; World Health Organisation, 1972, 1976; Clarkson *et al*, 1984; GESAMP, 1986a). Swallowed methylmercury is absorbed with a very high efficiency (95-100%) in the intestines, whether administered as a salt, or bound to tissue as in consumed fish muscle (Swedish Expert Group, 1971; World Health Organisation, 1976; George, 1991). Animal studies also support the idea that virtually all orally ingested methylmercury is absorbed into the bloodstream in the digestive tract (Clarkson *et al*, 1984). There is, by contrast, extensive absorption of inorganic mercury to the intestinal cell surface, and uptake efficiency is low (3-14 %; George, 1991).

Methylmercury has an extremely high affinity for sulphydryl (-SH) groups (Carty and Malone, 1979). Most of the methylmercury in blood is bound to the SH groups of the cysteine residues of serum proteins, and of glutathione in erythocytes (George, 1991). The rapid transport of methylmercury to the body tissues suggests, however, that a diffusible form of methylmercury exists (Lakowitz and Anderson, 1980), possibly as a transient association of methylmercury with chloride. Both inorganic mercury and methylmercury have, under experimental conditions, been shown to form neutral complexes with chloride ions. These complexes diffuse rapidly through cell membranes, although the permeability of methylmercury (Lakowitz and Anderson, 1980) is much greater than that of inorganic mercury (as mercuric chloride: Gutknecht, 1981).

Following uptake, methylmercury is rapidly distributed throughout the body tissues, the distribution phase being complete in approximately three to four days (GESAMP, 1986a). Methylmercury passes the blood-brain "barrier" into the central nervous system (Berlin, 1963 cited in GESAMP, 1986a). It also readily crosses the placenta and is found in all foetal tissues (Swedish Expert Group, 1971; Clarkson *et al*., 1984). About 10% of an ingested burden of methylmercury ends up in the brain, and about 7% in the blood (GESAMP, 1986a).

Methylmercury is accumulated and concentrated in human hair at the time of follicular formation of the hair. The concentration in the newly-formed hair has a constant ratio of approximately 250:1 to the simultaneous blood concentration (Clarkson *et al*., 1984). The mercury concentration of a length of hair will remain constant during the residence of the hair on the head and, as such, offers an effective means of studying previous blood concentrations. Hair mercury concentrations have been widely used in studies of populations exposed to methylmercury (WHO, 1976). Methylmercury in human tissues slowly undergoes cleavage of the carbon-mercury bond to release inorganic mercury (Hg^{2+}) into the body tissues (Clarkson *et*

al., 1984).

1.4.2 Effects of mercury on human health

Methylmercury is neurotoxic to humans (Takizawa, 1979). The major symptoms reported in people suffering from Minimata disease included sensory disturbances, ataxia (or uncoordinated movement), hearing impairment, and constriction of the visual field (Takizawa, 1979). It appears that the toxic effects of methylmercury primarily occur in damage to the sensory part of the nervous system (GESAMP, 1986a).

The World Health Organisation (WHO, 1976) reported that 5% of an adult population can be expected to show overt symptoms when the blood concentration of total mercury is between 0.2 and 0.5 µg/g. This would correspond to 50-125 µg/g in hair, or to a long-term intake of 3-7 µg of methylmercury/kg body weight (GESAMP, 1986a). Victims of the Niigata methylmercury poisoning incident in 1965 had high mercury levels (Takizawa, 1979), in both hair (57-570 µg/g) and blood (6.4-90.8 µg/dl).

The initial clinical symptom of methylmercury poisoning is an abnormal sensation or numbness (paresthesia) in hands, feet and around the mouth (GESAMP, 1986a). Increased exposure causes the development of the Minimata disease symptoms described above, then to paralysis, and general physical and mental debilitation, and death due to interference with the central nervous system in severe cases (Clarkson *et al.*, 1984; GESAMP, 1986a)

Damage to the nervous system by methylmercury is irreversible (Clarkson *et al.*, 1984), although clinical improvement is possible due to the adoption of some of the functions of damaged neurons by others. Apart from the nervous system effects, methylmercury has no other known effects of relevance to the marine food chain (GESAMP, 1986a), and is not known to be carcinogenic.

As mentioned above, the best recorded and scientifically studied incidents of methylmercury poisoning occurred in Japan (Takizawa, 1979), at Minimata (1953-1960) and Niigata (1965). About 2200 cases of Minimata disease have been officially diagnosed, of which 750 have been fatal. In the Niigata incident, 669 cases were recognised, with 55 deaths. Another outbreak of methylmercury poisoning, primarily the result of rural people eating seed grain which had been previously dressed with organic mercury as a pesticide, occurred in Iraq in 1972. A large increase in the number of patients developed into the most catastrophic epidemic on record, with 6350 persons hospitalised, of whom 459 died (Takizawa, 1979).

1.4.3 Human dietary exposure to methylmercury

Mercury has no known biological function in humans. As described previously, the majority of

mercury in fish tissues is in the form of methylmercury, the most toxic form. Even low concentrations of methylmercury in the human body may, therefore, be considered potentially harmful (GESAMP, 1986a). As methylmercury is readily absorbed by ingestion, consumption of seafood could lead to exposure levels sufficiently high to be of health concern. Ever since the occurrence of Minimata disease in Japan, there has been considerable interest in the availability of mercury to humans from dietary sources.

The principal, if not sole, source of human exposure to methylmercury, excluding accidental exposure or misuse of artificially-produced methylmercury, is the aquatic food chain (Clarkson *et al.*, 1984). It is difficult to estimate accurately the dietary intake of chemical food contaminants in the general population (GESAMP, 1986a). There is a large degree of variability in both the environmental contaminant levels and food consumption rates. The importance of seafood as a source of mercury in the human diet is dependent on several factors. These include the quantity of seafood consumed, whether the intake is long- or short-term, the particular species involved, the chemical form(s) of mercury in the tissue, and the presence of possible protective or toxicity-enhancing substances in the consumed tissue (GESAMP, 1986a).

The level of seafood consumption varies widely, both between and within countries. Island or coastal based populations may be particularly dependent on seafood, as fish, shellfish, or marine mammal tissues (Smith and Armstrong, 1975; Gras and Mondain, 1980; Turner *et al.*, 1980). Aquatic plants, used as both human food and domestic animal fodder, have also been shown to pose a potential health risk with regard to mercury content (Suckcharoen, 1978).

Most fish of commercial importance have methylmercury concentrations in the edible muscle in the range < 0.1 to 0.3 $\mu g\ Hg\ g^{-1}$ fresh weight (GESAMP, 1986a; MAFF, 1990, 1991; Leah *et al.*, 1991; Brown, 1992). Some countries (e.g. Italy and Spain) require imports of fish and shellfish to be accompanied by certificates of mercury content, in response to the possible health risk of consuming food containing elevated levels of mercury. In Scotland, these certificates are issued by Environmental Health Departments to exporting companies, usually in association with the health certificates required for each consignment (Brown, 1992). The data on which the mercury certificates are based are provided by the Scottish Office Agriculture and Fisheries Department (SOAFD), and are updated twice each year. Mercury levels in seafood products from Scottish waters are very low (maximum concentrations generally less than $0.2\ \mu g\ g^{-1}$). This is shown in Table 1.7, the values produced by SOAFD for Autumn 1991 (Brown, 1992). Much higher mercury concentrations (up to 100 $\mu g\ g^{-1}$ fresh weight) were measured in fish caught in the polluted Minimata and Niigata areas of Japan (Swedish Expert Group, 1971).

Elevated methylmercury concentrations (0.5 to 16 $mg\ kg^{-1}$ fresh weight) are also found in some commercially-fished predatory and long-lived species (Phillips, 1980; GESAMP, 1986a). Such levels are found in tuna (Miller *et al.*, 1972; Petersen *et al.*, 1974; Bernhard *et al.*, 1982),

Table 1.7 Summary of mercury concentrations (wet weight) in commercial fish and shellfish species landed at Scottish ports in Autumn 1991 (from Brown, 1992)

Species		Hg Concentration ($\mu g.g^{-1}$ wet weight)		
Commercial name	Specific name	Mean	Min.	Max.
Demersal Fish:				
Angler (Monkfish)	*Lophius piscatorius*	**0.05**	0.01	0.08
Cod	*Gadus morhua*	**0.07**	0.04	0.09
Haddock	*Melanogrammus aeglefinus*	**0.07**	0.04	0.09
Hake	*Merluccius merluccius*	**0.04**	0.01	0.08
Lemon Sole	*Microstomus kitt*	**0.06**	0.01	0.13
Ling (<80cm)	*Molva molva*	**0.10**	0.05	0.14
Ling (80-123 cm.)	*Molva molva*	**0.58**	0.23	0.77
Megrim	*Lepidorhombus whiffiagonis*	**0.05**	0.02	0.08
Plaice	*Pleuronectes platessa*	**0.04**	<0.01	0.07
Pollack	*Pollachius pollachius*	**0.10**	0.06	0.15
Saithe	*Pollachius virens*	**0.07**	0.04	0.12
Skate	*Raja batis*	**0.07**	0.01	0.17
Turbot	*Scophthalmus maximus*	**0.18**	0.04	0.40
Pelagic Fish:				
Herring	*Clupea harengus*	**0.07**	0.03	0.16
Mackerel	*Scomber scombrus*	**0.07**	<0.01	0.12
Whiting	*Merlangius merlangus*	**0.06**	0.04	0.12
Shellfish:				
Cockles	*Cerastoderma edule*	**0.02**	0.02	0.02
Crab	*Cancer pagurus*	**0.10**	0.01	0.12
Lobster	*Homarus gammarus*	**0.10**	0.05	0.14
Mussels	*Mytilus edulis*	**0.02**	0.01	0.04
Nephrops	*Nephrops norvegicus*	**0.08**	0.04	0.15
Oysters	*Ostrea edulis*	**0.03**	0.02	0.04
Queen Scallops	*Chlamys opercularis*	**0.05**	0.02	0.23
Scallops	*Pecten maximus*	**0.03**	<0.01	0.07
Squid	*Loligo* spp.	**0.04**	<0.01	0.08

swordfish (Miller *et al.*, 1972; Freeman *et al.*, 1973; Barber and Whaling, 1983; Monteiro and Lopes, 1990), marlin (Schultz and Crear, 1976; Schultz *et al.*, 1976; Mackay *et al.*, 1975; Barber and Whaling, 1983) and shark (Topping and Graham, 1976; Walker, 1976; McKie and Topping, 1982; Marcovecchio *et al.*, 1986; Leah *et al.*, 1991; Hornung *et al.*, 1993), even in fish caught remote from mercury-polluted areas. It is notable, perhaps, that the commercial importance of shark fisheries is currently increasing worldwide (Vas, 1991).

Freshwater fish may also act as a significant source of exposure of humans to dietary mercury. A recent study by Tariq and co-workers (Tariq *et al.*, 1992) of mercury levels in two commercially-exploited freshwater fish species, *Catla catla*, and *Chela chanius*, from Rawal Lake in Pakistan, indicated that total mercury was present, at least in larger specimens, in concentrations well in excess of 1 μg Hg.g^{-1} wet weight. Such levels led the authors to express concern about the marketing of these fish.

In 1970, the United States Food and Drug Administration (FDA) began testing many commercial fish species for mercury content (Peterson *et al.*, 1973). Nearly 4% of the canned tuna on the wholesale market contained mercury in excess of the FDA guideline of 0.5 μg.g^{-1} fresh weight, with maximum levels of 1 μg.g^{-1} fresh weight. Similarly, all but 42 of 853 samples of swordfish had mercury levels in excess of 0.5 ppm. Concern over these elevated mercury levels led to a wide-scale seizure of swordfish, and a lesser but considerable confiscation of tuna, by the FDA in 1971 (Miller *et al.*, 1972; Peterson *et al.*, 1973).

The Joint FAO/WHO Expert Committee on Food Additives established, in 1972, a provisional tolerable weekly intake (PTWI) of 0.3 mg total mercury, of which no more than 0.2 mg should be methylmercury, per person per week (WHO, 1972). The Committee considered that a sustained intake at this rate would give rise to an equilibrium level in whole blood well below 0.2 μg.ml^{-1}, which is the lowest concentration believed to be associated with toxic effects (Swedish Expert Group, 1971). This evaluation was based on relationships between the intake of methylmercury from the diet, and the levels of mercury in hair, whole blood, and blood cells in human subjects in Japan and Sweden (WHO, 1972).

It is often possible to identify, within a population, certain critical groups who have a particularly high seafood consumption (GESAMP, 1986a), and who may thus be at a higher risk from mercury in seafood dietary sources. Examples might be workers in the fishing and seafood industries and their families, or certain ethnically isolated groups, such as Inuits and other native people, who subsist on marine mammals or fish. Studies of methylmercury intake in general population groups have been carried out on several occasions in different parts of the world. These studies have either calculated intakes from seafood consumption data, combined with data on mercury concentrations in seafood, or carried out biological monitoring using a good indicator tissue such as hair or blood, where a good linear correlation exists between

mercury intake and tissue mercury concentration (cf. Sherlock *et al.*, 1984).

In a Peruvian population, which was chronically exposed to methylmercury from long-term consumption of ocean fish, the weekly fish intake averaged 10.1 kg for an average family of 6.2 persons (Turner *et al.*, 1980). Blood mercury concentrations in the population ranged from 11 to 275 ng.l^{-1} (or 0.011 to 0.275 ppm), with a mean concentration of 82 ng.l^{-1} (or 0.082 ppm). These levels were in contrast to those of a nearby control population, where an average family of 6.4 persons consumed only 1.9 kg of fish per week. In this population, blood mercury levels were in the range 0.003-0.025 ppm, with a mean of 0.001 ppm. No symptoms or signs which could be attributed to methylmercury intoxication were observed in any individual in either population (Turner *et al.*, 1980).

A similar study was made of two Senegalese populations, with different rates of seafood consumption (Gras and Mondain, 1980). A population which consumed only 3-4 fish meals per week had a mean mercury concentration in hair of <4 ppm, and a mean blood mercury concentration of 0.030 ppm. Much higher mean levels of mercury, of 7.33 ppm, were found in hair of fishermen who ate 2-3 fish meals per day, and blood mercury concentrations were a little higher, at 0.035 ppm.

These blood mercury levels were similar to those measured in Inuit people from North West Territories in Canada (Smith and Armstrong, 1975), whose principal food items, ringed and bearded seals, had elevated mercury levels compared to other major food sources (fish and caribou). Mean mercury concentrations of 0.034 and 0.038 parts per million (ppm) were reported for these people which, although slightly elevated, are well short of the 0.2 ppm toxic effects level of the Swedish Expert Group (1971). It is probable that the low mercury levels reported for Arctic Charr and caribou, the main winter and spring food, makes the overall diet appear safe. The authors point to a potentially dangerous situation if these Inuit people are forced to rely entirely on a diet of seals for extended periods.

The evidence presented above, of the potential for accumulation of mercury in certain vulnerable groups of humans, indicates the necessity for monitoring of mercury levels in the marine environment. In particular, there is a continuing requirement for the monitoring of such levels in marine biota, especially those organisms likely to be utilised as a food source for human populations, or organisms representative of environments where such organisms are obtained. The work presented hereafter in this thesis explores aspects of the use of a single species of fish, the eelpout (*Zoarces viviparus* L.), for the monitoring of mercury in an estuarine environment.

Chapter 2

Variability of mercury in tissues of the eelpout, *Zoarces viviparus* (L.), from the Forth Estuary and Firth of Forth, Scotland

2.1 Introduction

2.1.1 Mercury in the Forth Estuary, Scotland: current knowledge reviewed

2.1.1.1 The Forth Estuary, Scotland: a general introduction

The Forth Estuary and Firth of Forth form a major geographical feature on the east coast of Scotland, running from Stirling in Central Scotland, to join the North Sea some 100 km downstream (McLusky, 1987). The area of the Forth Estuary and Firth of Forth, including the major centres of conurbation, is shown in Figure 2.1. The Forth Estuary is generally considered to include all tidal waters upstream of the Forth Bridges at Queensferry, with the Firth of Forth comprising all waters downstream from South Queensferry to the Isle of May (McLusky, 1987).

There are major shipping routes to the ports of Leith and Grangemouth, to petrochemical terminals in the upper Firth, and to naval facilities in the lower estuary , and the tidal waters of the Forth are used for cooling four power stations (Leatherland, 1987). Scotland's major petrochemical complex, responsible for approximately 50% of Central Scotland's Gross Domestic Product , is situated in the middle Forth Estuary, at Grangemouth. The complex comprises British Petroleum (BP) companies, Imperial Chemical Industries (ICI), and others. The location of the Grangemouth complex is shown in Figure 2.1. Long-term studies of the impact of discharges from Grangemouth on the intertidal benthic community have been summarised by Bagheri and McLusky (1982), and McLusky (1987). The Forth also receives the domestic effluent from Edinburgh and other coastal towns, plus brewery and distillery wastes from several sources (McLusky, 1987).

The Forth Estuary has been described as one of the most-contaminated coastal areas in Scotland, with respect to trace metals (Davies, 1987). Despite this contamination, the Forth Estuary and Firth of Forth is an important recreational resource with several water-sport centres and many popular beaches. The Forth also has important commercial fishery resources, including an active salmon fishery, and catches of the Norway Lobster, *Nephrops norvegicus*, and crabs and lobsters. The estuary is an important overwintering area for shoals of sprat and herring and, including the coastal fringes of the Firth, a nursery and feeding area for demersal fish species (Leatherland, 1987; Elliott *et al.*, 1989).

2.1.1.2 Discharges of mercury to the Forth Estuary: inputs and legislative control

The Forth Estuary is unique in Scotland since it has, for several decades, received an industrial discharge of inorganic mercury compounds (Elliott and Griffiths, 1986). The mercurial compounds are a waste product of a catalytic procedure in an organic chemical manufacturing

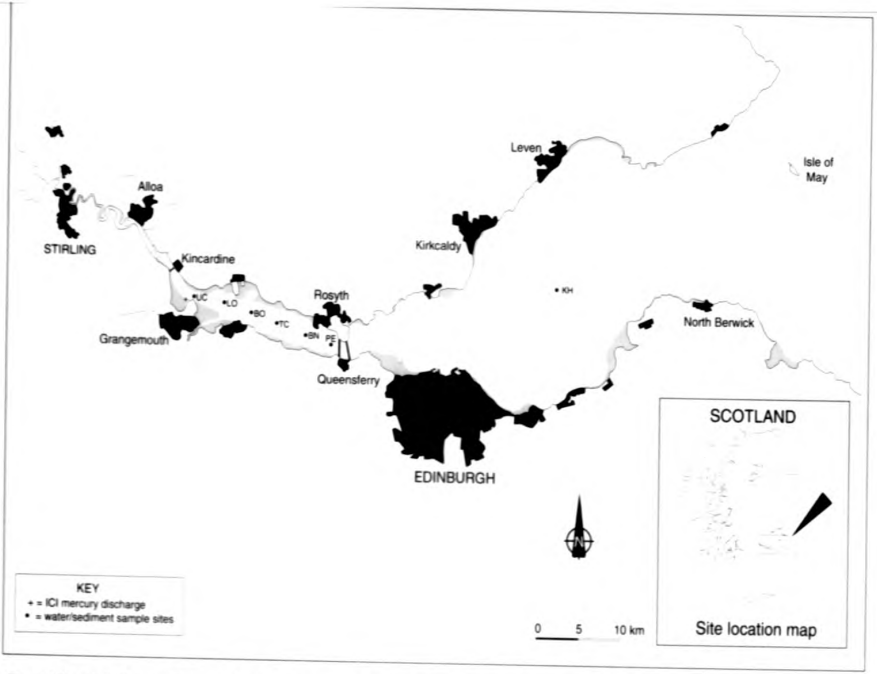


Fig. 2.1 The Forth Estuary and Firth of Forth, showing conurbations, mercury discharge and water and sediment mercury sampling sites

process at ICI (Imperial Chemical Industries, known as Zeneca since 1992), in the Grangemouth industrial complex (Dr. T. Leatherland, FRPB, pers. comm.). Discharge has been made via a sub-littoral long-sea outfall to the main estuary channel since 1975 (see Fig. 2.1), and prior to this via an outfall to the tidal section of the River Carron (Elliott and Griffiths, 1986). The early discharges to the River Carron have left a legacy of significantly contaminated sediments in the vicinity of the original discharge (FRPB, 1987). The installation of a catalyst-recovery plant in the early 1970s led to a considerable reduction in the input of mercury (Davies *et al.*, 1986).

The discharge of mercury to the Forth Estuary and Firth of Forth from various land-based sources is regulated by the Forth River Purification Board (FRPB) under Part II of the Control of Pollution Act (1974), through a system of legal consents and post-consent monitoring of effluent quality (Leatherland, 1987). Regular chemical monitoring of consented discharges by the FRPB to assess compliance of effluent quality with consent conditions allows an estimation of the total inputs of mercury to the Forth Estuary from point source discharges. Table 2.1 shows the estimated inputs of mercury to the Forth Estuary and Firth from different sources for the year 1988 (FRPB, 1991). The estimate of atmospheric input (not given as year-specific) was obtained from Davies (1987). Almost 90% of the mercury entering the Forth tidal waters in 1988 was in the point-source discharge of industrial effluents from ICI (now Zeneca) at Grangemouth.

Figure 2.2 shows the total tonnage of mercury discharged to the Forth each year from 1975 to 1992 (unpublished data kindly supplied by the FRPB). Each annual value is based on the mean mercury concentration of 12-24 samples of effluent at each individual source, collected through the year. The 1992 value is based on samples collected over the first 11 months only. Inputs of mercury in the period 1975-1992 peaked in 1981, with the discharge of more than 6 tonnes of mercury. The great majority of this was the result of operational problems in the ICI plant; Dr. S. Hull, FRPB, pers. comm.).

In 1981, mercury concentrations in the range 0.66-0.96 $\mu g.g^{-1}$ were measured in the muscle of flounders collected close to the discharge (FRPB, 1982). These levels were particularly disturbing as they exceeded the level considered, at that time, to be safe for human consumption (0.5 $\mu g.g^{-1}$). It was stated by FRPB at that time that mercury discharges to the Forth would have to be reduced (FRPB, 1982). Accordingly, ICI were required by FRPB to tighten up control of their mercury catalyst recovery plant, and the discharge consent conditions were subsequently revised by FRPB, specifying tighter limits on the discharge of mercury. The operation of the ICI plant was improved significantly (FRPB, 1987) and mercury inputs fell steadily to the present date. Recently, (December, 1992), the input of mercury from ICI was noted to be very low, approaching a "zero" input (Dr. S. Hull, FRPB, pers. comm.). Further reductions in mercury input are likely with the commissioning of a biological treatment plant at Zeneca, due around 1995 (Dr. A. Griffiths, FRPB, pers. comm.).

Table 2.1 Inputs[1] of Mercury to Forth Tidal Waters for the Year 1988 (after FRPB, 1991).

Source	Input (Kg Hg.Year^{-1})	% of total
ICI Grangemouth	680	87.2
BP Refinery/Chemicals	5	0.6
Other Industrial Sources	10	1.3
Sewage Treatment Works	70	8.9
Atmospheric Deposition	15	1.9
Total	780	100

Note. [1]. Estimates based on 12-24 samples per annum per source, except for atmospheric deposition value, which was taken from Davies (1987).

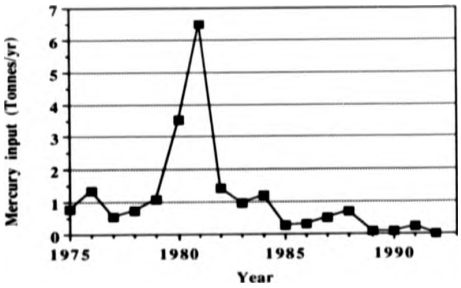


Fig. 2.2 Point source discharge inputs of mercury to the Forth Estuary from all sources,1975-1992. Information kindly supplied by the Forth River Purification Board.

Apart from the previously-mentioned discharge, consented and monitored under UK legislation (Control of Pollution (1974) Act), there is also a European Community Directive Environmental Quality Standard in operation, of 300 ng $Hg.l^{-1}$, covering the discharge of mercury and mercury compounds: Directive 84/156/EEC on limit values and quality objectives for mercury discharges by sectors other than the chlor-alkali industry (European Communities, 1984). A consequence of the implementation of this Directive is a requirement for monitoring to assess compliance with a number of Environmental Quality Standards (Anon., 1991).

The monitoring of mercury by the FRPB has been based on principles and recommendations from the International Council for the Exploration of the Seas (ICES). The ICES recommendations on sampling and analytical methodology have been generally adopted by the Joint Monitoring Group (JMG) of the Oslo and Paris Commissions (ICES, 1978; FRPB, 1987) to which the FRPB makes regular submissions of mercury monitoring data (FRPB, 1987). The JMG formulates policies to eliminate and reduce pollution, and to prevent further contamination of the marine environment (Marine Forum, 1991).

2.1.1.3 Studies of mercury in the Forth Estuary

Effective and rational control requires a good knowledge of the resource (Leatherland, 1987). Much research interest has, therefore, been focused on the distribution of mercury in different components, biotic and abiotic, of the Forth estuarine ecosystem. Detailed studies have been made, especially by the Forth River Purification Board (FRPB) and the Department of Agriculture and Fisheries for Scotland (DAFS; now called Scottish Office Agriculture and Fisheries Department, SOAFD), in support of their statutory roles. Attention has focused principally on the physical and chemical relationships with mercury in the estuary, and on bioaccumulation of mercury by biota in the estuary.

Mercury concentrations are measured annually in the Forth Estuary by the FRPB to assess EQS compliance, in water (as 'dissolved' mercury), in the common mussel (*Mytilus edulis* L.), and in skeletal muscle of the flatfish, flounder (*Platichthys flesus* L.). Measurements are also made of mercury levels in sediment (in the <63 μm diameter fraction). The European and Paris Commissions have adopted an EQS for mercury concentrations in fish flesh which requires that the mean concentration of mercury in the flesh (skeletal muscle) of a representative sample of fish, locally caught from areas receiving significant inputs of mercury, shall not exceed 0.3 $mg.kg^{-1}$ on a wet weight basis (European Communities, 1984; MAFF, 1991). Results of FRPB monitoring programmes for mercury in water, sediment and biota are presented later.

As described earlier, when mercury is discharged to the estuarine environment, it becomes rapidly associated with particulate material, and its subsequent transport and fate is controlled by sediment flux processes. The sediments of the lower Forth Estuary are relatively mobile. Not

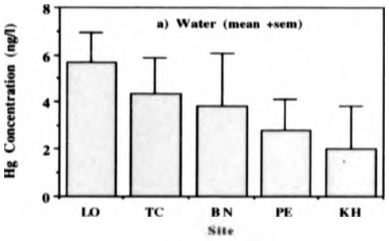
only is there a massive natural resuspension and redistribution during storm events, but approximately 800 000 tonnes of sediment are dredged each year by the port authorities from the approaches to Grangemouth Docks (FRPB, 1989). This dredge spoil is pumped back into the water column only a few kilometres downstream from the dredge site (J. McManus, FRPB, pers. comm.; author, pers. obs.). The net natural movement of sediments into or out of the lower Forth Estuary is a significant unknown (FRPB, 1989), although it appears probable that the total inventory of sediment in the estuary is increasing.

The role of suspended particulate material in the distribution and speciation of mercury in the Forth Estuary was investigated by DAFS (Davies, 1980), who found that mercury from the ICI discharge rapidly becomes associated with particulate material. It was also suggested that reactive mercury (the dominant form of mercury in seawater following addition of inorganic mercury), in most of the estuary, is derived from particulate material, although mercury concentrations in water near Grangemouth may be more directly affected by the discharge. Furthermore, suspended solids were found to control the total mercury concentrations in water, other than in the immediate vicinity of the discharge. Total mercury concentrations were, however, not directly related to salinity.

There is good evidence, therefore, to show that the mercury discharged into the Forth Estuary is rapidly removed from solution by adsorption to particulate material and its subsequent movements are dominated by transport in the particulate phase. While total mercury concentrations in the water column may be 20-200 ng.l^{-1}, the actual dissolved component is usually only 1-4 ng.l^{-1} (Davies, 1987). Work by the FRPB (FRPB, 1987) indicates that fine suspended particulate material is well-mixed throughout the lower estuary, at least with respect to its mercury concentration. As in many estuaries,there is a net upstream pumping of sediments in the Forth (FRPB, 1987), and the estuary contains extensive mudflats, natural depositional areas. A net upstream tidal pumping of sedimentary material in the upper Forth Estuary may be responsible for higher sedimentary levels of mercury in this area than in the lower estuary (FRPB, 1991).

The net loss of mercury bound to particulate material from the estuary by tidal exchange with the Firth of Forth was investigated and found to be very small, in the order of 4 kg/day (Davies *et al.*, 1986), which represents 5-10% of the mercury in suspension in the estuary, or about 0.01% of the mercury in deposited sediments. It has been estimated (Elliott and Griffiths, 1986) that approximately 97% of the standing mass of mercury in the Forth Estuary ecosystem is contained in the sediments, demonstrating the role of sediments as a sink for pollutants in estuaries like the Forth.

Some results of FRPB monitoring for dissolved mercury concentrations in water and total mercury in sediments are shown in Figs. 2.3 and 2.4, for sites shown in Fig. 2.1. Although



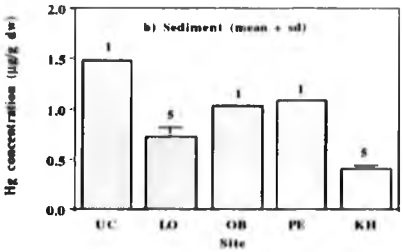
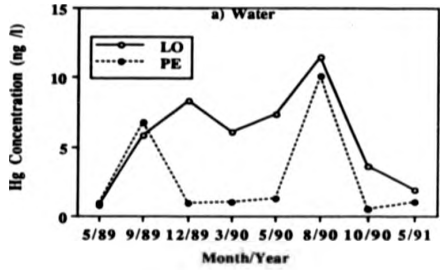


Fig. 2.3 Spatial variation of: a) Dissolved Hg concentrations in water (0.45 µm filtered, 5/89-5/91), and b) Total Hg concentrations in sediment (≤ 63 µm fraction), October 1992. Site locations are shown in Fig. 2.1.



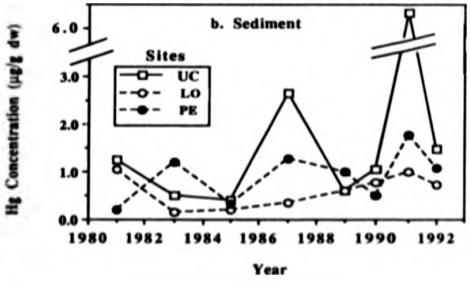


Fig. 2.4 Temporal variations of: a) dissolved Hg concentrations in water (0.45 µm-filtered), and b) total Hg concentrations in sediment (< 63 µm fraction)

there is considerable variation about the mean values for dissolved mercury in water, there is a clear trend of decreasing concentrations with distance away from the mercury discharge at Grangemouth. Concentrations of dissolved mercury may show variability of an order of magnitude between sampling times. It is to be expected that a large number of factors will influence the concentration in water. Such factors might include the magnitude of mercury inputs, relative flows of river and sea water, degrees of vertical and horizontal mixing of estuarine water, and the degree of scavenging of mercury by organic and inorganic particulate material, depending in turn on composition and concentration of particulate material, and factors affecting its transport, resuspension and settlement. Mercury levels in water are usually at least two orders of magnitude less than the previously described EQS of 300 ng.l^{-1}.

Mercury levels in sediment may also be highly variable, the results in Fig. 2.4b demonstrating that there is considerable variability between annual samples collected near the ICI discharge at Grangemouth (site UC), although there is a general trend of decreasing concentrations with distance from the outfall (Fig. 2.3b). As described earlier, the distribution of mercury in the estuary is probably highly dependent on the transport of suspended particulate material. The lack of clear temporal trends in mercury concentrations in sediment, despite the major reduction of mercury inputs (Fig. 2.2) may reflect the constant redistribution of sediment which takes place within the Forth, resulting both from natural processes, and from human activities (eg dredging, and alterations to natural hydrodynamics resulting from engineering works within the estuary). The great natural variability of mercury concentrations in abiotic components of such aquatic systems, as shown here, has led to the development of the use of biological indicators to reflect more accurately the availability of mercury in these environments (Phillips, 1977, 1980; Bryan *et al.*, 1985; Elliott *et al.*, 1988).

2.1.1.4 Bioaccumulation monitoring of mercury concentrations in Forth Estuary biota

The assessment of the degree of contamination at the individual level requires the use of common, representative indicator organisms (Elliott *et al.*, 1988). The use of quantitative biological indicators to monitor metal levels in the aquatic environment has been extensively and comprehensively reviewed by Phillips (1977, 1980), and Bryan *et al.*, (1985). From these reviews, it is possible to identify the following as ideal, important characteristics for an indicator species for metals in the aquatic environment:

1. An indicator organism is required to be representative of the area of collection i.e it must be sedentary, or have a restricted home range.
2. The organism should be sufficiently long-lived to allow sampling of more than one year class.
3. The organism should be large enough to provide adequate tissue for analysis.
4. Any proposed indicator organism should be abundant and easy to collect.

5. A major requirement to avoid spurious conclusions (Phillips, 1977) is that all organisms in a survey exhibit the same correlation between their metal contents and those in the environmental compartment of interest, at all locations studied, under all conditions.

Monitoring of mercury levels in representative estuarine biota has been undertaken by the FRPB on a regular basis since 1982. As stated above, data on mercury concentrations in mussels and flounder, collected annually to assess for compliance with the EQS values of various EC Directives, are submitted, in addition, to the JMG of the Oslo and Paris Commissions. Studies have also been made by FRPB for several years of mercury concentrations in the brown alga, *Fucus vesiculosus*, as a potential guide to dissolved mercury concentrations in the water column (Phillips, 1977; Bryan *et al.*, 1985). The mussel, *Mytilus edulis*, is monitored to assess primarily the bioaccumulation of mercury in the particulate phase. Mussels, as filter-feeding bivalves, obtain trace metals not only from food and solution, but also from ingestion of inorganic particulate material (Phillips, 1977).

Analysis of mercury in fish skeletal muscle is arguably one of the best ways of monitoring mercury bioaccumulation (Phillips, 1977; Bryan *et al.*, 1985). Among estuarine fish, the flounder (*Platichthys flesus*), exhibits many of the above-mentioned characteristics necessary in indicator organisms for bioaccumulation studies (Phillips, 1980; Bryan *et al.*, 1985; Elliott *et al.*, 1988). Flounder is widely used in Northern Europe to monitor estuarine mercury contamination (Preston and Portmann, 1981; Kiorboe *et al.*, 1983; McKie, 1983; Kohler *et al.*, 1986; Jensen and Chang, 1987; Elliott *et al.*, 1988; Clark and Topping, 1989; FRPB, 1990; MAFF, 1990, 1991; Stronkhorst, 1992), and a related species, the winter flounder (*Pseudopleuronectes americanus*) has been similarly used recently in North America (Vitaliano and Zoanowicz, 1992).

Results of FRPB monitoring studies on bioaccumulation in biota, kindly made available by FRPB for this review, are shown in Figures 2.5 a-c. Annual mean values (1982-1990) of mercury concentration in the thallus of *Fucus vesiculosus* are shown in Fig. 2.5a. The annual mean mercury concentrations in *Mytilus edulis* tissue are shown in Fig. 2.5b, for the period 1982-1992. Fig. 2.5c shows the annual mean mercury concentrations in skeletal muscle of a restricted size range (180-230 mm) of flounder (*Platichthys flesus*), for the years 1982 to 1991. Data for the years 1982-1990 were taken from FRPB (1991). Data for later years was kindly supplied by FRPB (Dr. S. Hull, pers. comm.).

Mean mercury concentrations in all three species, collected in the vicinity of the ICI mercury discharge (*Fucus*, *Mytilus* at Grangemouth; *Platichthys* at Longannet, LO), showed the same general pattern with time. Generally higher, or peak mercury concentrations were measured in the early 1980's, followed by an overall reduction in the following years. Mean mercury concentrations in mussels and flounder collected at Port Edgar, some 20 km downstream from

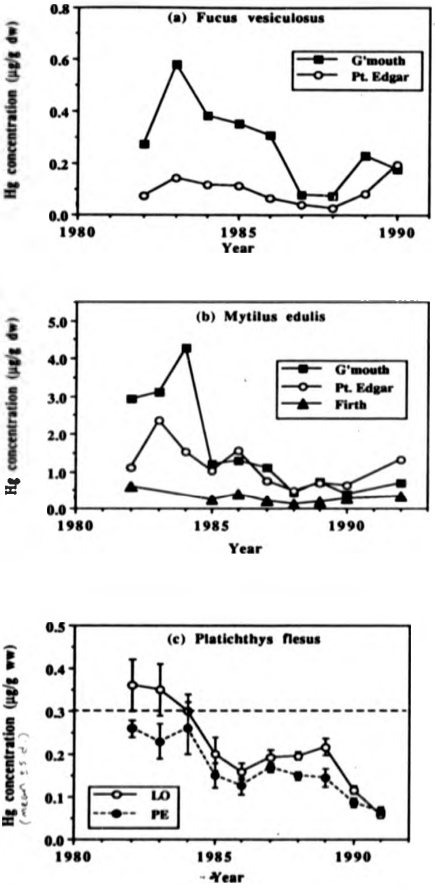


Fig. 2.5 Temporal variability of mercury concentrations in three species from the Forth Estuary and Firth of Forth.

the ICI discharge, followed the same general pattern as those from the upstream site, but with lower mean values in the early 1980's. Despite the different life habits and feeding strategies of mussels and flounders, the apparent trends in mercury content are remarkably similar, both spatially and temporally. Mean mercury concentrations in flounder muscle at Longannet exceeded the previously-mentioned EQS for mercury in fish muscle in only 1982 and 1983, and not at all at Port Edgar.

The mean mercury concentration in *Fucus* from Port Edgar showed no clear pattern during the period 1982 to 1990, except that mercury concentrations were always lower than those in *Fucus* from Grangemouth. Mercury concentrations in mussels collected from the Firth of Forth coast (Cockenzie) during the years 1982 to 1992 were always very low relative to the other sites, and showed no noteworthy variations during the period. Comparison of annual mean mercury levels in biota (Figs. 2.5 a-c) with the annual mercury inputs to the estuary (Fig. 2.2) shows that a reduction in the total amount of mercury discharged annually to the estuary appears to be reflected in a reduction of mercury levels in biota within the estuary.

2.1.2 Use of the teleost fish, eelpout (*Zoarces viviparus* L.), as a monitoring organism, in estuarine environments

2.1.2.1 Biology and ecology of the eelpout

The eelpout is a viviparous, demersal teleost fish, with a moderately elongate body, as shown in Fig. 2.6. The species is a true estuarine resident, abundant in the Forth Estuary, and common around the North Sea and the Baltic (Wheeler, 1969; Elliott and Taylor, 1989). Although individuals in excess of 500 mm are recorded in the literature (Andriashev, 1986), the maximum length recorded in the Forth Estuary is approximately 300 mm (Dr. M. Elliott, pers. comm.). Sexual maturation takes place towards the end of the second year of life (Andriashev, 1986).

The female eelpout has a single median ovary in which, following internal fertilisation of the ovae in late summer, the embryos develop for a period of about four months (Kristoffersson *et al.*, 1973). The young then emerge from the mother at a length of 45-50 mm, between December and March. Fig. 2.7 shows a gravid female eelpout from the Forth Estuary, and a brood dissected from a female of similar size, both from April 1992. The size of these broods varies between 10 and 400 young per female (Andriashev, 1986), the number being correlated with the size of the mother (Gotting, 1976; Elliott and Griffiths, 1986). A visible external yolk sac is present on each embryo during the first two months of pregnancy, after which it is reabsorbed into the embryonic abdomen. There are no special supplementary structures between the mother and the embryos, which lie free in the ovarian cavity during the whole period of pregnancy, surrounded by an ambient nutritive fluid, the

Fig. 2.6 Eelpout (*Zoarces viviparus* L.) from the Forth Estuary, Scotland, March 1992

Fig. 2.7 Female eelpout carrying intra-ovarian brood, and a brood from female of similar size, April 1992

embryotrophe (Kristoffersson *et al.*, 1973).

The eelpout is an important component of the fish assemblage in the Forth Estuary, accounting for 9.3% of fish occurrence in trawl catches. Production of eelpout in the Forth Estuary varied, between 1982 and 1985, from 750 to 1000 kg wet weight.km^{-2}.yr^{-1}, which accounted for 17 to 23% of total fish production (Elliott and Taylor, 1986). Trawling of the subtidal and intertidal areas of the Forth has shown that eelpout, in common with several other fish species, enter the intertidal areas of the estuary at high water to feed, returning to the subtidal areas when the tide falls (Poxton, 1987). The species shows a bimodal annual abundance pattern, with peaks during spring, possibly due to changes in behaviour leading to increased susceptibility to capture, and summer, as Year Class 1 fish reach a size at which they are retained in trawl nets (Elliott *et al.*, 1990). Although not currently captured as a food species in the Forth, historical records show that the species was once collected in the Forth Estuary, and sold for food in Edinburgh (Day, 1884). The species has also been fished for food more recently in the Baltic (Soin, 1968).

Eelpout is a predatory species, feeding on a wide range of macroinvertebrate and meiofaunal species. Recorded prey items include crustaceans (brown shrimp, *Crangon crangon*; shore crab, *Carcinus maenas*; barnacles; amphipods: *Corophium volutator*, *Jaera albifrons*, and *Gammarus* sp), polychaetes (ragworm, *Nereis diversicolor*; *Pygospio elegans*; *Capitella capitata*), molluscs (common mussel, *Mytilus edulis*; baltic tellin, *Macoma balthica*; *Hydrobia ulvae*), and meiofauna (harpacticoid copepods, ostracods, and foraminiferans). Fish eggs and fry are also reportedly taken (Andriashev, 1986; Hall and Raffaelli, 1990; author, personal observations).

2.1.2.2 Eelpout as a monitoring organism for pollutants in estuarine environments

As a resident estuarine fish species, the eelpout has been proposed as, and successfully demonstrated to be, a suitable organism to monitor environmental levels of persistent bioaccumulating substances, and to study the environmental effects of such substances. Mercury levels in eelpout skeletal muscle were shown to be related to environmental mercury levels in the Ems Estuary in the Netherlands, and it was observed that a substantial reduction in mercury inputs to the estuary over time was reflected by a similar reduction of tissue mercury levels (Essink, 1980, 1985, 1988). In response to the fundamental requirements of an indicator species, as established by Phillips (1977, 1980) and Bryan *et al.* (1985), Jacobsson *et al.* (1986) summarised the reasons why the eelpout should be a suitable indicator species for estuarine environments in northern Europe as:

1. It has a relatively small home range during the entire life cycle;
2. Single specimens are large enough to provide samples for chemical and

physiological analysis;

3. Specimens are relatively easy to catch, and are available all year round;

4. A high abundance within a wide geographical area makes monitoring easier and broadens the application of the system;

5. The organism is easily aged, using growth marks on the otolith bones;

6. The relatively long life span (commonly up to 5 years, occasionally longer) allows the integration of effects over time, and may reveal the effects of long-term exposure;

7. The viviparous mode of reproduction provides the opportunity to observe accumulation or effects of a substance over more than one generation.

The same workers subsequently used the eelpout to assess successfully the effects of petrochemical waste emissions on reproductive success of fish in the locality of discharges, where a clear effect of reduced brood survival was observed in fish caught close to the effluent discharges, compared with fish from clean control sites (Jacobsson and Neuman, 1991). The species has also been proposed as a suitable organism to monitor sub-lethal effects of oil components on resident fish in coastal waters, via the activity levels in liver tissue of aryl hydrocarbon hydroxylase (AHH), a component of the cytochrome P_{450} system (Jensen and Knudsen, 1983).

The eelpout clearly has potential as an indicator organism for the assessment of bioaccumulation of persistent pollutants, and thereby of the availability to the biota of such substances in the estuarine environment. Mercury levels in females and the young from the brood have been studied previously in Forth Estuary eelpout to assess the effects of mercury contamination on fecundity (Elliott and Griffiths, 1986). After standardising for length and weight of female fish, no significant difference was observed in fecundity, despite a significant difference in the degree of maternal mercury contamination between sites.

2.1.3 Rationale and aims of study

The principal hindrance to the use of a fish species as a representative indicator or sentinel organism for the assessment of contaminant levels in a particular habitat was summarised by Elliott *et al.* (1988) as follows:

"Contaminant levels in fish will vary with season, physiological and reproductive condition, sex, size and age, in addition to contaminant exposure. Therefore, the variability due to all but the latter has to be reduced or quantified in order to produce a high signal-to-noise ratio such that valid spatial and/or temporal trends can be distinguished". With regard to temporal variation of mercury, following a limited study of mercury in saithe (*Pollachius*

virens), ICES (as Topping *et al.*, 1975) recommended that studies of seasonal variation should be undertaken for all fish species that are selected for monitoring programmes, in order to identify the time period when changes in metal levels of tissues are minimal. This proposal has been largely disregarded, with respect to mercury, since that time. These statements provide the basis for the work presented in this chapter. The aims of the study are as follows:

1. To assess the degree of mercury contamination in several tissues, in particular the skeletal muscle and liver tissues, of the eelpout (*Zoarces viviparus* L.) from populations in the Forth Estuary and Firth of Forth;

2. To quantify variability of mercury concentrations in tissues of eelpout, in relation to a number of biological parameters (sex, length, weight, year class);

3. To quantify spatial variability of mercury concentrations in tissues of eelpout from a number of sites in the Forth Estuary and Firth of Forth, along a historical environmental gradient of mercury contamination;

4. To quantify temporal variability of mercury concentrations in tissues of eelpout, both seasonal and annual, within individual sites, and to relate this, where possible, to environmental or biological factors;

5. To assess the utility of the analysis of covariance (ANCOVA) statistical technique for comparison of the mercury content of samples, where there is a covariance between contaminant level and size or age of fish.

2.2 Materials and Methods

2.2.1 Sampling Procedures

2.2.1.1 Sampling Sites

Eelpout were collected as a by-catch of routine demersal fish population monitoring surveys carried out by the Forth River Purification Board (FRPB), at five subtidal sites in the lower Forth Estuary, and one site in the Firth of Forth. The estuary was sampled five times annually, and the Firth site four times during these surveys. The locations of all sites are shown on Figure 2.8, with the distance of the mid-point of each trawling site from the industrial mercury discharge at Grangemouth given in Appendix 2.1.

When present in catches, eelpout were collected from the two sites, Longannet (LO) and Port Edgar (PE), to permit the study of mercury contamination from sites previously demonstrated, by Elliott and Griffiths (1986), to have different degrees of mercury contamination. Of the five trawl sites in the lower estuary, these two sites had also been found previously to have the largest catches of eelpout (Dr. M. Elliott, pers. comm.). Trips were also made, weather conditions and available boat time permitting, to collect eelpout samples from these two sites when routine fish population surveys were not scheduled. Catches of eelpout from the intermediate sites, Bo'ness (BO) and Tancred (TC), had previously been low during routine surveys and, similarly, in this study eelpout were absent or only present in low numbers from these intermediate sites on most sampling occasions. Blackness (BN) occasionally provided relatively large catches of eelpout.

The Firth of Forth is regarded as generally uncontaminated with mercury (Davies, 1987). Eelpout were collected, therefore, at Kingstone Hudds, from the routine demersal fish population surveys carried out at this site during the study period, to provide a comparison with fish from the Forth estuary sites.

2.2.1.2 Sampling Dates

Samples were collected from the Forth Estuary sites and the Firth of Forth reference site between November 1989 and April 1992. A sample of eelpout was also obtained from the Firth of Clyde in June 1991. These fish were caught intertidally by hand at Ballochmartin Bay, near Millport, on the Isle of Great Cumbrae (Ordnance Survey grid reference NS/182570). Three visits were made to attempt to catch eelpout specimens from the Ythan Estuary, north of Aberdeen (December 1989 and June 1990), but a combination of beam trawling and eel-fyke trapping failed to capture any specimens of eelpout from this estuary.

2.2.1.3 Trawling Procedure

Fish were collected in the estuary by Agassiz trawling at slack water, using an Agassiz frame

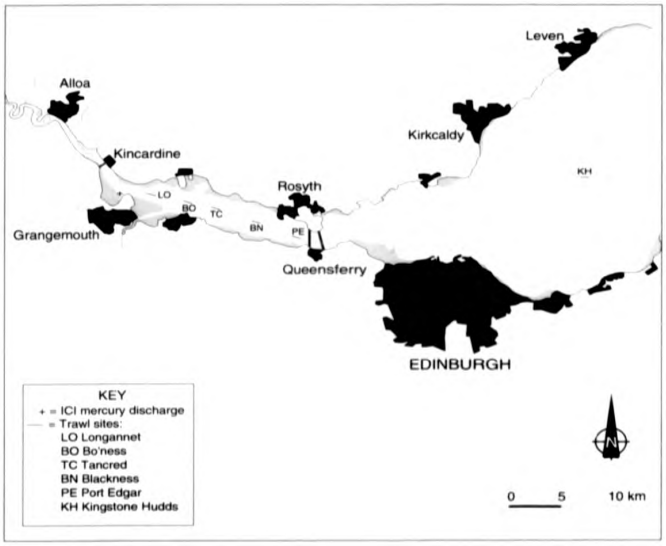


Fig. 2.8 Location of trawl sample sites, in relation to mercury discharge at Grangemouth

with a two metre mouth width, and a 15 mm stretched-mesh net. The net was towed over trawls of 0.8 km length at a speed of 2.5 knots by the S.V. *Forth Ranger*. Trawl sites were located on each occasion using a standard DECCA Plotter navigation system. At the end of each trawl, the net was hauled and the entire catch removed from the cod-end of the net to a fish box for sorting. Eelpout were removed from the catch at this stage. Fish were caught at the site in the Firth of Forth by a combination of Agassiz and Otter trawling. Trawls of 1 km length were carried out at this site, and fish collection was performed as described above.

2.2.1.4 Fish Sacrifice and Allometric Measurements

Fish were sacrificed on board the boat by a blow to the head. The total body length, from the tip of the top lip to the end of the tail, was recorded to the nearest millimetre.Each fish was then wrapped in a separate, sealable polythene bag labelled with a number coded to the length measurement record. Fish samples were then kept in a refrigerator at 4° C until the boat returned to port. On return to the laboratory (maximum 1 hour after removal from the refrigerator on board the vessel), the fresh weight of each fish was recorded to the nearest 0.01 grammes on an electronic balance. If fish were not to be dissected immediately, they were re-bagged and placed in a freezer at -20° C until dissection was performed. Otoliths (ear bones) were taken, during dissection, from the head of fish from November 1989 to April 1990, and used to assign fish to a year class, as outlined in Appendix 2.2.

2.2.1.5 Collection of Tissue Samples for Total Mercury Analysis

A skeletal muscle sample was collected from the tail of each eelpout, following removal of the skin. Tail muscle samples were dissected from the same position on each fish, after removal of the skin, as shown in Figure 2.9. Liver tissue, usually the whole organ, was also collected for mercury analysis. As the liver of *Zoarces viviparus* is very homogeneous in composition (Pekkarinen, 1980), sub-samples were collected from very large livers (liver weight >1.5 g). The whole kidney was removed from fish captured during Summer 1990 and in the period Summer 1991 to Spring 1992. Very small fish were found to have insufficient kidney material to permit accurate mercury analysis. As a result, kidney samples were generally collected only from older fish (greater than 120 mm in length). The kidney in eelpout lies along the mid-line of the dorsal side of the body cavity. When a kidney tissue sample was to be collected, it was necessary first to dissect the gonad tissue, followed by a mesenteric tissue skin covering the kidney tissue proper. No attempt was made to differentiate between the head and tail regions of the kidney. The whole kidney was removed, weighed and frozen as described in section 2.2.1.6.

Gonadal and reproductive tissues were collected from most fish from April 1990 onwards. Male *Z. viviparus* have paired-lobed testes (medial on the posterior dorsal wall of the body cavity. These were dissected out of the animal and weighed before freezing, as described in Section 2.2.1.6. Female *Z. viviparus* have a single ovary attached medially to the posterior

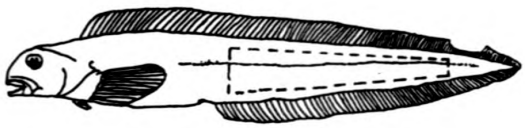


Fig. 2.9 Location of skinned skeletal muscle fillet collected for mercury analysis, indicated by the dashed box

dorsal wall of the body cavity. From non-gravid female eelpout (fish not carrying embryonic fish within the ovarian sac), the ovary was dissected out whole and weighed, then frozen at -20°C. Female fish carrying embryos or larval fish were frozen before dissection. The whole ovarian sac was dissected while still frozen solid, and total weight recorded before thawing.

Ovarian fluid constitutes a highly variable percentage of the total weight of the ovary during development of the brood (see Chapter 3 for details). From gravid female fish collected between November 1990 and April 1992, ovarian fluid was allowed to drain from each thawing ovary to collect in a weight-tared polythene petri dish, and wet weight was recorded to the nearest 0.01g. Details of mercury analysis of ovarian fluid will be presented elsewhere (see Chapter 3). The total number of embryos in each gravid female fish ovary was recorded. The total weight of each brood was recorded to the nearest 0.001g. The length of each individual embryo was measured to the nearest 1 mm. From September 1991 to April 1992, the wet weight of each individual embryo was also recorded to the nearest 0.001g. For early stage broods (from September and October each year), the entire brood was frozen for later mercury analysis. In later broods, with larger individual embryos, a sub-sample of 6-10 embryos was taken at random from each brood, total weight of sub-sample measured to the nearest 0.001g, and frozen for later mercury analysis. After collection or sub-sampling of the ovarian fluid and brood, the wet weight of the remaining ovarian sac tissue was recorded to the nearest 0.001g, and frozen for later mercury analysis.

2.2.1.6 Tissue Sample Treatment and Storage

On dissection, each tissue sample was placed into a weight-tared, clean polypropylene petri dish marked with the sample's identifying number, and the wet weight of the sample was measured on an electronic balance to the nearest 0.001 g. Samples were then stored in a freezer at -20° C until mercury analysis was performed. To minimise the chance of operator bias with samples from sites with suspected differing degrees of mercury contamination, all tissue samples were treated anonymously after collection (by sample number only) until final tissue mercury concentrations were calculated.

2.2.2 Mercury Analysis Procedure

2.2.2.1 Solutions and Reagents Preparation

All chemicals used in this analytical procedure were of "Analytical" grade or equivalent. De-ionised distilled water (DDW) produced in the laboratory of the Forth River Purification Board by a Elgastat laboratory deioniser contains no detectable level of mercury and is used routinely for trace metal analytical work in the FRPB laboratory. This source of DDW was used, therefore, for all solution preparation in mercury analytical work carried out by the author at the FRPB laboratory.

Tissue digestion and cleaning of apparatus was carried out in the laboratory at University of Stirling. Milli-Q filtered water (Millipore), produced by filtration of deionised water to remove ionic and organic components, was found to contain no detectable mercury and was used for solution preparation and cleaning procedures (see section 2.2.2.2). Analytical grade nitric acid (BDH "Analar") is of sufficient purity for use in trace metal analysis of fish tissues (Harper *et al.*, 1989; Philips, 1989) and was used, therefore, as a less expensive alternative to "Aristar" or "Superpure" grades in this work.

Tin (II) chloride (stannous chloride, $SnCl_2$ 2-hydrate) was used as a reductant solution (22.5% vol/vol with 50% hydrochloric acid, HCl) and was prepared as follows. A 225 g sample of tin (II) chloride 2-hydrate (BDH Spectrosol) was dissolved completely in 1000 cm^3 of concentrated hydrochloric acid (HCl, 36% w/w, BDH "Analar") and then made up to 2000 cm^3 with DDW. The solution was then bubbled vigorously with nitrogen gas (N_2, oxygen-free, BOC) for three to four hours to remove any traces of mercury contamination (Dr. D. Harper, pers. comm.).

2.2.2.2 Cleaning of Analytical Apparatus and Minimising Contamination

All experimental vessels and storage containers were cleaned by the procedures of Harper *et al.* (1989) before each use. All vessels and containers, plastic and glass, were soaked overnight (or for a minimum of eight hours) in a solution (5% volume/volume with Milli-Q filtered water) of Decon 90™ detergent (Decon Laboratories, Hove, Sussex), in a non-circulating water bath at approximately 30°C. The bath was covered by "Cling-Film" to minimise airborne contamination during the soaking process. The equipment was then thoroughly rinsed several times with Milli-Q filtered water. Finally, equipment was soaked in a solution of nitric acid to remove surface trace metal contamination.

Glassware was soaked in a 10% nitric acid solution (HNO_3, volume/volume with Millipore-Q filtered water) overnight and rinsed thoroughly before use. Plastic apparatus and containers soaked in a 5% nitric acid solution (v/v with Millipore-Q filtered water), as stronger acid solutions may have the effect of activating the surface of the plastic, creating a greater risk of contamination (Philips, 1989). Containers with lids were stored sealed until required, and other apparatus was stored after wrapping in "Cling-Film". The acid-soaking procedure was not carried out for the polytetrafluoroethene pressure digestion vessels used for sample dissolution. A final cleaning stage (described in section 2.2.2.3) was carried out instead, prior to their use for tissue digestion.

To reduce potential contamination by other users, the nitric and hydrochloric acids used in the above procedure, and in the tissue digestion procedure described later (see section 2.2.2.3) were taken from bottles dedicated to these uses (Philips, 1989). Labelled beakers and vessels were retained for use with specific individual solutions (i.e. DDW, or stannous chloride, or 50% nitric acid solutions) to reduce the risk of cross-contamination.

2.2.2.3 Cold Vapour Atomic Absorption Spectrophotometry for Total Mercury

(a) Tissue Digestion Apparatus

Pressure dissolution of tissues was performed using polytetrafluoroethene (ptfe, or Teflon) pressure digestion vessels, obtained from Valtech Plastics plc (Thirsk, North Yorkshire, UK). Each consists of a vessel (10 ml volume) with a screw-threaded rim, a pressure seal gasket, and a cap fitted with a screw thread. A laboratory oven (MacFarlane Robson Ltd) was used to heat the digestion vessels for pressure dissolution. The oven was operated inside a fume cupboard.

(b) Cold Vapour Atomic Absorption Spectrophotometry Apparatus

Cold vapour atomic absorption spectrophotometric measurements for mercury were carried out using a Data Acquisition Cold Vapour Atomic Absorption Spectrophotometer (model number DA-1500 DP6, Data Acquisition Ltd., Stockport, Cheshire, England). This machine has a single mercury vapour lamp, and operates a negative pressure pump system to draw mercury vapour into the beam of the lamp in a glass absorbance tube. The absorbance, by mercury vapour in the tube, of light from the lamp is measured at 253.65 nm, and converted to a numerical value which is displayed in real-time on a digital read-out. This system quantifies the absolute mass of mercury released from the volume of solution assayed.

A novel apparatus, shown in Fig. 2.10, was designed by Dr. D. Harper (FRPB) to facilitate the reduction of mercuric ions in solution, to elemental mercury vapour for quantification by CVAAS. The system, by minimising the volume of the reaction vessel and tubing to minimise the retention time of mercury vapour in the system, produces well-defined absorbance peaks, even for sample solutions with low mercury concentrations (Dr. D. Harper, pers. comm.). The reaction vessel was constructed from a 30 ml screw-topped polypropylene tube (Sterilin), with two holes drilled in the cap to take tubes for air and mercury vapour transport. Narrow, stiff plastic piping (2 mm.) was fitted through these holes, and the airtightness of the holes was improved using ptfe tape.

Mercury vapour was removed from the reaction vessel by negative pressure and taken, by the minimum practical length of silicone rubber tubing (5 mm diameter), to a drying tube. This contained granular magnesium perchlorate ($Mg(ClO)_4$, BDH Analytical) as a drying agent, packed under glass wool, to remove moisture and nitric acid vapour. Mercury vapour was then taken, again by the minimum practical length of silicone tubing, to the "Sample-In" port of the cold vapour atomic absorption spectrophotometer. This system was found to improve the sensitivity of the method when used for lower concentration samples (Dr. D. Harper, pers. comm.).

(c) Analytical Procedure for total mercury determination

(i) Pressure Dissolution Procedure for Tissue Samples

Prior to use, ptfe digestion vessels were given a final cleaning by pressure heating for one hour,

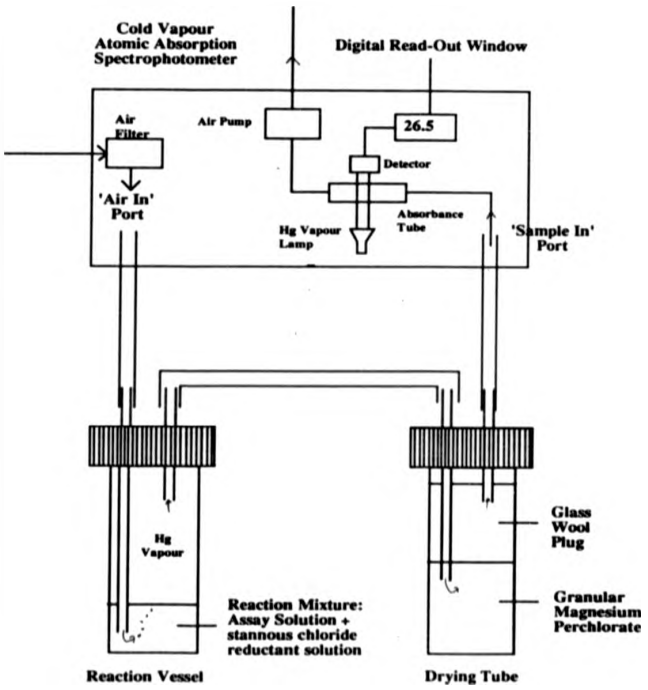


Fig. 2.10 Cold Vapour Atomic Absorption Spectrophotometry system for determination of total mercury.

in a laboratory oven at 110°C, with 5 ml of concentrated nitric acid (BDH 'Analar'). Vessels were then removed from the oven, left to cool for one hour, and then the insides were rinsed several times with Milli-Q filtered water. Vessels were stored with caps on to reduce the risk of airborne contamination. The dry weight of each vessel was then determined by oven-drying to constant weight. The time to reach constant weight was usually 1-2 hours at 110°C. The dry weight of a digestion vessel was standardised, therefore, as the weight after 2 hours drying at 110°C.

Tissue samples for digestion were crudely homogenised by fine chopping while frozen. A single sample was then placed in each digestion vessel and dried to constant weight at 110°C. Each vessel with tissue was weighed after two hours, and then every hour until a constant weight was reached. Large tissue samples (> 2 g) were dried overnight. The dry weight of the tissue was calculated by subtraction of the dry weight of the vessel from the dry weight of vessel + dried tissue.

It was found that use of smaller volumes of acid with small tissue samples (<0.1 g), by increasing the concentration of mercury in solution, increased the sensitivity of the method for use with these samples, resulting in very few tissue samples with mercury concentrations below the limit of detection. Samples of weight > 0.1 g were digested with 5 ml of concentrated nitric acid acid, while 2.5 ml of acid was added to smaller samples. The caps and pressure seals were added to the vessels, and hand-tightened. The vessels were then placed in a laboratory oven (in a fume cupboard), at 110°C for 1 hour, then removed, and left to air cool for at least 1 hour. Digest solutions were then diluted 1:1 with DDW in pre-marked, acid-cleaned polypropylene or polythene storage tubes, labelled with the tissue sample reference number. These tubes were stored in batches in clean, resealable polythene bags for up to one month until analysed for total mercury.

(ii) Cold Vapour Atomic Absorption Spectrophotometry (CVAAS) procedure for total mercury determination

The absorbance reading on the spectrophotometer was zeroed, at a wavelength of 253.6 nm, by aspirating 2 ml.of DDW in the reaction vessel. The background absorbance of the solution matrix was established as the mean absorbance of 6 assays of 4 ml of the matrix solution (2 ml of 50% nitric acid solution + 2 ml of 22.5% stannous chloride solution).

An aliquot (2 ml) of the sample assay solution was pippeted into the reaction vessel. An aliqot (2 ml) of the 22.5% stannous chloride reductant solution was then added, and the cap screwed on immediately. A fine stream of air bubbles was immediately drawn into the solution by negative pressure from a continuously-running internal pump in the spectrophotometer, and mixing was aided by gentle manual shaking of the vessel. The elemental mercury vapour (Hg^{o}), released by reduction of mercuric ions (Hg^{2+}), was aspirated out of solution by the bubbled air. After passing through the drying tube, and into the absorbance tube of the

spectrophotometer, the mercury vapour caused an absorbance of the light from the mercury lamp. The magnitude of the absorbance was registered as a value on the digital read-out of the spectrophotometer. The solution was shaken until a peak value of absorbance was reached and had begun to decrease. The cap was then removed, the assayed solution poured into a waste solution beaker, and the vessel was rinsed out several times with tap water. Duplicate assays were performed on each tissue sample solution. The zero of the machine was reset by aspirating with DDW approximately every 10 samples.

(iii) Calibration of CVAAS analysis for mercury

Absorbance measurements were related to quantitative masses of mercury by calibration with inorganic mercury solutions. A stock solution of mercuric nitrate was prepared using a spectrophotometric standard mercury solution. One ml of 1×10^3 mg Hg^{2+} $litre^{-1}$ mercuric nitrate solution (BDH Spectrosol™ Spectroscopy Standard Solution) was made up to 100ml, to produce a solution of 10 mg $Hg^{2+}.litre^{-1}$. One ml of this stock solution was made up to 100 ml with 50% nitric acid solution, to produce a calibration solution of 0.1 mg $Hg^{2+}.litre^{-1}$ (100 $\mu g.litre^{-1}$). The stock solution was kept for up to two weeks before a new solution was prepared. The calibration solution was prepared freshly for each day on which mercury analysis was carried out.

Absorbance readings were related to mercury masses using a calibration graph, prepared fresh each day. A volume of 10 µl of the 100 µg $Hg.l^{-1}$ working solution contained 1 ng of Hg^{2+} ions in solution. A series of assays was made, measuring the absorbances of different volumes of working solution, and hence different, known masses of mercuric ions. The range of masses covered was usually 1-100 ng mercury (i.e. 10-1000 µl of working solution). The total assay volume was always 2 ml, the difference in each case being made up with 50% nitric acid, to maintain the same acid matrix as that of the digested tissue samples.

Triplicate assays were made of each mercury mass, the mean background absorbance value (A_B) was subtracted from each, and a calibration graph was drawn with known mercury mass as the independent variable, and background-corrected absorbance (A_C) as the dependent variable. The calibration graph was linear over the range of masses used on every occasion. A best-fit line was fitted to the data by least-mean squares linear regression using a desktop calculator. The correlation coefficient of the relationship between mercury mass and absorbance was greater than 0.97 on every ocassion. The linear regression equation was of the form:

$$\text{Background-corrected Absorbance, } A_C = a + b \times M \qquad \text{(Equation 2.1),}$$

where a and b are constants, M is Hg mass (ng). The calculation of tissue sample mercury concentrations from absorbance readings is described in Appendix 2.3.

2.2.2.4 Method Testing

(a) Recovery of mercury (accuracy)

The accuracy of the whole analytical procedure, defined as the proximity of a measured value to the true value (Philips, 1989), was tested by measuring the recovery of known masses (5 ng) of mercury from digested samples. The recovery of mercury was measured from samples of concentrated nitric acid spiked with known masses (5ng) of mercury (in 50 ul aliquots of the calibration solution), followed by the pressure digestion stage and dilution with DDW. A mean recovery of 102.1 ± 8.97 % of the spiked mercury was obtained, indicating the satisfactory operation of the method for an inorganic solution matrix.

The possibility existed that the presence of a complex mixture of organic materials in the digest solution might interfere with the efficiency of either the digestion procedure or analytical procedure. The recovery of mercury from tissue samples was also tested, therefore, using a standard analytical Certified Reference Material, DORM-1, a dried, powdered dogfish muscle sample with a total mercury concentration guaranteed to be within a certain range (National Research Council of Canada). Accurately-weighed sub-samples of DORM-1 were subjected to the full mercury analytical procedure described above, as advised by Hamilton (1991). The sub-samples were dried to constant dry weight, pressure digested with concentrated nitric acid at 110°C for one hour, diluted (1:1 vol./vol. with DDW), and analysed for total mercury by CVAAS. Values of recovery greater than 100% have been included in the calculation of mean recovery (Bloom, 1992).

The mean recovery of total mercury from DORM-1, of 0.740 ± 0.080 $\mu g.g^{-1}$ (equal to 92.8 % recovery, with a relative standard deviation of 10.8 %), lies within the limits of variability of the certified total mercury concentration of DORM-1 (0.798 ± 0.074 $\mu g.g^{-1}$). It was established, therefore, that the method outlined above gave a satisfactory recovery of total mercury from fish tissues.

(b) Detection Limit of CVAAS method (precision)

The limit of detection (precision) of the method was established according to the procedure of Philips (1989). Ten replicate assays for total mercury were performed on small volumes (100 µl) of calibration solution (100 $\mu g\ Hg^{2+}\ l^{-1}$), containing known and detectable small masses of mercury (10 ng). The total assay volume was mad up to 2 ml with 50% nitric acid (v/v with DDW) and assayed for total mercury by CVAAS. The mean Hg mass and standard deviation of the ten replicate assays was 9.9 ± 0.52 ng. The limit of detection was taken as the value of two standard deviations i.e. a mass of approximately 1 ng of Hg. As most assays were carried out on 2ml of a 10 ml sample, this is equivalent to a tissue concentration of approximately 5 $ng.g^{-1}$, the lower range of detection reported by Harper *et al.* (1979), on whose methodology much of the above is based.

2.2.3 Data Treatment and Statistical Analysis

2.2.3.1 Treatment of mercury concentrations below Limit of Detection

Using the CVAAS method described here, the value for limit of detection (LD) of the method relates to an actual mass of mercury in the assay. Jensen (1982), and Hansen (1982), both suggested that samples with a mercury mass below the LD could be assumed to contain a mass of mercury half that of the LD. This argument is based on the premise that if a sample is below the LD, then the true value of the mass of mercury contained must lie between the LD and zero It was stated that this estimation of values below LD was statistically safe, introducing no serious bias, as long as such samples accounted for no more than 5 % of the total. They argued that a more serious bias would be introduced if values below LD were excluded completely. As only a very small number of samples in this study were found to be below the LD value, they were assumed to contain a mass of 0.5 ng, ie half the LD value, and tissue concentrations were calculated using this value.

2.2.3.2 Seasonal Pooling of Samples

In order to provide sufficiently large sample sizes to permit robust statistical comparisons, and to assess temporal variability of mercury concentrations or burdens through an annual cycle, individual sampling dates were pooled into one of four three-month periods, or seasons, defined as follows:

Autumn: September to November
Winter: December to February
Spring: March to May
Summer: June to August

These periods were selected as they represented both clearly identifiable divisions with respect to water temperature in the Forth Estuary (see Appendix 2.4), and biologically significant periods in the repoductive cycle of the eelpout. In the 'Summer' period, for example, the ovae in the female ovary undergo vitellogenesis, and the male testes increase rapidly in size. The weight of the brood increases only slowly through the 'Autumn' period, and rapidly towards departure of the brood from the ovary ('partus') in the 'Winter' period. The terms Autumn, Winter, Spring and Summer are used hereafter to represent the three-month periods defined above.

2.2.3.3 Wet and dry weight mercury concentrations

It was observed that, for fish with a known fresh weight, on re-weighing after freezing for up to four months at -20°C, there is a weight loss due to dehydration. The magnitude of this loss is negatively correlated with the original fresh weight, as shown in Fig. 2.11. The data showed

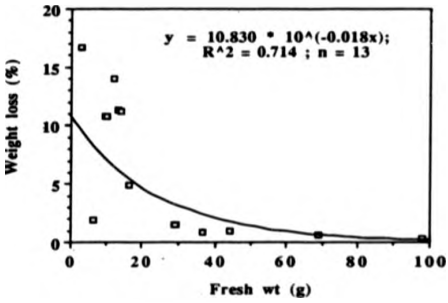


Fig. 2.11 Weight loss on freezing for 4 months at -20degC: relation to fresh weight for eelpout from Forth Estuary, November 1991

Table 2.2 Water content[1] of eelpout skeletal muscle

Site	Date of Collection	Sample Size	Water Content (%) Mean	sd
LO	14-06-91	26	77.21	2.000
PE	" "	21	77.47	1.983
KH	31-07-91	11	75.56	3.176
Pooled[2]	-	58	76.99	2.326

Notes: [1]. As measured by loss of weight after drying to constant weight at 110°C.
[2]. No significant differences between sites (ANOVA: F= 2.831, P= 0.068).

a good fit to an exponential decay curve, although there is considerable scatter in the data for smaller fish. The loss appeared to be due to water loss, probably predominantly from the skeletal muscle, as a skin of ice formed around the carcass, to be lost on thawing. As the internal organs were generally frozen in fluid in the body cavity, it was assumed that weight loss from organs and tissues, other than skeletal muscle, was minimal.

Wet weight mercury concentrations of skeletal muscle are likely, therefore, to show a bias, if muscle samples are not collected from freshly killed fish and weighed before freezing. Wet weight concentrations in muscle samples from small fish may be potentially more than 15% greater than the fresh weight value. It was decided, therefore, in the interest of accuracy, to use the dry weight concentrations for comparisons between samples. This has the added advantage of allowing an easy comparison with previous studies of mercury in components of the Forth estuary ecosystem, which also cited dry weight concentrations (Elliott and Griffith, 1986).

The water content of skeletal muscle was measured accurately in Summer 1991 in samples of skeletal muscle from freshly-killed eelpout from three sites. Water content was measured as the loss of weight on drying to constant weight at 110°C. Results, presented in Table 2.2, show no significant differences between sites. Wet weight mercury concentrations of skeletal muscle, where presented in this study, were estimated by correction of the dry weight concentration, using the mean water content:

Estimated Hg conc (wet weight) = Observed Hg conc (dry weight) * (100-76.99/100).

Although a small error is introduced by this correction, due to the variability of water content between individual fish, the magnitude is much less than that arising from the bias due to size-related weight loss on freezing.

2.2.3.4 Statistical Analysis

All variables in analyses were assessed for fit to a normal distribution by means of a frequency histogram. If a variable was not normally-distributed, transformations were used to attempt to obtain normal distributions (Zar, 1984). In cases where a variable showed a non-normal distribution, a $\log_{10}$ transformation was found to normalise the distribution. Parametric statistical techniques were used for normally-distributed data sets (Zar, 1984). If transformation did not produce a normal distribution, then non-parametric analyses were used (Sokal and Rohlf, 1981). Non-parametric analyses were also used when sample sizes were insufficiently large to permit description of the distribution of a variable. Statistical tests were carried out using either the SPSSx™ statistical package, on a Hewlett-Packard mainframe computer system, or the Statview™ statistical package, on an Apple MacIntosh personal computer.

Where mercury values showed a linear covariance with a biological parameter, such as length or

weight, a linear regression was calculated, and sample regressions were compared using an Analysis of Covariance (ANCOVA: Zar, 1984). Sums of squares were calculated for the ANCOVA using the SPSSx package, and subsequent calculations of test-statistics were performed manually. When two regressions were compared, the t-statistic was calculated, and a two-tailed test was performed, the difference between regressions being accepted as significant at $P< 0.05$. More than two regressions were compared firstly, using the F-statistic, for overall coincidence of regressions, with a one-tailed test of significance. If the differences between regressions were significant at the 5% level ($P< 0.05$), the slopes and elevations of the regressions were compared separately, and significant differences were identified using Tukey Multiple Comparison Tests (Zar, 1984).

2.3 Results

2.3.1 Total mercury concentrations in eelpout tissues

2.3.1.1 Mean total mercury concentrations in eelpout tissue

The results of mercury analysis in six tissues of eelpout are shown by individual sites, and for the whole study, in Table 2.3. The maximum mercury concentrations measured in the study were, on a wet weight basis, in skeletal muscle at Longannet, and on a dry weight basis, in liver tissue at Port Edgar. In general, mercury concentrations were lower in reproductive tissues (testes, ovary, brood) than in non-reproductive tissues (muscle, liver, kidney), and lowest of all in brood tissues. Occasional unrecorded tissue sample weights (wet or dry) resulted in some different sample sizes for wet and dry weight concentrations.

2.3.1.2 Correlations between tissue total mercury concentrations

The correlation of total mercury concentrations in seven tissues of eelpout is presented in Table 2.4. Mercury concentrations generally show significant positive correlations between tissues. There is a considerable degree of variability in the data, as seen from the generally low correlation coefficients, despite correlations being highly significant in many cases. The mercury concentration of whole ovary is not significantly correlated with those of skeletal muscle nor liver. Ovarian tissue levels also show no correlation with muscle levels. Notably, while the mercury concentrations of brood tissues and skeletal muscle are significantly correlated, those of brood and ovarian tissues are not.

2.3.2 Variability of mercury concentrations in eelpout skeletal muscle

2.3.2.1 Variability related to biological parameters

The total mercury concentration of eelpout skeletal muscle was examined, on a dry weight basis, for individual sexes within sites in each season, for relationships with a number of biological variables: length, weight, and age (as year class). As shown for fish from Longannet (LO) in Winter 1989/89 (see Fig 2.12, 1a-3a), mercury concentration increases with increasing length, weight and year-class. The increase is not linear, however, with length or year class. A transformation of the Hg concentration data to $\log_{10}$(Hg concentration) has, in this case, the dual advantage of producing a set of values which shows both a good fit to a normal distribution (see Materials and Methods: 2.2.3.3), and a linear relationship with length, weight or year class, as shown in Fig. 2.12, 1b-3b.

Seasonally-pooled single-sex samples were assessed for linear relationships between the $\log_{10}$ of mercury concentration and length, weight, or year class. Linear regression equations for

Table 2.3 Summary statistical parameters for mercury concentrations in seven tissues of eelpout from sites in the Forth estuary[1], Firths of Forth[2] and Clyde[3], and for all sites pooled

Tissue	Site	Total Hg Concentration									
		Total Hg (μg.g⁻¹ wet weight)					Total Hg (μg.g⁻¹ dry weight)[4]				
		Mean	sd	Min	Max	n	Mean	sd	Min	Max	n
Muscle	LO	0.114	0.087	0.01	0.75	275	0.495	0.378	0.04	3.26	275
	BO	0.183	0.190	0.05	0.72	11	0.797	0.826	0.22	3.14	11
	TC	0.135	0.080	0.04	0.35	20	0.585	0.347	0.18	1.52	20
	BN	0.118	0.094	0.01	0.52	66	0.514	0.407	0.05	2.28	66
	PE	0.094	0.063	0.01	0.43	241	0.408	0.272	0.04	1.87	241
	KH	0.073	0.046	0.02	0.25	59	0.319	0.201	0.07	1.09	59
	MP	0.054	0.040	0.02	0.16	11	0.233	0.174	0.08	0.68	11
	All sites	0.104	0.081	0.01	0.75	683	0.454	0.350	0.04	3.26	683
Liver	LO	0.109	0.110	<0.004	0.67	229	0.328	0.346	<0.004	2.91	233
	BO	0.278	0.459	<0.004	1.09	5	0.728	1.176	<0.004	2.80	5
	TC	0.099	0.070	0.02	0.24	8	0.319	0.276	<0.004	0.87	10
	BN	0.107	0.060	0.01	0.21	36	0.387	0.313	<0.004	1.07	45
	PE	0.102	0.08	<0.004	0.64	174	0.356	0.390	<0.004	3.81	182
	KH	0.095	0.074	0.01	0.34	39	0.313	0.234	0.04	1.05	39
	MP	0.106	0.024	0.07	0.18	14	0.332	0.121	0.24	0.67	13
	All sites	0.107	0.102	<0.004	1.09	505	0.345	0.363	<0.004	3.81	527
Kidney	LO	0.105	0.096	0.01	0.48	33	0.436	0.421	0.04	1.69	33
	BN	0.126	0.079	0.03	0.27	20	0.545	0.414	0.11	1.77	21
	PE	0.082	0.036	0.02	0.14	22	0.389	0.188	0.09	0.82	22
	KH	0.072	0.026	0.03	0.14	28	0.398	0.199	0.12	0.92	28
	MP	0.071	0.035	0.04	0.13	5	0.392	0.109	0.22	0.50	5
	All sites	0.094	0.069	0.01	0.48	108	0.436	0.324	0.04	1.77	109
Testes	LO	0.086	0.096	<0.004	0.44	64	0.355	0.268	<0.004	1.43	67
	BN	0.079	0.059	0.01	0.18	20	0.429	0.365	0.08	1.39	20
	PE	0.082	0.076	0.01	0.36	60	0.360	0.308	0.06	1.70	60
	KH	0.052	0.052	0.01	0.20	20	0.288	0.250	0.05	0.90	20
	MP	0.023	0.024	0.01	0.04	2	0.189	0.045	0.16	0.22	2
	All sites	0.081	0.083	<0.004	0.45	183	0.372	0.321	<0.004	2.05	186
Whole ovary[5]	LO	0.053	0.050	<0.004	0.20	37	0.245	0.210	<0.004	0.89	38
	BN	0.080	0.069	0.02	0.18	8	0.228	0.137	0.09	0.41	8
	PE	0.056	0.056	<0.004	0.22	36	0.243	0.214	<0.004	0.79	35
	KH	0.060	0.044	<0.004	0.18	24	0.264	0.180	<0.004	0.71	24
	MP	0.048	0.023	0.03	0.07	3	0.238	0.097	0.16	0.35	3
	All sites	0.057	0.051	<0.004	0.22	112	0.248	0.194	<0.004	0.89	112
Brood	LO	0.039	0.047	0.01	0.22	24	0.196	0.169	0.02	0.75	24
	BN	0.041	0.021	0.02	0.08	10	0.159	0.136	<0.004	0.42	16
	PE	0.029	0.028	<0.004	0.14	31	0.148	0.125	0.01	0.54	35
	KH	0.050	0.022	0.02	0.08	8	0.179	0.070	0.10	0.28	8
	All sites	0.036	0.034	<0.004	0.22	74	0.164	0.137	<0.004	0.75	86

Table 2.3 contd over/

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Ovarian tissue[6]	LO	0.026	0.017	0.01	0.07	10	0.183	0.092	0.11	0.36	10
	BN	0.034	0.022	0.02	0.08	8	0.245	0.134	0.11	0.50	8
	PE	0.038	0.041	0.01	0.17	16	0.283	0.356	0.05	1.47	16
	KH	0.037	0.019	0.01	0.08	9	0.163	0.070	0.08	0.30	9
	All sites	0.034	0.029	0.01	0.17	43	0.228	0.232	0.05	1.47	43

Notes: [1] Forth estuary sites: LO, BO, TC, BN and PE; [2] Firth of Forth site: KH; [3] Firth of Clyde site: MP; [4] Mean water content of tissues (% loss of weight on drying to constant weight at 110° C.): Muscle, 77.99; Liver, 69.97; Kidney, 78.44; Testes, 78.53; Ovary, 79.30; Embryos, 78.26. [5] Whole ovary of non-gravid females; [6] Ovarian sac tissue of females carrying brood in ovarian cavity.

Table 2.4 Correlation of mercury concentrations (dry weight) in seven tissues of eelpout from the Forth estuary, and Firths of Forth and Clyde (Pearson correlation coefficient r, sample size n, two-tailed probability P)

		Tissue						
Tissue		Skeletal Muscle	Liver	Kidney	Testes	Whole Ovary[1]	Ovarian Tissue[2]	Brood
Skeletal Muscle	(r)	•	0.3165	0.615	0.495	0.112	0.164	0.499
	(n)	•	516	109	185	112	42	84
	(P)	•	<0.001	<0.001	<0.001	0.241	0.298	<0.001
Liver	(r)		•	0.463	0.190	0.074	0.463	0.344
	(n)		•	100	120	90	36	75
	(P)		•	<0.001	0.038	0.485	0.004	0.003
Kidney	(r)			•	0.398	0.419	0.462	0.271
	(n)			•	42	42	17	17
	(P)			•	0.009	0.006	0.062	0.293
Testes	(r)				•	n/a	n/a	n/a
	(n)				•	-	-	-
	(P)				•	-	-	-
Whole Ovary	(r)					•	n/a	n/a
	(n)					•	-	-
	(P)					•	-	-
Ovarian Tissue	(r)						•	-0.009
	(n)						•	41
	(P)						•	0.954

Notes: [1] Whole ovary from female fish containing no larval fish or embryos in ovarian cavity

[2] Ovarian tissue from female fish containing larval fish or embryos in ovarian cavity

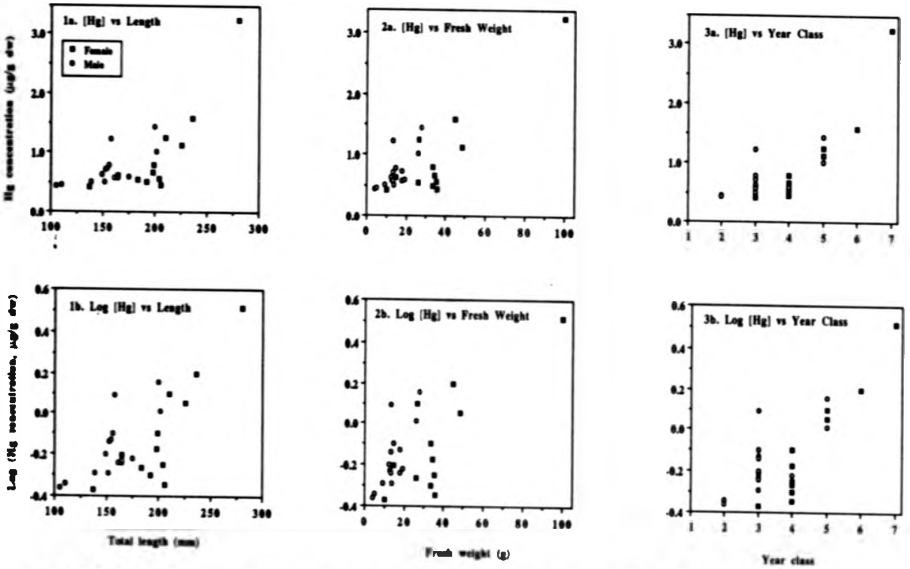


Fig. 2.12 Variation of mercury concentration and $\log_{10}$(mercury concentration) of skeletal muscle with biological parameters (length, weight and year class) for male and female eelpout from Longannet, Forth Estuary, Winter 1989/1990)

Table 2.5a Seasonal linear regression[1] and ANCOVA statistics for $\log_{10}$(Hg conc, $\mu g.g^{-1}$) with total length (mm) for single sex samples of eelpout skeletal muscle from Longannet in the Forth Estuary.

Season	Sex	Sample Size	a	b	r	P	t_s[2]	v_s	P_s	t_c[2]	v_c	P_c
Autumn 1989	F	2	-	-	-	-	n/a					
	M	8	-1.5852	0.0086	0.681	0.0628						
Winter 1989/90	F	12	-1.3859	0.0064	0.856	0.0004	1.56	23	>0.10	2.96	24	<0.01
	M	15	-0.8353	0.0043	0.732	0.0019						
Spring 1990	F	17	-1.4153	0.0065	0.648	0.0045	1.54	56	>0.10	-1.55	57	>0.10
	M	43	-0.753	0.0031	0.335	0.0282						
Summer 1990	F	39	-0.8990	0.0021	0.325	0.0433	0.40	70	>0.50	0.47	71	>0.50
	M	35	-0.9700	0.0027	0.546	0.0007						
Autumn 1990	F	6	-1.3770	0.0050	0.518	0.2928	n/a					
	M	6	-1.0300	0.0040	0.957	0.0028						
Spring 1991	F	7	-1.1019	0.0050	0.811	0.0267	0.44	12	>0.50	0.98	13	>0.20
	M	9	-1.3860	0.0062	0.758	0.0180						
Summer 1991	F	13	-1.3914	0.0036	0.703	0.0074	1.04	21	>0.20	2.07	22	>0.10
	M	12	-1.7440	0.0056	0.748	0.0051						
Autumn 1991	F	9	-0.5979	0.0021	0.591	0.0936	1.42	15	>0.10	0.82	16	>0.20
	M	10	-1.0021	0.0051	0.739	0.0145						

Notes: [1]. Linear regression equation takes the form Log_{10}(Hg conc.) = a + b(length).

[2]. t_s and t_c are values of t-statistic used for testing similarities of slopes and elevations of regression lines by Analysis of Covariance (ANCOVA). v_s and v_c are the degrees of freedom at which critical values are read when testing slopes and elevations respectively. Significance levels P_s and P_c are from a two-tailed test.

Table 2.5b Seasonal linear regression[1] and ANCOVA statistics for $\log_{10}$(Hg conc, $\mu g.g^{-1}$) with fresh weight (g) for single sex samples of eelpout skeletal muscle from Longannet in the Forth Estuary.

Season	Sex	Sample Size	a	b	r	P	t_s[2]	v_s	P_s	t_e[2]	v_e	P_e
Autumn 1989	F	2	-	-	-	-	n/a					
	M	8	-0.6872	0.0209	0.516	0.1908						
Winter 1989/90	F	12	-0.4437	0.0095	0.812	0.0013	1.40	23	>0.10	2.38	24	<0.05
	M	15	-0.4240	0.0176	0.741	0.0016						
Spring 1990	F	17	-0.6304	0.0170	0.502	0.0400	0.87	56	>0.20	1.53	57	>0.10
	M	43	-0.4019	0.0098	0.290	0.0591						
Summer 1990	F	39	-0.7099	0.0057	0.355	0.0265	0.79	70	>0.20	0.84	71	>0.20
	M	35	-0.7304	0.0094	0.528	0.0011						
Autumn 1990	F	6	-0.8631	0.0173	0.591	0.2162	n/a					
	M	6	-0.6842	0.0162	0.897	0.0152						
Spring 1991	F	7	-0.5813	0.0154	0.757	0.0489	0.66	12	>0.50	0.64	13	>0.50
	M	9	-0.7309	0.0219	0.737	0.0236						
Summer 1991	F	13	-1.0018	0.0085	0.657	0.0147	0.34	21	>0.50	0.89	22	>0.20
	M	12	-0.9608	0.0102	0.616	0.0330						
Autumn 1991	F	9	-0.3802	0.0165	0.705	0.0338	1.12	15	>0.20	0.25	16	>0.50
	M	10	-0.4485	0.0138	0.645	0.0441						

Notes: [1]. Linear regression equation takes the form Log_{10}(Hg conc.) = a + b.(fresh weight).

[2]. t_s and t_e are values of t-statistic used for testing similarities of slopes and elevations of regression lines by Analysis of Covariance (ANCOVA). v_s and v_e are the degrees of freedom at which critical values are read when testing slopes and elevations respectively. Significance levels P_s and P_e are from a two-tailed test.

Table 2.5c Seasonal linear regression[1] and ANCOVA statistics for $\log_{10}$(Hg conc, μg.g^{-1}) with year class for single sex samples of eelpout skeletal muscle from Longannet in the Forth Estuary.

Season	Sex	Sample Size	a	b	r	P	t_s[2]	v_s	P_s	t_e[2]	v_e	P_e
Autumn 1989	F	2	-	-	-	-	n/a					
	M	8	-0.9403	0.1935	0.388	0.3427						
Winter 1989/90	F	12	-1.0249	0.2106	0.928	0.0000	2.07	23	>0.05	2.77	24	<0.02
	M	15	-0.5802	0.1287	0.723	0.0023						
Spring 1990	F	17	-0.9898	0.19526	0.453	0.0677	n/a					
	M	43	-0.4542	0.0627	0.2260	0.145						
Summer 1990	F	39	-0.7692	0.0627	0.3323	0.0388	0.32	70	>0.50	0.33	71	>0.50
	M	35	-0.7912	0.0763	0.512	0.0017						
Autumn 1990	F	6	-1.6131	0.3107	0.586	0.222	n/a					
	M	6	-0.8739	0.1384	0.862	0.0270						
Spring 1991	F	7	-1.0171	0.1912	0.815	0.0256	0.09	12	>0.50	0.32	13	>0.50
	M	9	-1.0471	0.1902	0.697	0.0367						
Summer 1991	F	13	-1.0887	0.0925	0.728	0.0048	0.50	21	>0.50	0.28	22	>0.50
	M	12	-1.1709	0.1121	0.777	0.0030						
Autumn 1991	F	9	-0.5303	0.0784	0.798	0.0100	0.72	15	>0.20	0.36	16	>0.50
	M	10	-0.6586	0.1216	0.677	0.0317						

Notes: [1]. Linear regression equation takes the form Log_{10}(Hg conc.) = a + b(year class).

[2]. t_s and t_e are values of t-statistic used for testing similarities of slopes and elevations of regression lines by Analysis of Covariance (ANCOVA). v_s and v_e are the degrees of freedom at which critical values are read when testing slopes and elevations respectively. Significance levels P_s and P_e are from a two-tailed test.

Longannet samples in eight seasons, from Autumn 1989 to Autumn 1991, are presented in Table 2.5, a-c (no samples were obtained from LO in Winter 1990/91). In all seasons, where a sufficient size range was represented, $\log_{10}$(mercury concentration) increased in a linear manner with specimen length, weight and year class. Regressions were significant in most cases (two-tailed $P < 0.05$), although the regression with length for female fish from Autumn 1991 was only weakly significant ($P<0.10$). Only two female specimens were captured in Autumn 1989, preventing any analysis of relationships as above. No significant relationship was observed for female fish from Autumn 1990, where a small sample size covered a restricted size range.

Sex-related variability of mercury concentration in eelpout skeletal muscle was studied by comparing the single-sex linear regressions of $\log_{10}$(Hg conc.) versus length, weight, or year class. The linear regression equations of female and male fish were compared by analysis of covariance (ANCOVA) using Student's t-statistic (Zar, 1984). ANCOVA statistics for the comparison of regression slopes and elevations between sexes are also presented in Tables 2.5, a-c. Comparison between-sexes was not possible in Autumn 1989 or Autumn 1990 (see above). There were no significant differences between the slopes of male and female regressions in the six seasons tested, whether comparing covariance in terms of length, weight or year class. Neither were there significant differences between the elevations of the male and female regression lines, except in Winter 1989/90, when the elevation of the female line was significantly lower ($P<0.01$) than that of the males.

As a difference in mercury concentrations of skeletal muscle between sexes was eliminated as a possible source of variability, data from the two sexes were pooled and a common regression equation calculated for each site within seasons. Table 2.6 summarizes the regression statistics of sex-pooled samples from individual sites in each season. The distribution of log-concentrations with length is shown for individual sites in each season in Fig. 2.13. The best-fit lines described by the regression equations in Table 2.6 are also shown for individual sites, and labelled by site.

2.3.2.2 Site-related (spatial) variability

Spatial variability in mercury concentrations of eelpout skeletal muscle was investigated within seasons by comparing the linear regressions of $\log_{10}$(Hg concentration) and length from individual sites (Table 2.6), using analysis of co-variance (ANCOVA). Only regressions significant at $P<0.10$ were included in the ANCOVA, although sites with non-significant regressions are included in Table 2.6. ANCOVA statistics for spatial comparisons are summarised in Table 2.7. Comparisons were possible in seven of nine seasons. There was no significant difference between sites in Autumns of 1989 or 1990, in terms of a comparison of overall regressions. There were also no significant differences between the slopes of regressions in the remaining five seasons. In each of these five seasons, however, there was a significant difference between the elevations of regression lines. The differences were identified

Table 2.6 Seasonal linear regression statistics[1] for $\log_{10}$(Hg conc., $\mu g.g^{-1}$) versus length (mm) in eelpout skeletal muscle, for sex-pooled samples.

Year	Season	Site	Sample Size	a[1]	b[1]	R^2	P
1989	Autumn	LO	10	-1.1092	0.0046	0.292	0.107
		BN	10	-1.4756	0.0058	0.640	0.0055
1989/90	Winter	LO	27	-0.8434	0.00400	0.539	<0.0001
		PE	28	-0.6099	0.00170	0.108	0.0872
1990	Spring	LO	60	-0.9975	0.00447	0.218	0.0002
		PE	33	-0.3144	-0.0003	0.0022	0.7972
1990	Summer	LO	76	-0.9379	0.00243	0.173	0.0002
		PE	53	-0.9179	0.0022	0.165	0.0026
		KH	23	-1.5996	0.0050	0.318	0.0051
1990	Autumn	LO	11	-0.8689	0.0026	0.565	0.0077
		PE	19	-1.2195	0.0041	0.6093	0.0001
1990/91	Winter	BN	11	-1.3362	0.00466	0.522	0.0120
1991	Spring	LO	16	-1.1771	0.0052	0.571	0.0007
		BO	5	-1.3902	0.0079	0.988	0.0006
		TC	11	-1.032	0.0046	0.579	0.0065
		KH	8	-1.447	0.0058	0 662	0.0141
1991	Summer	LO	25	-1.4707	0.00417	0.465	0.0002
		PE	12	-1.0164	0.0042	0.569	0.0046
		KH	11	-0.8546	0.0026	0.164	0.2162
		Clyde	11	-1.2819	0.0045	0.580	0.0065
1991	Autumn	LO	19	-0.8162	0.00360	0.412	0.0031
		PE	18	-1.240	0.0043	0.713	0.0001
		KH	9	-1.1921	0.0025	0.428	0.0557
1991	Spring	BN	12	-0.8562	0.0344	0.4344	0.0197

Notes. [1]. Linear regression equation takes the form: $\log_{10}$(Hg concentration) = a + b (length)

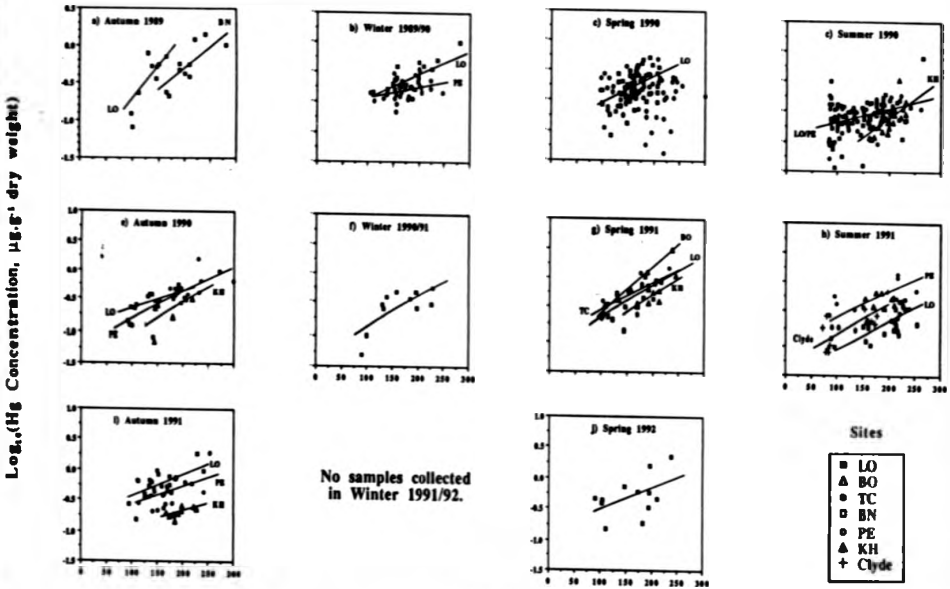


Fig. 2.13 Log_{10}(mercury concentration) of skeletal muscle related to total length for eelpout from the Forth estuary and other sites

Table 2.7 Comparison of individual site regressions of $\log_{10}$(Hg in muscle) on length within seasons, using Analysis of Covariance*

Season	Sites	Overall ANCOVA				Btwn-slopes ANCOVA				Btwn-elevations ANCOVA			
		F	DoF	Sig	P	F	DoF	Sig	P	F	DoF	Sig	P
10/89	LO, BN	3.23	2, 16	ns	>0.05								
1/90	LO, PE	9.32	2, 51	sig	<0.0005	3.36	1, 51	ns	>0.05	14.70	1,51	sig	<0.0005
4/90	No comparison												
7/90	LO, PE, KH	2.77	4,152	sig	<0.05	0.86	2, 152	ns	>0.50	4.69	2, 152	sig	<0.01[1]
*10/90	LO, PE	2.28	1, 26	ns	>0.20								
1/91	No comparison												
4/91	LO, BO, TC, KH	3.48	6, 32	sig	<0.05	0.83	3, 32	ns	>0.50	6.23	3, 32	sig	<0.025[2]
7/91	LO, PE, MP	9.44	4, 42	sig	<0.0005	3.23	2, 42	ns	>0.50	19.74	2, 42	sig	<0.0005[3]
10/91	LO, PE, KH	14.90	4, 40	sig	<0.0005	0.13	2, 40	ns	>0.50	30.95	2, 40	sig	<0.0005[4]

Notes: * Analysis of covariance (ANCOVA) carried out after Zar (1984)

[1] Tukey multiple comparison tests: LO vs PE p>0.05; PE vs KH p>0.05; LO vs KH q= 4.207, DF = 152, 3, P< 0.01

[2] Tukey multiple comparison tests: BO vs KH q= 5.42, DF = 32, 4, p<0.005; TC vs KH q = 3.94, DF = 32, 4, p<0.05; all other comparisons, p>0.05

[3] Tukey multiple comparison tests: LO vs PE q= 8.45, p<0.0001; LO vs MP q= 3.71, p<0.05; PE vs MP q= 4.20, p<0.025; all DF = 23, 4

[4] Tukey multiple comparison tests: LO vs PE q= 4.11, p<0.025; LO vs KH q= 14.58, p<0.001; PE vs KH q= 11.25, p<0.001; all DF = 42,3

using Tukey multiple comparison tests, the results of which are also summarised in Table 2.7. In summary, differences between sites were the result of differences between regression elevations, while differences between the slopes of regression were not significant in all cases. Notably, the comparison of sites in the Forth Estuary with a site in the Firth of Clyde in Summer 1991 revealed no differences in the degree of mercury accumulation with length (slope), the regression for Clyde fish being intermediate between those for fish from LO and PE.

2.3.2.3 Seasonal variability of skeletal muscle mercury concentration and relation to fish condition

As LO was the site with the most complete set of seasonal results for skeletal muscle mercury concentrations, the seasonal and annual variability of muscle mercury concentration was studied at this site. In addition, any sites for which regressions of $\log_{10}$(Hg concentration) with length had been shown to be not significantly different from the regression for LO in a particular season (see section 2.4.1.2) were included in a common regression for that season. The common linear regression statistics for each season, and the sites included in the regression, are summarized in Table 2.8. As no samples were collected from LO in Winter 1990/91, the significant linear regression from BN (see Table 2.6) was used to complete the seasonal record.

Seasonal variability in the degree of mercury contamination in skeletal muscle (as the slope of the regression of $\log_{10}$(Hg conc.) with length) is shown in Fig. 2.14. The figure describes results for the periods Autumn 1989 to Summer 1990, and Autumn 1990 to Summer 1991. Both years showed seasonal differences in the value of slope. The two years showed a generally similar pattern, with an overall increase in the value of the slope from Autumn to a peak value in the following Spring, followed by a sharp decrease between Spring and Summer. The similarity of the seasonal values of the regression slope was tested within each year using an analysis of covariance based on the F-statistic (Zar, 1984). For the periods Autumn 1989-Summer 1990 ('1990'), and Autumn 1990-Summer 1991 ('1991'), there were no significant differences between the seasonal regression slopes ('1990': $F=1.944$, $v_e= 3, 226$, $P>0.20$: '1991': $F=1.683$, $v_e=3, 91$, $P>0.20$).

The allometric condition factor, K', was calculated for the same fish (from LO and associated sites) which were used to study seasonal variability. Details of the calculation of K' are given in Appendix 2.5. The condition factors of male and female fish were compared within each seasonal sample using two-tailed t-tests (Zar, 1984). No significant differences were observed between sexes within seasons (all $p> 0.05$; test results not presented), and sexes were pooled for calculation of mean values. Figure 2.15 presents the seasonal mean allometric condition factors for each season from Autumn 1989 to Summer 1990, and Autumn 1990 to Summer 1991. Seasonal variability was observed in the value of K', with lowest values found in Winter or Spring, followed by an increase in condition to a maximum value in Summer of each year.

Table 2.8 Seasonal linear regressions[a] between $\log_{10}$(Hg conc., $\mu g.g^{-1}$ dw) and length, for eelpout from Longannet and associated sites in the Forth Estuary.

Year	Season	Sites included	n	a	b	R^2	P[b]
1989/90	Au	LO, BN	20	-1.0663	0.00422	0.409	0.0024
	Wi	LO	27	-0.8434	0.00400	0.539	0.0000
	Sp	LO	60	-0.9975	0.00447	0.218	0.0002
	Su	LO, BN	127	-0.9321	0.00238	0.183	0.0000
1990/91	Au	LO, PE	31	-1.0875	0.00351	0.503	0.0000
	Wi	BN[c]	11	-1.3362	0.00466	0.522	0.0120
	Sp	LO, BO, TC	32	-1.2160	0.00579	0.628	0.0000
	Su	LO	25	-1.4707	0.00417	0.465	0.0002
1991/92	Au	LO	19	-0.8162	0.00360	0.412	0.0031

Notes. a. Linear regression equation takes the form: $\log_{10}$(Hg conc.) = a + b.(length).

b. Probability from a two-tailed t-test.

c. No samples obtained from LO during Winter 1990/91.

Table 2.9 Annual comparisons of seasonal regressions by ANCOVA

Seasons/ Years	ANCOVA Comparisons
Autumn 1989 vs 1990 vs 1991	Slopes: no significant differences (F=2.89, degrees of freedom=2, 34, P>0.05) Elevations: no significant differences (F= 3.13, df = 2, 35, P>0.05)
Winter 1989 vs 1990	Slopes: no significant difference (t= 0.47, df = 34, P>0.50) Elevations: significant difference (t= 4.51, df = 35, P<0.001)
Spring 1990 vs 1991	Slopes: no significant difference (t= 0.95, df = 88, P>0.20) Elevations: no significant difference (t= 0.08, df = 89, P>0.50)
Summer 1990 vs 1991	Slopes: no significant difference (t= 1.29, df = 148, P>0.20) Elevations: significant difference (t= 3.32, df = 149, P<0.002)

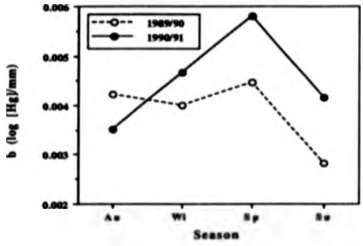


Fig. 2.14 Seasonal variation in the regression coefficient (b) of log (muscle Hg conc.) in skeletal muscle, on length of eelpout from sites in the Forth Estuary, Scotland.

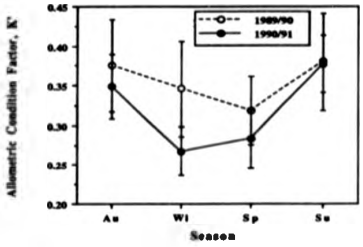


Fig. 2.15 Seasonal variation in mean allometric condition factor (± sd) for eelpout from sites in the Forth Estuary

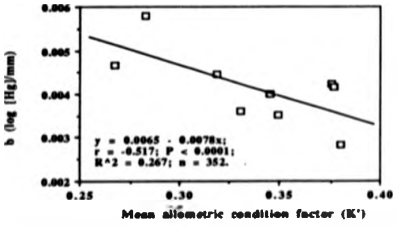


Fig. 2.16 Relationship between fish condition and the regression coefficient (b) of log (skeletal muscle Hg conc.) with length for seasonal samples from Longannet in the Forth Estuary.

The degree of mercury contamination of skeletal muscle, as the seasonal regression slopes, shows an inverse linear relationship to the seasonal mean condition factor, as shown in Figure 2.16. Although the value of slope is shown plotted against the mean value of K', the regression equation in the figure was calculated using the actual values of K'. The slope of the regression is negatively correlated with the condition factor, and the relationship is statistically significant.

2.3.2.4 Annual variability of muscle mercury concentration assessed by ANCOVA

The between-years' variability of eelpout muscle mercury concentrations was assessed within individual seasons, by comparison of the seasonal linear regressions of $\log_{10}$(Hg conc) on length, in Table 2.8. Between-years' comparisons were made, using ANCOVA, for Autumn (1989/90/91), Spring (1990/91), Summer (1990/91) and Winter (1989/90) samples. ANCOVA statistics are summarised in Table 2.9. No significant differences were observed between the regression slopes in any season, although significant differences were observed between the elevations of the regressions for Winter (1989 vs 1990) and Summer (1990 vs 1991).

2.3.2.5 Summary

The $\log_{10}$ of mercury concentrations in eelpout skeletal muscle from the Forth Estuary, Firth of Forth and Firth of Clyde, show significant linear correlations with length, weight or age class of the fish in most samples. The linear regression of $\log_{10}$(concentration) on length, weight, or year class is also statistically significant in most cases. The rate of increase is not significantly different between sexes in almost all samples, and sexes were pooled for subsequent analysis of spatial and temporal variability. When linear regressions of $\log_{10}$(concentration) on length for single sites were compared within seasons, there were no significant differences between the slopes of samples from different sites within any single season. There were, however, some statistically significant differences between sites in terms of regression elevations in some seasons.

The slope of the linear regression of $\log_{10}$(mercury concentration) on length (i.e. the rate of increase of $\log_{10}$(concentration) with length) for eelpout from Longannet in the Forth Estuary showed a seasonal pattern of variation, which was similar in both years studied. The value of slope generally increased from Autumn to a maximum in Spring, then fell sharply in Summer samples. The differences between seasons were not significant, however, in either year. Fish condition, as the allometric condition factor, showed a pattern of seasonal variation which was generally the inverse of that observed for the slope of $\log_{10}$(mercury concentration) on length, with a minimum value in Spring. The seasonal slope of $\log_{10}$(mercury concentration) on length showed a significant negative correlation with the seasonal value of allometric condition factor. With regard to annual variability, there was little difference between the years studied when the regressions of $\log_{10}$(mercury concentration) on length for LO fish were compared within single seasons.

2.3.3 Total mercury concentrations and burdens in eelpout livers

2.3.3.1 Variability of Liver Somatic Index and liver mercury concentration

The Liver Somatic Index (LSI, weight of liver as a percentage of body weight) was calculated for all Longannet and Port Edgar fish analysed for liver mercury concentration. Provided juvenile fish of Year-Class 1 ("young-of-the-year" fish) were excluded from the Summer samples, no significant correlations were observed within-season for single-sex samples between LSI or mercury concentration and size (as length or weight; all Pearson correlations, $p>0.05$; test results not presented). The mean mercury concentrations and mean LSI values of adult male and female fish were compared at both of the two sites within each season, and no significant differences of mean values were observed between the sexes (Two-tailed t-tests, all $P>0.01$; test results not presented), and sexes were pooled for further data analysis.

Table 2.10 summarises the seasonal variation of mean liver somatic index (Table 2.10a) and liver mercury concentration (Table 2.10b) for samples of adult eelpout (Year-Class >1) from Longannet (LO) and Port Edgar (PE) in the Forth estuary. A clear seasonal maximum value can be seen in the mean LSI in both years in each site (Table 2.10a), with the liver accounting for up to a mean value of 2.9 % of body weight. Also shown in Table 2.10a are test statistics (Analysis of variance, F-statistic) comparing mean LSI values of LO and PE within each season. The mean LSI was significantly higher at PE than LO in Spring 1990, while mean LSI was significantly higher at LO in Summer of both years. There were no other significant differences in mean LSI between the sites in any season.

Seasonal mean LSI values were compared within-site in each year by ANOVA. For Longannet samples in 1990, a highly-significant difference was indicated between seasons ($F=35.006$, between-groups degrees of freedom (DF)=3, within-groups DF=129, $P=0.000$). A Tukey Multiple Comparison test revealed the Summer mean LSI to be significantly higher ($P< 0.05$) than the means of the other three seasons. The Winter sample was excluded from a comparison of seasonal means for Longannet in 1991 due to a very small sample size (n=1). An ANOVA revealed another highly-significant difference between seasons for Longannet in 1991 ($F=46.065$, DF=2, 59, $P=0.0000$), with Summer being significantly higher than the other two seasons (Tukey Multiple Comparison Test, $P< 0.05$).

In 1990, an ANOVA revealed a significant difference between seasonal means at Port Edgar ($F=5.1696$, DF=3, 97, $P=0.0023$), with the Summer mean significantly higher than the Winter (Tukey test, $p< 0.05$). In 1991 at Port Edgar, a significant difference between means ($F=5.9022$, DF=3, 50, $P=0.0016$) lay between Summer and Autumn means (Tukey test; $P < 0.05$).

A seasonal pattern, similar in each case, was also observed in the mean liver mercury

Table 2.10 Seasonal variation of mean liver somatic index and liver Hg concentration (dry weight) for adult eelpout from two Forth Estuary sites. Analysis of variance statistics are given for the comparison of sites within seasons.

a. Liver Somatic Index (%)

Year	Season	Longannet Mean	Longannet sem	Longannet n	Port Edgar Mean	Port Edgar sem	Port Edgar n	Between-site Comparison (ANOVA) F-statistic	Btwn-grp D of F	Within-grp D of F	P (2-tailed)
1990	W	1.255	0.074	31	1.194	0.075	31	0.343	1	60	0.5603
	Sp	1.214	0.043	45	1.380	0.058	34	5.585	1	77	0.0206
	Su	2.310	0.114	52	1.626	0.096	32	17.377	1	82	0.0001
	A	1.518	0.249	5	1.380	0.166	4	0.189	1	7	0.6767
1991	W	1.750	-	1	1.289	0.307	4	not possible	-	-	-
	Sp	1.361	0.180	9	1.823	0.247	5	2.318	1	12	0.1538
	Su	2.883	0.126	34	2.061	0.127	27	20.663	1	59	0.0000
	A	1.220	0.130	19	1.340	0.125	18	0.4367	1	35	0.5131

b. Total Hg Concentration ($\mu g.g^{-1}$ d.w.)

Year	Season	Longannet Mean	Longannet sem	Longannet n	Port Edgar Mean	Port Edgar sem	Port Edgar n	Between-site Comparison (ANOVA) F-statistic	Btwn-grp D of F	Within-grp D of F	P (2-tailed)
1990	W	0.690	0.109	31	0.612	0.130	31	5.953	1	60	0.6497
	Sp	0.443	0.038	45	0.416	0.056	34	0.165	1	77	0.6854
	Su	0.184	0.031	53	0.247	0.028	32	1.884	1	83	0.1736
	A	0.341	0.173	5	0.264	0.050	4	0.149	1	7	0.7114
1991	W	0.489	-	1	0.166	0.085	4	not possible	-	-	-
	Sp	0.316	0.076	9	0.156	0.050	5	2.106	1	12	0.1724
	Su	0.202	0.031	34	0.284	0.042	27	2.562	1	59	0.1148
	A	0.395	0.049	19	0.386	0.054	18	0.015	1	35	0.9028

Table 2.11 Liver mercury and liver size for year class 1 eelpout from two sites in the Forth estuary

Year	Site	LSI (%)		Hg conc. ($\mu g.g^{-1}$ dw)		Sample
		Mean	sd	Mean	sd	Size
1990	LO	**1.518**	0.352	**0.205**	0.165	14
	PE	**1.352**	0.504	**0.366**	0.156	9
1991	LO	**1.665**	0.293	**0.118**	0.047	11
	PE	**1.186**	0.163	**0.159**	0.027	6

Notes. See Results text (Section 2.7.7) for details of statistical comparisons between sites

concentration in both years at Longannet (LO), and in 1990 at Port Edgar (PE). In each year, highest mean mercury concentrations were measured in Winter, with successively lower means in Spring and Summer, followed by an increase in Autumn. At PE, in 1991, mean values were lower in Winter and Spring, than in Summer and Autumn.

When these differences were tested statistically for LO samples, within-site comparisons of mean mercury concentrations between-seasons indicated a statistically-significant difference between seasonal means ($P<0.05$) in 1990 (ANOVA: $F = 13.071$, $DF = 3, 130$, $P = 0.0000$). A Tukey Multiple-Comparison Test revealed that the Summer mean was significantly lower ($P<0.05$) than the Winter and Spring means, and the Spring mean also significantly lower than the Winter value. In 1991 at LO, the Spring, Summer and Autumn means were compared, revealing a significant difference (ANOVA: $F=5.994$, $DF=2, 59$, $P = 0.0043$). A Tukey test indicated that the Summer mean was significantly lower ($P<0.05$) than the Autumn value.

An ANOVA revealed a statistically-significant difference between seasonal means at PE in 1990 ($F=3.528$, $DF = 3, 97$, $P=0.0178$). The Summer mean mercury concentration was significantly lower (Tukey test, $P<0.05$) than the Winter mean. There were no significant differences between seasonal mean mercury concentrations in livers of PE fish in 1991 (ANOVA: $F=2.334$, $DF= 3,50$, $P=0.0852$).

Results for juvenile (Year-class 1, Summer only) eelpout from Longannet (LO) and Port Edgar (PE) are presented separately, in Table 2.11. In 1990, although there was no significant difference between LO and PE in terms of liver somatic index (Mann-Whitney $U=41.0$, two-tailed $P = 0.179$), there was a significant difference between sites in relation to mean mercury concentration of the liver ($U=31.0$, $df= 21$, $P=0.046$). Mean concentration was highest in juvenile fish from PE. In 1991, the livers of LO fish were significantly larger relative to body size than those of PE fish (Mann Whitney $U=4.0$, $P=0.0019$), and mercury concentrations were significantly lower (Mann Whitney $U= 12.5$, $P= 0.036$).

2.3.3.2 Between-year comparisons of Liver Somatic Index and liver mercury concentrations

Individual seasonal means of LSI and liver total mercury concentration from 1990 and 1991 (see Table 2.10 a and b) were compared between-years, within-site and season, to determine if there was any significant annual variation. At LO, the only significant annual difference in the value of LSI was in Summer, with a significantly higher mean LSI in 1991 than in 1990 ($F=10.851$, $DF=1,84$, $P= 0.0014$). Similarly, mean LSI values at PE in Spring and Summer 1991 were significantly higher than those of the same seasons in 1990 (Spring: $F = 6.343$, $DF=1,37$, $P=0.0162$; Summer: $F = 7.690$, $DF=1,57$, $P=0.0075$). Analysis of variance (ANOVA) revealed no statistically-significant differences in liver total mercury concentrations between-years at either LO or PE, when comparisons were made within-site and season (all $P>0.05$, full test statistics not presented).

2.3.3.3 Relationship between Liver Somatic Index and liver total mercury concentration

The relationship between Liver Somatic Index and total mercury concentration of liver, for the eight seasons described above, is presented for each site in Figure 2.17a and b, along with the regression and correlation statistics for each relationship. The $\log_{10}$ of liver mercury concentration shows a highly-significant negative correlation, and a linear decrease, with increasing liver somatic index (LSI) for eelpout from Longannet (Fig. 2.17a), with a large degree of scatter evident in the data (R^2= 0.29). There is also a negative correlation between liver mercury concentration and LSI for fish from Port Edgar (Fig. 2.17b), although the relationship is not statistically significant (0.10>P>0.05), and the calculated linear regression shows no fit to the data (R^2= 0.02). The range of LSI values is more restricted at PE than LO, with less difference apparent between seasonal means.

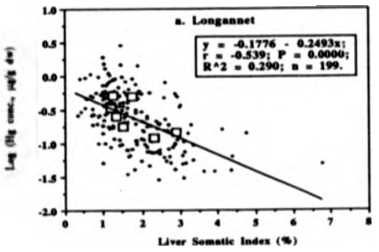
2.3.3.4 Total mercury burdens of eelpout livers

(a) Relationships with biological parameters (length, sex)

The total mercury burden of eelpout liver was found to increase in a linear fashion, as $\log_{10}$(Hg burden, μg), with $\log_{10}$(Total fish length, mm). Most within-season, within-site, single-sex samples showed a significant linear regression of $\log_{10}$(Hg burden) on $\log_{10}$(length). The linear regression statistics for single-sex samples are summarised in Table 2.12. In many cases the regressions were highly significant (P<0.001, eg all samples in Spring 1990, and Summer 1991). Data showed a good fit to the calculated regression line in most cases. Values of the coefficient of determination (r^2), for the significant regression lines, ranged from 0.298 to 0.932. Only a few samples (males, PE, Winter 1989/90; females, KH, Summer 1990; females, KH, Autumn 1991) showed non-significant regressions (P>0.05), and in most of these cases only a very restricted range of lengths was represented in catches.

Regression lines of male and female samples were tested for similarity using an analysis of covariance (ANCOVA) procedure, based on the t-statistic (Zar, 1984). The statistics of t-tests are summarised in Table 2.12, for samples where testing between sexes was possible. Differences between slopes of regressions within-sites within-seasons tested non-significant (P>0.05, two-tailed test) in all cases where a comparison was possible (all P>0.20). In only one case (LO, Summer 1990) was there a difference, at the 5% significance level, between the elevations of male and female regressions.

Zar (1984) points to the risk of repeated t-tests leading to the false acceptance of differences as significant, when the difference exists only as a chance occurrence (Type 1 error). If, as suggested by Zar, the level at which a difference was accepted as significant was lowered to 1% (P<0.01) when performing multiple t-tests, to reduce the risk of committing a Type 1 error, then there were no significant differences between sexes with regard to the elevations of regression



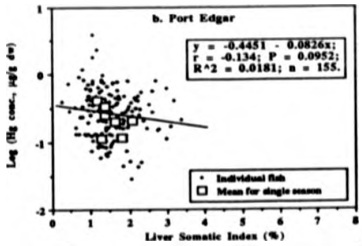


Fig. 2.17 Variation of liver mercury concentration with liver somatic index for eelpout from two sites in the Forth Estuary. Regression lines calculated on individual points for each site.

Table 2.12 Seasonal linear regression statisticsa, and a comparison between sexes, for the regression of $\log_{10}$(Liver Hg burden, µg) on $\log_{10}$(Body Length, mm) for eelpout from the Estuary and Firth of Forth, and the Firth of Clyde.

Season	Site	Sex	n	a[a]	b[a]	R^2	P	t_s[b]	DoF	P	t_c[c]	DoF	P
Winter 1989/90	LO	F	13	-11.459	4.441	0.549	0.0038	1.038	27	>0.20	1.137	28	>0.20
		M	18	-7.902	2.926	0.404	0.0046						
	PE	F	16	-8.126	2.938	0.298	0.0288	n/a-	-	-	-	-	-
		M	15	-2.003	0.111	0.001	0.9265						
Spring 1990	LO	F	13	-10.360	3.877	0.666	0.0007	0.185	41	>0.50	0.829	42	>0.20
		M	32	-9.512	3.533	0.325	0.0007						
	PE	F	18	-7.096	2.445	0.384	0.0061	0.088	30	>0.50	1.037	31	>0.20
		M	16	-7.391	2.537	0.494	0.0024						
Summer 1990	LO	F	33	-8.440	3.005	0.859	0.0000	0.330	62	>0.50	2.040	63	<0.05
		M	33	-8.695	3.192	0.506	0.0000						
	PE	F	16	-10.378	3.991	0.726	0.0000	0.524	35	>0.50	0.144	36	>0.50
		M	23	-9.440	3.525	0.626	0.0000						
	KH	F	9	-4.824	1.503	0.022	0.6991	n/a-	-	-	-	-	-
		M	9	-22.597	9.244	0.640	0.0095						
Spring 1991	LO	F	3	-13.768	5.317	0.998	0.0192	0.249	5	>0.50	1.645	6	>0.10
		M	6	-12.741	4.996	0.814	0.0138						

(Table 2.12 contd. overleaf)

Table 2.12 (continued)

Season	Site	Sex	n	a[a]	b[a]	R^2	P	t_s[b]	DoF	P	t_e[c]	DoF	P
Summer 1991	LO	F	20	-11.077	4.210	0.653	0.0000	0.105	41	>0.50	1.687	42	>0.05
		M	25	-10.731	4.130	0.465	0.0000						
	PE	F	14	-13.610	5.422	0.854	0.0000	0.401	29	>0.50	0.913	30	>0.20
		M	19	-14.381	5.755	0.650	0.0000						
	KH	F	9	-10.958	4.286	0.314	0.0000	n/a	-	-	-	-	-
		M	2	-	-	-	-						
	Clyde	F	6	-9.802	3.327	0.906	0.0009	1.018	10	>0.20	1.490	11	>0.10
		M	8	-9.738	3.740	0.861	0.0000						
Autumn 1991	LO	F	9	-12.775	5.027	0.682	0.0007	0.700	15	>0.20	0.241	16	>0.50
		M	10	-11.185	4.281	0.726	0.0001						
	PE	F	11	-10.781	4.144	0.445	0.0021	0.781	14	>0.20	0.756	15	>0.20
		M	7	-13.099	5.146	0.549	0.0127						
	KH	F	9	-8.913	3.248	0.037	0.2385						
Spring 1992	BN	F	8	-14.081	5.532	0.659	0.0023	n/a					
		M	4	-2.918	0.038	0.001	0.988						

Notes:
a. Linear regression equation takes the form: Log_{10}(Liver Hg burden, µg) = a + b.Log_{10}(Length, mm)
b. Test statistics for comparison of regression line slopes between-sexes.
c. Test statistics for comparison of regression line elevations between-sexes.

lines within-sites within-seasons. At this level of significance, no significant difference was observed between the mercury burdens of male and female eelpout livers, with respect to body length. The single-sex samples were pooled and a common regression calculated for each site within each season. Regression statistics for these common regressions are summarised in Table 2.13. The distribution of $\log_{10}$(liver Hg burden) with $\log_{10}$(length) is shown for individual sites by seasons in Fig. 2.18.

(b) Comparisons between sites

Between-site differences in liver mercury burden were tested within each season by comparing the regression lines of $\log_{10}$(Hg burden) on $\log_{10}$(length) for individual sites (see Table 2.13) using analysis of covariance (ANCOVA: Zar, 1984). Comparisons between sites were possible in six seasons.

Results of the ANCOVA tests are summarised in Table 2.14. There were no significant differences between sites in Winter 1989/90, Spring 1990, Summer 1990, and Autumn 1991, indicating that mercury burdens in livers of fish from different sites in the Forth Estuary and Firth of Forth were similar, with respect to fish length. An ANCOVA test of similarity found no significant differences between either slopes ($t= 0.684$, $DF= 21$, $P>0.50$), or elevations ($t= 2.074$, $DF= 22$, $P>0.20$) of regressions for samples from the Firths of Forth (KH) and Clyde (MP) in Summer 1991 (see Table 2.13), indicating that liver mercury burdens were similar in fish from both these sea areas, when fish size was accounted for. In Spring 1991, however, the slopes of regressions for LO and PE samples were significantly different, and an overall ANCOVA test of similarity in Summer 1991 revealed a significant difference between the regressions for LO, PE and KH. Further ANCOVA testing revealed a significant difference between the slopes of the regressions. The slope of the LO regression was significantly different from the slopes of both the PE and the KH regressions. The regressions of the PE and KH samples were not significantly different.

(c) Temporal variability of eelpout liver mercury burdens

The seasonal variability of liver mercury burdens was compared using ANCOVA for the two sites with most-complete seasonal records, LO and PE. Comparisons were made of the seasonal regressions of $\log_{10}$(burden) on $\log_{10}$(length) for each site within an annual period. There were no significant differences between seasons in the regressions of $\log_{10}$(liver mercury burden) on $\log_{10}$(length), at either LO or PE, as shown by the summary of ANCOVA statistics in Table 2.15.

The annual variability of liver mercury burdens was investigated for eelpout from Longannet and Port Edgar, comparing single-site, single-season samples from Spring, Summer and Autumn 1990 and 1991. Sample sizes for Winter 1989/90 and 1990/91 were too small, or

Table 2.13 Statistics for the linear regression[1] of $\log_{10}$(total mercury burden, µg) on $\log_{10}$(length, mm) for livers of eelpout from the Estuary and Firth of Forth*

Season	Site	n	a	b	R^2	Significance
Autumn 1989	LO	6	-4.9852	1.4998	0.105	0.531
	BN	6	-16.8993	6.5848	0.671	0.013
Winter 89/90	LO	31	-8.7517	3.2877	0.212	0.000
	PE	35	-2.1749	0.4195	0.000	0.159
Spring 1990	LO	45	-9.5642	3.5462	0.170	0.000
	PE	34	-7.5121	2.6144	0.214	0.000
Summer 1990	LO	66	-8.3550	3.0002	0.420	0.000
	BN	11	-10.2300	3.8001	0.120	0.057
	PE	39	-9.8416	3.7237	0.448	0.000
	KH	18	-11.8513	4.5498	0.033	0.078
Autumn 1990	LO	5	-3.5234	0.6506	0.007	0.896
	PE	4	-8.1537	2.9093	0.818	0.096
	LO/PE	9	-8.4803	3.0075	0.465	0.003
Winter 1990/91	LO/BN/PE	9	-11.3328	4.2514	0.281	0.027
Spring 1991	LO	9	-13.4074	5.2520	0.721	0.000
	PE	4	-13.8046	5.4852	0.933	0.013
Summer 1991	LO	45	-10.8079	4.1313	0.539	0.000
	PE	33	-14.0513	5.6105	0.753	0.000
	KH	11	-10.6745	4.1624	0.270	0.012
	MP*	14	-9.2396	3.4684	0.920	0.000
Autumn 1991	LO	19	-11.8996	4.6213	0.714	0.000
	BN	7	-13.9325	5.4395	0.767	0.002
	PE	18	-11.1973	4.3198	0.483	0.000
	KH	9	-8.9128	3.2484	0.037	0.239
Spring 1992	BN	12	-12.9300	5.0249	0.884	0.0000

Notes. [1]. The regression equation takes the form: $\log_{10}$(liver Hg burden) = a + b.$\log_{10}$ (length)

*. The site MP is in the Firth of Clyde

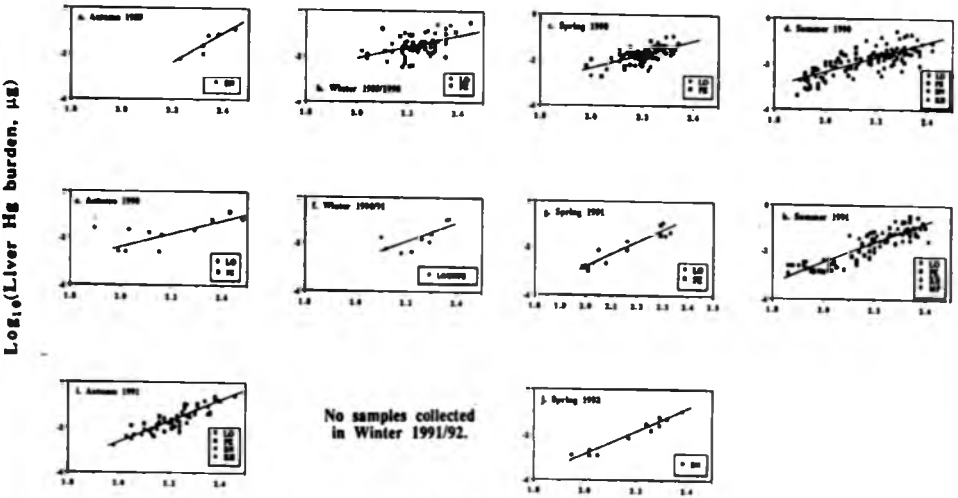


Fig. 2.18 Seasonal Relationships between Length and Liver Hg burden for eelpout, Autumn 1989 to Spring 1992.

Table 2.14 Comparison of individual site regressions of $\log_{10}$(liver Hg burden, µg) on $\log_{10}$(length, mm) within seasons for eelpout from sites in the Forth estuary and Firth of Forth, using ANCOVA[a]

Season	Sites	Overall ANCOVA				Btwn-slopes ANCOVA				Btwn-elevations ANCOVA			
		F	DoF	Sig	P	F/t	DoF	Sig	P	F/t	DoF	Sig	P
1/90	LO/PE	-	-	-	-	0.228 (t)	43	ns	>0.50	0.935 (t)	44	ns	>0.05
4/90	LO/PE	-	-	-	-	1.140 (t)	75	ns	>0.20	0.333 (t)	76	ns	>0.50
7/90	LO/BN/PE/KH	0.615	6, 216	ns	>0.25	-	-	-	-	-	-	-	-
4/91	LO/PE	-	-	-	-	3.142 (t)	9	sig	<0.02	-	-	-	-
7/91	LO/PE/KH	2.565	4, 83	sig	<0.05	3.777 (F)	2, 83	sig	<0.05[b]	-	-	-	-
10/91	LO/BN/PE	0.961	4, 38	ns	>0.25	-	-	-	-	-	-	-	-

Notes: [a] ANCOVA: Analysis of co-variance according to Zar (1984)
[b] Significant differences identified using Tukey multiple comparison tests:

LO vs PE: q= 11.364, p<0.001, df = 83, 3
LO vs KH q= 6.315, p<0.001, df = 83, 3
PE vs KH q= 3.124, p>0.05, df = 83, 3

Table 2.15 Comparison of seasonal regressions of $\log_{10}$(liver Hg burden) on $\log_{10}$(length) for eelpout from two Forth estuary sites, Longannet (LO) and Port Edgar (PE), using ANCOVA[a]

Year	Site	Seasons included	Value of F statistic	Degrees of Freedom	Significance Level (one-tailed)
1990	LO	Wi, Sp, Su	3.725	4, 136	<0.05[b]
	PE	Sp, Su, Au	2.126	4, 77	> 0.05
1991	LO	Sp, Su, Au	0.941	4, 67	> 0.25
	PE	Sp, Su, Au	0.651	4, 49	> 0.25

Notes: [a]. ANCOVA: Analysis of co-variance according to Zar (1984). [b]. Significant difference between elevations (F=7.258, df=2, 138, P<0.0025); differences identified by Tukey Multiple Comparison Test: (Wi vs Sp: q=4.923, P<0.005; Wi vs Su: q=4.993, P<0.005; Sp vs Su: q=0.170, P>0.50, all tests, degrees of freedom= 3, 136).

represented too short a range of length measurements to permit comparisons to be made. The regressions of $\log_{10}$(liver mercury burden) on $\log_{10}$(length) were compared between years using an analysis of covariance (ANCOVA), based on the t-statistic (Zar, 1984).

There was no statistically-significant difference between the regressions for Longannet samples in Spring 1990 and 1991, for either slopes (t=1.663, degrees of freedom (df)=50, P>0.10), or regressions (t=1.068, df=51, P>0.20). There was a highly-significant difference between the slopes of the regressions for Longannet samples in Summer 1990 and 1991 (t=10.960, df=105, P<0.001). The Autumn regressions for Longannet 1990 and 1991 showed no statistically significant differences between slopes (t=1.676, df=20, P>0.10), or elevations (t=0.409, df=21, P>0.50). The liver mercury burdens of Longannet eelpout in Spring and Autumn were, therefore, similar in the two years, with respect to length. The rate of increase in the liver burden with length was, by contrast, greater in Summer of 1991 than in 1990.

There was more annual variability of liver mercury burdens for Port Edgar samples, with a significant difference between the slopes of Spring 1990 and 1991 samples (t=2.980, df=35, P<0.01), and between the slopes of Summer 1990 and 1991 samples (t=3.188, df=68, P<0.005). There was, however, no significant difference between the regressions of Port Edgar samples from Autumn 1990 and 1991, whether considering slopes (t=1.657, df=18, P>0.10) or elevations (t=1.204, df=19, P>0.20) of the regressions. The rate of increase of liver mercury burden with length was, therefore, higher in both Spring and Summer of 1991 than in the same seasons in 1990, although the increase of liver burdens with length was similar in Autumn of both years.

2.3.3.5 Summary

Both liver somatic index (LSI) and liver mercury concentrations of adult eelpout from the Forth Estuary showed no significant correlation with length, weight or year class in single site, single season sample, and differences between sexes were not significant. A statistically significant increase was observed in LSI in Summer of both years in eelpout from both Longannet (LO) and Port Edgar (PE) in the Forth Estuary. In contrast, liver mercury concentration showed the opposite pattern, with minimum mean values in Summer in both years at LO, and in 1990 at PE. Liver mercury concentration (as the $\log_{10}$ value) showed a highly significant linear correlation with LSI for fish from LO. There was also a negative correlation between these variables for PE, although the relationship was not statistically significant.

Total mercury burden (i.e. mass of mercury in liver per fish) increased in a linear fashion with body length, when both variables were logged ($\log_{10}$), and linear regressions were significant in most samples. There was no significant difference between the sexes in almost all samples. There were also no significant differences between the regressions of different sites in all

seasons except Spring and Summer 1991. There were no significant differences between seasons in the regressions of $\log_{10}$(mercury burden) on $\log_{10}$(length) at either LO or PE, although there were significant differences between years in Summer at LO, and Spring and Summer at PE.

2.3.4 Total mercury concentrations and burdens in kidney tissues of eelpout

2.3.4.1 Variability of kidney somatic index, and kidney mercury burden

The mean kidney somatic index, KSI (weight as a % of body weight), kidney mercury concentration ($\mu g.g^{-1}$ dry weight), and kidney mercury burden (μg Hg 100 g^{-1} body weight) are presented in Table 2.16, for a number of sites in different seasons. In single-season, single-site samples, none of these parameters was significantly correlated with length or weight of the fish (Pearson correlation, all $P>0.05$). The temporal and spatial variability of KSI, mercury concentration and mercury burden was studied using one-way analysis of variance (ANOVA).

(a) Temporal variability within sites

Longannet (LO): The KSI in Summer 1991 was significantly lower than in Summer 1990 and Autumn 1991 ($F=18.482$; total degrees of freedom=32; $P=0.0000$; Tukey multiple range tests: $P<0.05$). Kidney mercury concentration was significantly higher in Autumn 1991 than in the other two seasons ($F=22.294$; $P=0.000$), and a significant seasonal difference in kidney mercury burden was the result of a significant difference between Summer and Autumn 1991 ($F=13.260$; $P=0.0001$; Tukey: $P<0.05$).

Blackness (BN): There were no significant differences between seasons, in terms of KSI ($F=1.154$; total DF=19; $P=0.342$), kidney mercury concentrations ($F=2.124$; $P=0.148$), or kidney mercury burdens ($F=2.668$; $P=0.097$).

Kingstone Hudds (KH): Seasonal differences were observed in all three measures at this site. A significant difference between seasonal mean KSI values ($F=5.806$; total DF=27; $P=0.0039$) was due to a significant difference between Spring and Autumn 1991, the highest and lowest values (Tukey: $P<0.05$). A significant seasonal difference in mean kidney mercury concentrations ($F=8.108$; $P=0.0007$) was the result of significant differences between Spring 1991 and both Summer 1990 and Autumn 1991 (Tukey: $P<0.05$). The mean kidney mercury burden of Autumn 1991 was significantly lower than the means of the other three seasons ($F=7.006$; $P=0.0015$).

(b) Spatial variability of kidney somatic index, and kidney mercury burden

Summer 1990: There was no significant spatial variability in KSI ($F=7.199$; total DF=20; $P=0.194$), mercury concentration ($F=1.604$; $P=0.230$) or mercury burden ($F=0.0063$;

Table 2.16 Spatial and temporal variability of somatic index (% of body weight), mercury concentration ($\mu g.g^{-1}$ dry weight), and mercury burden (μg Hg. 100 g^{-1} body weight) in eelpout kidney tissue

Site	Season/Year	Sample Size	Kidney Somatic Index		Hg Concentration		Hg Burden	
			mean	se	mean	se	mean	se
Longmeet	Summer 1990	7	0.329	0.037	0.340	0.043	0.044	0.004
	Summer 1991	20	0.474	0.016	0.266	0.048	0.024	0.003
	Autumn 1991	6	0.276	0.034	1.114	0.208		
Blackness	Summer 1990	7	0.402	0.028	0.367	0.079	0.046	0.007
	Autumn 1991	6	0.319	0.038	0.809	0.128	0.053	0.008
	Spring 1992	8	0.315	0.043	0.503	0.190	0.028	0.008
Port Edgar	Summer 1991	22	0.391	0.017	0.389	0.040	0.029	0.003
Kingstone Heath	Summer 1990	7	0.412	0.045	0.325	0.022	0.033	0.003
	Summer 1991	6	0.491	0.038	0.416	0.060	0.030	0.005
	Autumn 1991	8	0.264	0.036	0.256	0.032	0.014	0.002
Millport	Summer 1991	5	0.322	0.027	0.392	0.049	0.024	0.006

P=0.994) between LO, BN and KH in this season.

Summer 1991: There was a significant difference in KSI between sites (F=8.428; total degrees of freedom, DF=52; P=0.0001). Tukey multiple range tests revealed that both LO and KH values were significantly higher (P<0.05) than Port Edgar (PE) and Millport (MP) samples. There was, however, no significant difference between sites in terms of kidney mercury concentration (F=1.930; P=0.137), or kidney mercury burden (F=1.279; P=0.292).

Autumn 1991: There was no significant difference between the mean KSI values of LO, PE, and KH fish in this season (F=0.621; total DF=19; P=0.549). A statistically-significant difference between sites, in both mercury concentration (F=12.342; P=0.0005), and mercury burden (F=10.524; P=0.0011), was due to a significantly lower value in KH fish (Tukey tests: P<0.05).

2.3.4.2 Summary

Kidney somatic index, mercury concentration and mercury burden were not significantly affected by size or age of the fish. In addition, no consistent patterns of either temporal or spatial variability are obvious from the results presented above, although kidney somatic index shows both greater temporal and spatial variability than either mercury concentration or mercury burden.

2.3.5 Total mercury concentrations and burdens of eelpout testes

2.3.5.1 Testes total mercury concentrations and Testes Somatic Index

As total mercury concentration of testes tissue was found to be independent of fish size (length and weight) within sites in each season (scatterplots, Spearman Rank Correlation, test results not presented), the arithmetic mean value was calculated for each within-site, within-season sample. Where sample sizes were sufficiently large ($n \geq 4$ in each sample), a comparison of total mercury concentrations in testes was performed between sites.

Comparisons were possible for Spring 1990 (LO vs PE, Mann-Whitney U-test, M-W), Summer 1990 (LO vs BN vs PE; Kruskal-Wallis, K-W), Spring 1991 (LO vs BO vs TC, K-W), Summer 1991 (LO vs PE, M-W U), and Autumn 1991 (LO vs BN vs PE, K-W). In only one case, Spring 1990, was the difference between sites significant at the 5% level (M-W, P= 0.038), with fish from Longannet having a higher median total mercury concentration than fish from Port Edgar. In all other cases, the value of p was greater than 0.05. The mean mercury concentration of eelpout testes showed a seasonal pattern in both years, as shown in Fig. 2.19a (with standard error and sample size). A decrease in mercury concentration was observed in

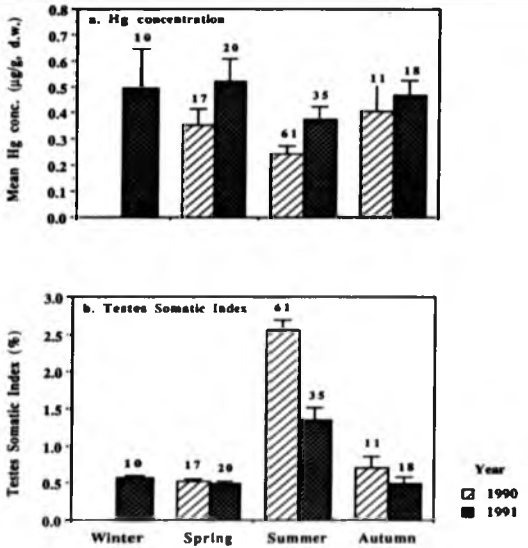


Fig. 2.19 Seasonal and annual comparisons of: a) testes Hg conc., and b) Testes Somatic Index for eelpout from the Forth Estuary

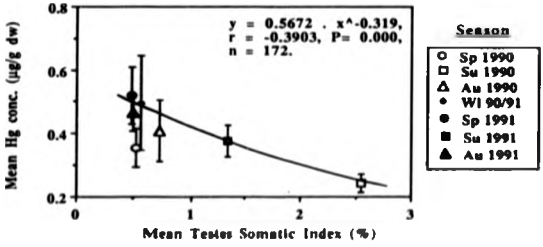


Fig. 2.20 Testes mercury concentration (mean ± s.e.m.) related to testes somatic index for eelpout from estuary and Firth of Forth. Regression equation calculated on individual data from all seasons pooled.

both Summers. The magnitude of the decrease was largest in 1990, when the increase in TSI was greatest. Similarly, mean mercury concentration was lower in Autumn 1990 than in Autumn 1991, in contrast to the TSI values which showed the opposite pattern. The mercury concentration of testes was negatively correlated with the testes somatic index, as illustrated in Fig. 2.20. The data, plotted as seasonal mean values for clarity, showed a poor fit ($r = -0.39$) to the exponential decay model shown on the plot.

The value of Testes Somatic Index (TSI, weight of testes as a percentage of body weight) was calculated for all male fish analysed for testes mercury concentration. When TSI values were tested within-season for association with size (length and weight), no significant correlations were observed within any season (all Pearson correlations, $p>0.05$; full test results not presented).

The mean TSI value (with standard error of the mean and sample size) is shown in Fig. 2.19b for each season during two years (seven seasons from Spring 1990 to Autumn 1991 inclusive). Both years showed the same pattern of seasonal variation in TSI. Testes accounted for approximately 0.5% of the male fish body weight in Spring. A large increase in TSI was observed in Summer in both years. The increase was greater in Summer 1990 (TSI ≈2.5%) than in Summer 1991 (TSI ≈1.5%). TSI values decreased to approximately 0.5% in Autumn in both years, and a similar value was observed in Winter 1990/91.

2.3.5.2 Testes total mercury burdens related to biological parameters

The total mercury burden of eelpout testes, as $\log_{10}$(Hg burden, ug), generally increased in a linear fashion with length and weight of fish. This is illustrated, in Fig. 2.21, for the increase of $\log_{10}$(Hg burden) with length for each season, from Spring 1990 to Autumn 1991. Linear regression statistics, describing the relationships for within-season samples, are shown in Table 2.17, along with regression statistics describing the regressions of $\log_{10}$(Hg burden) on fresh weight (not illustrated).

In all seasons, except Spring and Autumn 1990, the regression of $\log_{10}$(Hg burden) on length was statistically significant at the 5% level ($P<0.05$). Indeed, in Summer 1990, and Spring, Summer and Autumn 1991, the regressions were highly significant ($P < 0.0001$). In those seasons with a significant regression, the value of r^2, the coefficient of determination, showed that the fitted regressions explained between 42.5 and ≈91% of the total variation in testes mercury burdens ($r^2 = 0.425$ - 0.908). In Spring 1990 the regression of $\log_{10}$(Hg burden) on length was only weakly significant ($P<0.10$) and explained only about one-fifth of the variation in the data ($r^2 = 0.212$). In Autumn 1990, for a relatively small sample ($n=9$) of a relatively restricted length range (see Fig.2.21c), neither of the regressions of $\log_{10}$(Hg burden) on length nor weight were statistically significant ($P>0.20$ and $P>0.30$ respectively).

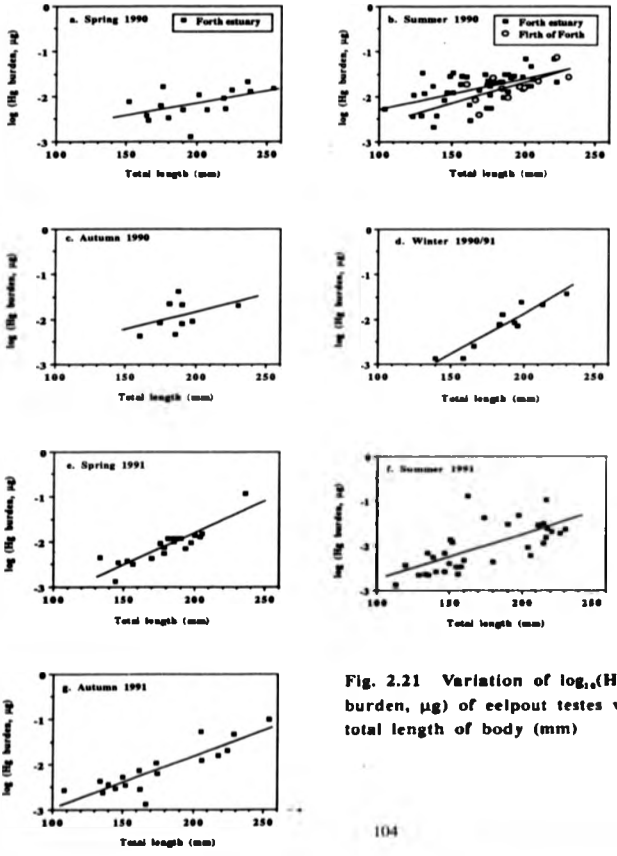


Fig. 2.21 Variation of $\log_{10}$(Hg burden, µg) of eelpout testes with total length of body (mm)

Table 2.17 Linear regression statistics for seasonal relationships between $\log_{10}$(total mercury burden, μg) of testes and total body length (mm) or fresh body weight (g) for eelpout from the estuary and Firth of Forth.

			Length				Weight			
Season	Site	n	a	b	R^2	Significance	a	b	R^2	Significance
Spring 1990	Estuary	17	-3.1477	0.0051	0.212	0.0635	-2.4990	0.0129	0.176	0.0943
Summer 1990	Estuary	49	-2.9673	0.0068	0.298	0.0000	-2.2791	0.0212	0.283	0.0001
	Firth	12	-3.5565	0.0093	0.425	0.0216	-2.4208	0.0219	0.358	0.0400
Autumn 1990	Estuary	9	-3.4039	0.0079	0.192	0.2377	-2.4356	0.0204	0.147	0.3085
Winter 1990/91	Estuary	10	-5.5000	0.0181	0.876	0.0001				
Spring 1991	Estuary	20	-4.5887	0.0139	0.785	0.0000	-2.8207	0.0354	0.704	0.0000
Summer 1991	Estuary	33	-3.7505	0.0099	0.470	0.0000	-2.5323	0.0210	0.378	0.0002
Autumn 1991	Estuary	18	-4.1021	0.0114	0.748	0.0000	-2.7284	0.0279	0.826	0.0000

Note. 1. Linear regression equation takes the form: $\log_{10}$(Hg burden) = a + b.(length or weight)

The relationships between fresh body weight and $\log_{10}$(testes Hg burden) were statistically significant ($P< 0.05$) in fewer seasonal samples than was the case for length. Nonetheless, linear regressions of $\log_{10}$(Hg burden) on fresh weight were still highly statistically significant ($P \leq 0.0001$) in Summer 1990, and Spring, Summer and Autumn 1991. In Winter 1990/91, while $\log_{10}$(Hg burden) showed a significant linear regression with length, the relationship with fresh weight was non-significant. In seasons with significant linear relationships, the fitted regressions explained between 28 and ~83% of the total variation in testes Hg burdens (r^2= 0.283-0.826). Values of r^2 were generally a little lower than the equivalent values for the regressions with length.

Testes mercury burdens were measured in a sample of male fish from the Firth of Forth site, Kingstone Hudds (KH), in Summer 1990 (see Table 2.17 and Fig. 2.21b). Linear regressions of $\log_{10}$(testes Hg burden) on both length and weight were statistically significant ($P<0.05$), and explained around 40% of the total variation in each case (r^2 for length = 0.425, r^2 for weight = 0.358).

2.3.5.3 Seasonal and annual comparisons of testes total mercury burdens

Testes total mercury burdens were compared between seasons within each year by comparing the seasonal regressions of $\log_{10}$(testes Hg burden) on length by an analysis of covariance (ANCOVA). Regressions on length, rather than fresh weight, were selected for the use in the ANCOVA procedure as more seasons showed a significant relationship with length than with weight. Regressions on length also showed higher values of the coefficient of determination (r^2), than was the case for regressions on fresh weight. The seasonal sample for Autumn 1990 was excluded from the comparison as the regression of $\log_{10}$(Hg burden) on length was not significant in this season.

In 1990, the regressions of $\log_{10}$(Hg burden) on length in Spring and Summer were significantly different (Overall test of regressions: $F= 16.544$; degrees of freedom (DF) = 2,62; $P<0.0005$). The difference between the regressions lay not between slopes ($F= 0.362$; DF = 1,62; $P>0.50$) but between elevations ($F= 32.983$; DF = 1,62; $P<0.0005$). The elevation of the Summer regression was significantly higher than that of the Spring regression. An overall test of the regression of $\log_{10}$(Hg burden) on length for Winter 1990/91, Spring, Summer and Autumn 1991 revealed no significant differences between the four seasonal regressions ($F= 1.919$; DF = 6,77; $P>0.25$).

2.3.5.4 Summary

While testes somatic index and mercury concentration are independent of fish size in single seasons samples, testes mercury burden (as $\log_{10}$ value) shows a highly significant positive linear correlation with both length and weight in most seasonal samples. Mercury

concentrations of testes tissue from a sample of Firth of Forth eelpout (Summer 1990) were not significantly different from those of fish from the Forth Estuary. While there were statistically significant differences between seasonal regressions of $\log_{10}$(mercury burden) on length in 1990 (as a result of differences between regression elevations), there were no differences between seasons in 1991.

2.3.6 Mercury in eelpout ovarian tissues and broods

2.3.6.1 Female eelpout reproductive cycle

Figure 2.22 shows the monthly variation in brood somatic index (brood weight as a percentage of whole body weight) and ovary somatic index (ovary weight as a percentage of whole body weight) of female eelpout. Monthly variation has been presented, rather than the seasonal divisions presented for other tissues, due to the very rapid changes observed in relative size of the female reproductive organs. These changes would be masked by use of longer temporal periods. The points represent all female fish caught in each month. Numbers by points represent numbers of fish in samples.

Broods of young fish were present in the ovarian sacs of female eelpout (gravid females) caught in the periods November 1989 to April 1990, October 1990 to January 1991, and September 1991 to April 1992. Broods were found only in ovaries of fish of year class 3 or older. Non-gravid females were captured in very low numbers or were absent from catches in winter months, numbers increasing again in catches in March to April each year. In gravid females, the relative weight of broods (Brood Somatic Index, BSI) increased rapidly between October/November and January/February in each year. The apparent reduction of BSI from March to April 1990 is dependent on a single value in the latter month. Variability in the relative size of the non-gravid ovary (Ovary Somatic Index, OSI) was much less than that observed for broods. The size of brood (number of young) increases in a linear fashion with length, weight and age (as year class) of the mother eelpout, as shown in Fig. 2.23 for fish from the Forth estuary in Autumn 1989. The least-squares linear regressions describing these relationships are all statistically highly significant (regression statistics are given on each figure).

2.3.6.2 Total mercury concentrations and burdens of maternal and offspring tissues

Mercury concentrations of whole ovaries of non-gravid females were not significantly correlated with length, weight or year class in any single-site, single-season sample (Pearson correlation, all $P>0.05$; full test results not presented). Seasonal mean mercury concentrations of whole ovaries are presented by site in Table 2.18. Non-parametric statistics (Mann-Whitney U-test, Kruskal-Wallis analysis of variance) are also presented in Table 2.18, for the comparison of whole ovary mercury concentrations from different sites within seasons, and of seasons between years. There were no significant differences between sites in any seasons. Nor were

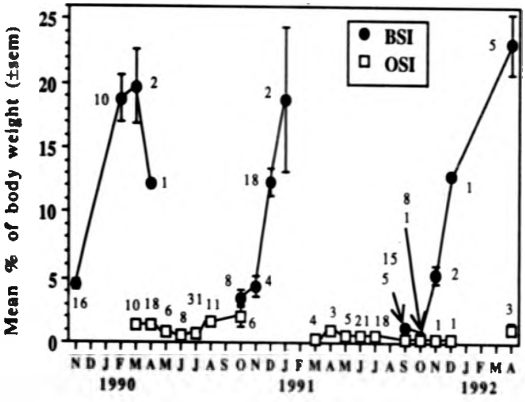
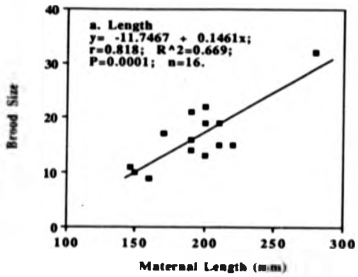
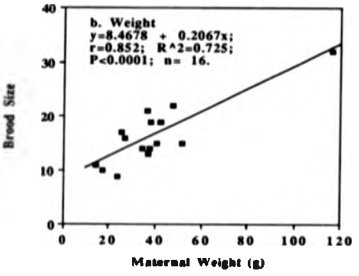


Fig. 2.22 Monthly variation in Brood and Ovary Somatic Indices of female eelpout





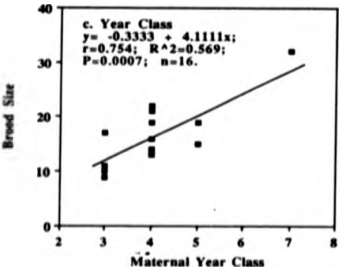


Fig. 2.23 Eelpout brood size related to length, weight and year class of mother fish, Forth estuary, Autumn 1989

Table 2.18 Seasonal mean mercury concentrations of eelpout whole ovaries at individual sites[1] in the Forth estuary and Firths of Forth and Clyde

Season[2]	Site	Sample Size	Mercury concentration ($\mu g.g^{-1}$ dry wt) mean	std error
Spring 1990	LO	6	0.258	0.073
	PE	18	0.211	0.058
Summer 1990	LO	19	0.273	0.055
	PE	4	0.309	0.049
	KH	7	0.212	0.046
Autumn 1990	LO	1	0.185	-
	PE	1	0.054	-
Spring 1991	KH	4	0.361	0.137
Summer 1991	LO	10	0.212	0.061
	PE	10	0.319	0.059
	KH	9	0.286	0.055
	MP (Clyde)	3	0.238	0.056

Notes: [1] Comparison of sites within seasons:

Spring 1990: LO vs PE, Mann-Whitney U-test, U= 31, p = 0.1252. No significant difference between the Hg concentrations of whole ovaries at the two sites.

Summer 1990: LO vs PE vs KH, Kruskal-Wallis, Degrees of freedom = 2, H' = 1.086, p = 0.581. No significant difference between the Hg concentrations of whole ovaries at the three sites.

Summer 1991: LO vs PE vs KH, Kruskal-Wallis, Degrees of freedom = 2, H' = 3.290, p = 0.193. No significant difference between the Hg concentrations of whole ovaries at the three sites.

[2] Comparisons of seasons between years:

LO: Summer 1990 vs Summer 1991, Mann-Whitney U-test, U' = 112, p = 0.435. No significant difference between the Hg concentrations of whole ovaries in the two years.

PE: Summer 1990 vs Summer 1991, Mann-Whitney U-test, U' = 21, p = 0.887. No significant difference between the Hg concentrations of whole ovaries in the two years.

KH: Summer 1990 vs Summer 1991, Mann-Whitney U-test, U' = 35, p = 0.179. No significant difference between the Hg concentrations of whole ovaries in the two years.

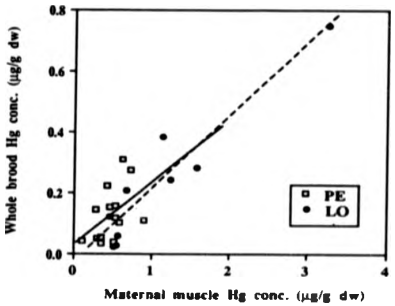


Fig. 2.24 Brood Hg concentration related to maternal muscle Hg concentration for two Forth Estuary sites, Winter 1989/90.

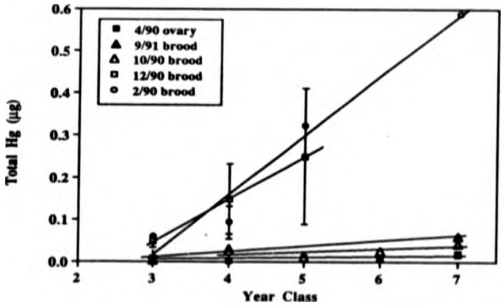


Fig. 2.25 Mercury content of Port Edgar eelpout ovaries and broods by year class

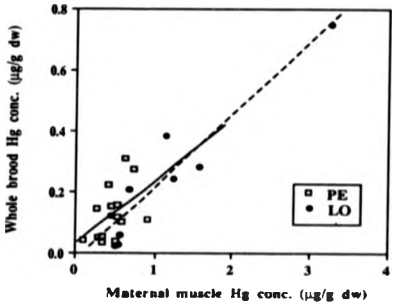


Fig. 2.24 Brood Hg concentration related to maternal muscle Hg concentration for two Forth Estuary sites, Winter 1989/90.

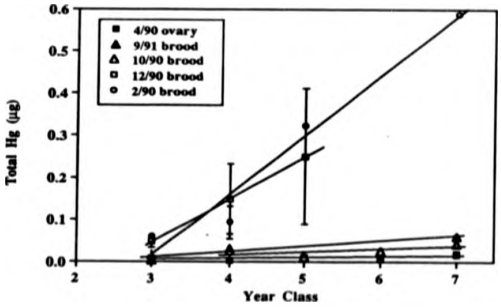


Fig. 2.25 Mercury content of Port Edgar eelpout ovaries and broods by year class

Table 2.19 Regression statistics for monthly relationships between year class of mother fish and total mercury content of whole ovary or brood, for eelpout from Port Edgar in the Forth estuary

Month	Sample Size	a	b	R^2	P
04/1990 Whole Ovary	11	-0.0006	0.00227	0.144	0.250
09/1991 Brood	8	-0.038	0.0143	0.930	0.0001
10/1990 Brood	4	-0.010	0.0065	0.468	0.316
12/1990 Brood	10	-0.259	0.1020	0.204	0.190
02/1990 Brood	5	-0.438	0.1456	0.937	0.0068

there any significant differences between years at any site, when comparing samples from the Summers of 1990 and 1991. The mercury concentrations of whole brood tissues are positively correlated with the mercury concentrations of the maternal skeletal muscle, as illustrated in Fig. 2.24 for eelpout from Longannet and Port Edgar in Winter 1898/90. The least squares linear regression equations and regression statistics describing these relationships are:

LO: $y = -0.0324 + 0.2401x$; $R^2 = 0.891$; $r = 0.944$; $P = 0.0001$; $n = 9$.

PE: $y = 0.0334 + 0.2028x$; $R^2 = 0.209$; $r = 0.457$; $P = 0.100$; $n = 14$.

The relationship for LO fish, covering a much wider range of maternal muscle mercury concentrations than PE fish, was statistically highly-significant, with a high degree of correlation. The relationship for PE fish was a poor fit to the data, and not significant at the 5% level. There was no statistically significant difference between the slopes of the regressions (ANCOVA: $t = 0.691$; degrees of freedom = 20, $p>0.20$), although comparison of the elevations revealed a statistically significant difference (ANCOVA: $t = 5.156$, degrees of freedom = 21, $p<0.001$), with the PE regression having the higher elevation.

The total amount of mercury (or mercury burden) of broods increases with the year class of the mother fish, as shown in Fig. 2.25 for fish from Port Edgar in four months covering early development of the brood in September or October, to the time of emergence of the broods (or 'partus') around February or March. The mercury content of post-partus ovaries, in April, is also shown for comparison. Linear regressions of mercury burden on year class were not significant for ovaries of non-pregnant fish from April, or in some samples of fish in early pregnancy, although the slope of these regressions increases in value through the period of brood development (Table 2.19). From Fig. 2.25, it is also seen that the mercury burden of broods increases within single year classes as the brood grows from Autumn to the following Spring. The increase of mercury in the brood through the period of its development is presented, more clearly for a single year class (4) from Longannet, in Fig. 2.26, thus controlling for the effects of year class on mercury content. There is little change in mercury content of the brood over the period September to November, followed by a rapid increase over the succeeding three months. This period of increasing mercury content coincides with the period of rapid increase in the relative weight of the brood (BSI) observed in each year, as was presented in Fig. 2.21. The mercury content of the broods in a single year-class increases in a non-linear fashion with increasing BSI, as shown in Fig. 2.27, the data showing a good fit ($R^2 = 0.747$) to an exponential model.

2.3.6.3 Summary

There is considerable variation in the size of the female ovary during an annual period, related mainly to the growth of an intra-ovarian brood of young. Brood somatic index (BSI) is highest in late winter females. The number of young in the brood shows a linear increase with the length, weight and year class of the mother fish. Ovary mercury concentrations did not show

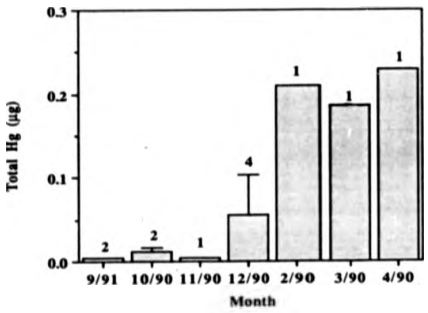


Fig. 2.26 Total mercury (µg) in broods of year class 4 eelpout from Longannet, Forth estuary

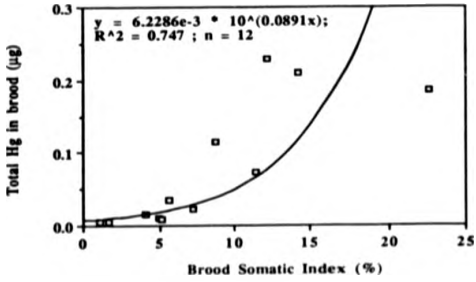


Fig. 2.27 Increase of brood mercury burden with Brood Somatic Index for eelpout (year class 4) from Longannet, Forth estuary

significant biological, spatial or temporal variation. The mercury concentration of brood tissues was positively correlated with the mercury concentration of maternal skeletal muscle. When the influence of maternal age was controlled, it was shown that mercury content of the brood increased only slowly from September to November, followed by a rapid increase over the next three months. Mercury content in broods of a single year class of mother fish increases in an exponential fashion with BSI.

2.4 Discussion

2.4.1 Mean mercury concentrations in eelpout tissues

The commonly-observed positive correlation between fish size or age, and mercury concentrations in skeletal muscle (see Chapter 1) means that a comparison of the mean mercury levels in fish from different sites is a relatively meaningless exercise (Leah *et al.*, 1991a). This procedure could, depending on the size distributions of individuals at different sites, produce misleading results. In this study, comparison of the arithmetic mean mercury concentrations in skeletal muscle between sites suggests a spatial trend of decreasing concentrations from Longannet, close to the industrial discharge of mercury at Grangemouth, to Kingstone Hudds in the Firth of Forth. Given, however, the observed significant positive linear regression of $\log_{10}$(mercury concentration) on length, weight and age in almost all seasonal samples, such a conclusion is risky if the variability due to fish size or age is not taken into account. A later section discusses the validity of the statistical procedures used to assess this covariability.

Presentation of arithmetic means of mercury concentrations does allow a general comparison with other studies, in terms of the overall levels and ranges encountered, and with legislative standards (Leah *et al.*, 1991). The mean mercury concentrations measured in eelpout muscle in this study are comparatively low in relation to the Environmental Quality Standard established by the European Community Directive on mercury in fish muscle (0.3 μg g^{-1} wet weight: see Chapter 1). Occasional muscle samples from individual fish in the Forth Estuary exceeded this EQS value. These were generally from the largest fish captured in the study. Comparison with other recent studies of mercury in fish tissues from UK waters shows that the levels recorded in muscle of eelpout in this study are relatively low (MAFF, 1990, 1991; Leah *et al.*, 1991a, b; Brown, 1992).

It is apparent from the mean and range of mercury concentrations presented for seven tissues of eelpout that there are considerable differences between tissues, in terms of degree of contamination. The highest mean concentrations are in the non-reproductive tissues, muscle, liver and kidney, although clear significant positive correlations exist between the concentrations of most tissues.

The observation, presented in Material and Methods, that there is a size-related loss of weight from the tissues on freezing at -20°C for four months, has some serious implications for the accuracy of mercury monitoring programmes. Reference is often made in the methodology of studies of mercury levels in fish muscle, to the collection of fish, and their subsequent freezing for a period of time, before tissue samples are collected for mercury analysis (e.g. Braune, 1987; Leah *et al.*, 1991a, b). These studies frequently present the mercury concentrations on a wet weight basis, and in relation to length or weight of the fish. Clearly, there is potential for

the introduction of errors related to the variable loss of water from tissues, with small fish potentially losing more water than large fish. In this study, small fish (around 1g) lost up to 17% of fresh weight on freezing. On the assumption that the majority of this weight loss is due to dehydration of the skeletal muscle, the concentrations in small fish could be seriously overestimated by this amount. In subsequent regression analysis of the relationship between mercury concentration and length (or weight), the error would result in a shallower regression slope with a higher elevation. The failure by many authors to properly describe the sample collection and handling methodology also introduces a certain element of uncertainty when they subsequently present mercury concentrations on a wet weight basis (e.g. Jensen and Cheng, 1987; Pellegrini and Barghigiani, 1989; MAFF, 1991). It was to avoid such uncertainty that mercury concentrations were considered on a dry weight basis in this study. Note that this assumes that the water content of the skeletal muscle is relatively constant through the year, an assumption which was not tested. Studies which either describe collection of tissues before freezing, or which present mercury concentrations on a dry weight basis, are relatively uncommon (e.g. Suns and Hitchin, 1990, and Elliott and Griffiths, 1986, respectively).

2.4.2 Use of regression and ANCOVA analysis in studies of mercury in fish

If, as is commonly observed, mercury concentrations in a tissue increase in a linear manner with some measure of size or age, such as length, weight or year class, then the mean concentration will be very dependent on the size distribution of specimens in the sample (Leah *et al.*, 1991). As a result, the resolution of spatial or temporal variability of mercury concentrations may be seriously compromised or biased (Evans *et al.*, 1993). Leah *et al.* (1991) also point out that, while allowing the summary of large amounts of data, the calculation of arithmetic means, perhaps over multi-species samples and large geographical areas, often masks the very heterogeneity that is of interest.

Calculation of the least squares linear regression describing relationships between mercury concentration and size (or age) effectively quantifies the effect of the size/age measure on mercury concentration. Regression statistics can also be combined with simple graphical methods (e.g. scatter plots) to provide detailed analysis, yet with a simplified presentation (Leah *et al.*, 1991). Analysis of covariance (ANCOVA) provides a technique for statistical comparison of samples, by comparing the covariance of the mercury concentration and size/age measure. ANCOVA has been used previously by a number of workers to compare least squares linear regression equations of mercury concentration on some biological parameter, and hence the degree of mercury contamination, of different samples (Menasveta and Siriyong, 1977; Tariq *et al.*, 1992; Evans *et al.*, 1993). Two or more regressions can be compared in terms of slopes or elevations of the regressions or, by sacrificing some statistical power, by an overall test for coincidental regressions (Zar, 1984).

Interpretation of regression functions is discussed by Zar (1984), who indicates that, while the slope of a regression quantitatively describes the rate of change of the dependent variable (Y) with a change in the independent variable (X), interpretation of the regression elevation in biological terms is not straightforward. Zar proposed that, for many regressions, the Y intercept, and consequently, the elevation, has no importance beyond helping to define the line, and the testing of hypotheses about the elevation often requires a risky extrapolation of the regression line far below the range of X for which data has been obtained.

In this work, most comparisons of samples have considered both slopes and elevations of the regressions being compared. This is the statistically-correct procedure (Zar, 1984), allowing the combination of regressions which are not significantly different, in a common regression (as with the seasonal comparisons of skeletal muscle mercury concentrations). The previously-mentioned problem of extrapolating beyond the range of the regression when considering the elevations, however, probably applies in all cases here, particularly when x values are considered on a logarithmic scale. It is possible, therefore, that only the comparison of slopes between regressions can be sensibly discussed in terms of biological significance.

2.4.3 Variability of mercury concentrations in eelpout skeletal muscle

2.4.3.1 Variability related to biological factors

The logarithm of the mercury concentration of skeletal muscle was linearly related to length, weight, and age of fish in almost all samples in this study. Such relationships have also been reported in a large number of studies on other species of fish in fresh, estuarine and brackish, and marine waters. A summary of such studies has already been presented in Chapter 1. With regard to biological parameters, the highest correlations and the most significant regressions in this study were observed between length and log mercury concentration. Length is also the easiest biological variable to record, especially in a field situation, and is likely to be less prone than weight to temporal (ie daily, seasonal) fluctuations. Analysis of variability in skeletal muscle mercury concentrations was carried out, therefore, using the regressions of log mercury concentration on length.

The increase of mercury concentration in muscle with increasing size and age of fish has been discussed by several workers. Cross *et al*. (1973) reported that concentrations of mercury increased significantly with size in white muscle of bluefish (*Pomatomus saltatrix*) and in the bathydemersal species *Antimora rostrata*. This study was particularly important as several metals were investigated and the differences between mercury and other elements, in terms of size-dependence, were well demonstrated. The results indicated that mercury was the only element amongst those studied to exhibit significant positive correlations between the concentration in white muscle and fish weight. It was suggested that copper, iron, manganese

and zinc were probably regulated by the fish, and that these metals were in equilibrium between the fish and the ambient environment. Mercury, by contrast, appeared not to be in equilibrium, presumably because excretion rates could not keep pace with uptake rates of this element. The consequence of this would be a positive uptake of mercury throughout the lifetime of the fish. Similar results were reported by McKie (1983) for flounders (*Platichthys flesus*) from the Ythan estuary (Scotland), in which muscle mercury concentrations increased with length of the fish. By contrast, copper concentrations in muscle were observed to decrease with increasing length.

Not all studies of mercury concentrations in fish muscle have reported a positive correlation between concentration and size or age. There appeared to be no relationship between mercury concentration and weight for samples of a number of fish species from the Canadian North Atlantic coast (Freeman *et al.*, 1972). Similarly, no correlation was observed between mercury concentration and size in yearling perch (*Perca flavescens*), in 15 of 16 lakes sampled by Suns and Hitchin (1990), a relationship which may be the result of the restricted size range obtained by sampling for a single year class. Braune (1987) observed that, while muscle mercury concentration was highly negatively correlated with both length and weight in yearling herring (*Clupea harengus*), correlations within subsequent year classes were poor or non-existent.

The increase of mercury concentration with size or age, as observed for eelpout in this study, may be the result of uptake of mercury by the fish at a greater rate than it can be excreted. Many experimental studies have shown that fish accumulate mercury, in both organic and inorganic forms, very rapidly from both solution and food (Ribeyre and Boudou, 1984; Boudou and Ribeyre, 1985; Pentreath, 1976 a,b,c). By contrast, excretion rates for mercury, especially methylmercury have been shown to be very low. The biological half-life of methylmercury, that is the time taken to lose half the body burden, or for the concentration in tissues to fall by half, has been observed in the range 231 to 1200 days (Jarvenpaa *et al.*, 1970; Ruohtula and Miettinen, 1975; Pentreath, 1976b). Indeed, following the rapid accumulation of methylmercury from seawater by thornback ray (*Raja clavata*) over a 91 day exposure period, no loss was observed over a subsequent 74 day period following transfer to clean seawater (Pentreath, 1976d). The result of the combination of these processes would be a net accumulation of mercury over the lifetime of the fish (Braune, 1987).

The amount of mercury accumulated by fish may be a function of a large number of factors, including environmental variables (eg water temperature, dissolved oxygen concentration, pH, salinity), mercury distribution in the environment (eg amount, speciation, and distribution among different physical components), and biological factors (eg age/duration of exposure, growth rate, size, metabolism, sex, prey selection, competition for food resource, availability/quality of food resource, reproductive state). As many of these factors are clearly related (eg pH and mercury speciation: Beijer and Jernelov, 1979; water temperature and feeding/growth/respiration: Winberg, 1958; Fonds *et al.*, 1989), the accumulation of mercury

by fish is likely to be a complex interaction of many of the above factors. Attempts have been made to construct predictive models of mercury accumulation by fish, taking these factors into account, and based on bioenergetic-type growth models (Fagerstom *et al.*, 1973,1974; Norstrom *et al.*, 1976; Braune, 1987). This type of model was first applied to a marine species, Atlantic Herring (*Clupea harengus*), by Braune (1987). The model accurately predicted the mercury levels which should be accumulated by these fish in the Bay of Fundy, Canada, at least for fish of 3 years and older, as confirmed by comparing the prediction with measurements in wild fish. Fish of a younger age were predicted to accumulate no mercury, a result which did not fit with the observed accumulation by wild fish. Braune proposed that this reflected clear differences in the metabolism and growth of young and older herring, which the model parameters had failed to address.

2.4.3.2 Sex-related variability in mercury concentrations of skeletal muscle

For eelpout from Longannet in the Forth Estuary, there is no statistically-significant sexual difference in muscle mercury concentrations with respect to body length, weight or age, in almost all samples where a comparison is possible. Essink (1980) reported a similar lack of significant sexual difference in muscle mercury content for eelpout from the Ems estuary in the Netherlands, although test statistics were not presented for the comparisons. As was done here, Essink pooled male and female samples when studying temporal and spatial variability of eelpout mercury content (Essink, 1980, 1985, 1988).

As was suggested by Essink (1980), it might be expected that the production of an intraovarian viviparous brood of young fish, on an annual basis, and to which a measurable amount of mercury is transferred, could provide the female fish with an extra mechanism by which it could eliminate some of the body burden of mercury. The lack of a significant difference between the mercury concentrations in muscle of male and female fish of a given length suggests that this method of mercury elimination may not be utilised to any extent. Alternative explanations could be:

1. In order to compensate for the energetic demands of producing a viviparous brood, the female fish has an elevated food intake, and/or gill ventilation rate, by which route(s) it accumulates more mercury than the male fish. In this way, the extra demands of the brood might lead to the accumulation of enough mercury to replace in the maternal tissues, the amount transferred to the ovary during the brood's development, or:

2. Following the parturition of the brood in March or April, any loss of mercury by the mother as a result of transfer to the brood, and its subsequent departure from the ovary at partus, might be replaced rapidly by mercury accumulated from the environment. The consideration of samples seasonally might cover too long a period to observe this loss, and

subsequent increase, of mercury burden.

Other workers have also demonstrated a lack of significant sexual difference in mercury content, for a number of fish species from a limited range of morphological groups and habitats. Some workers have, however, shown highly-significant sexual differences, usually in long-lived, large or predatory pelagic species. In samples of various fish species from estuarine or coastal habitats similar to that inhabited by eelpout in the Forth Estuary, there appears to be little evidence of any sexual difference in muscle mercury content. Pellegrini and Barghigiani (1989) observed no significant differences between male and female Tyrrhenian Sea flatfish, sole (*Solea vulgaris*) and megrim (*Lepidorhombus boscí*), in terms of the regressions of muscle mercury concentration on total length or on age. Initial studies by Jensen (1982, 1983) indicated no significant sexual differences in muscle mercury concentrations in another flatfish, flounder (*Platichthys flesus*), results which he confirmed in a later work (Jensen and Cheng, 1987).

While many species of large, predatory fish do exhibit sex-based differences on the degree of mercury contamination, Menasveta and Siriyong (1977) found no differences between males and females of four species of shark, nor between sexes of two species of tuna, yellowfin tuna (*Neothunnus albacora*) and bigeye tuna (*Parathunnus sibi*) from the Andaman Sea. Similarly, Barber and Whaling found no significant differences between sexes in three species of billfish, blue marlin (*Makaira nigricans*), white marlin (*Tetrapterus albidus*), and sailfish (*Istiophorus platypterus*), although pooling of sexes led to an increased coefficient of variation in the descriptive exponential models, compared to those of single sex samples.

The above results directly contradict those of Monteiro and Lopes (1990), who recorded that the rate of mercury accumulation was higher in males than females of another billfish species, swordfish (*Xiphias gladius*). They found that in medium-to-large specimens, mean mercury levels were higher in males than in females, although no differences were observed in small swordfish (length<125 cm.). Similar results have been reported for several species of shark: the gatuzo shark, *Mustelus schmitti* (Argentina, Marcovecchio *et al.*, 1986), the school shark, *Galeorhinus australis*, and gummy shark, *Mustelus antarcticus* (Australia, Walker, 1976), and the spiny dogfish, *Squalus acanthias* (British Columbia, Canada, Forrester *et al.*, 1973) In the latter case, the relationships for different sexes were independent of sampling location or environmental mercury concentrations. Long-lived freshwater fish species have also been shown to demonstrate sexual differences in the net rate of mercury accumulation. Tariq *et al.* (1992) found distinct differences between males and females of two commercially-fished species, *Catla catla* and *Chela chanius*, from the mercury-polluted Rawal Lake in Pakistan. With respect to both length and weight, mercury levels were generally higher in males of both species.

Reasons for sexual differences in mercury accumulation have been discussed by some of the above workers (Forrester *et al.*, 1972; Walker, 1976; Monteiro and Lopes, 1990; Tariq *et al.*, 1992), and in general by Phillips (1980). In most cases, lower mercury concentrations in females than males have been explained as the result of faster growth rates of female fish, leading to greater dilution of mercury by tissue growth. Walker (1976) referred to unpublished results which supported the suggestion of higher growth rates in female than male sharks. Monteiro and Lopes (1990) also discussed other possible causes. They considered the possibility of specific mechanisms of mercury elimination in female swordfish (*X. gladius*) which might result from the different chemistry of gonad and gamete, affecting the affinity of mercury and the loss of residues eliminated from the body in the sex products, or to a simple difference in the amounts of ova or sperm shed. They suggested that the evidence of Kai *et al.*, (1986), of higher selenium levels in female gonads of billfish, capable of forming excretable mercury-binding selenium proteins supported the former hypothesis.

Given the apparent importance of sex as a source of variability of mercury concentrations in certain species, a notably large number of studies in the literature on mercury accumulation by fish appear to have taken no account of possible differences between the sexes. There was no discussion of sex as a potential source of variation in, amongst others, studies by: Johnels *et al.* (1967), Zitko *et al.* (1970), Cocoros *et al.* (1973), Cross *et al.* (1973), Scott (1974), Dix *et al.* (1975), Brooks *et al.*, (1976), Topping and Graham (1978), Bull *et al.* (1981), McKie and Topping (1982), McKie (1983), Luten *et al.* (1987), Badsha and Goldspink (1988), Clark and Topping (1989), Chvojka *et al.* (1990), Cope *et al.* (1990), Grieb *et al.* (1990), and Suns and Hitchen (1990). Although not exhaustive, the list is long enough to indicate that ignorance of the possible influence of sex on mercury accumulation rates is widespread.

Even in an otherwise-comprehensive study of the biological factors controlling mercury accumulation in five species of freshwater fish in Canadian lakes, Jackson (1991) did not consider the possible effects of sex as a factor affecting mercury accumulation rates. A failure to account for such a difference could lead to considerable errors in the estimation of true mean mercury concentrations in samples, especially when larger, long-lived fish species are studied. The initial elimination, in this study, of sex as a source of biological variability in eelpout skeletal muscle mercury concentrations was, therefore, an important first step towards quantifying variability due to sources other than environmental levels, thus increasing the "signal-to-noise" ratio (Elliott *et al.*, 1988) to allow valid spatial and temporal comparisons.

2.4.3.3 Spatial variability of mercury concentrations of skeletal muscle

The spatial variability of skeletal muscle mercury concentrations was assessed by comparison of the single-site regressions of $\log_{10}$ (mercury concentration) on length. Differences between sites were minimal for eelpout in the Forth when compared with other recent studies from UK

waters. The within-species spatial variability of muscle mercury concentrations was assessed for three fish species from the North-east Irish Sea, using, as in this study, regressions of mercury concentration on length (Leah *et al.*, 1991 a, b). For all three species involved, plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), and lesser-spotted dogfish (*Scyliorhinus caniculus*), there was considerable variability between sites in the increase of mercury concentration with length. The magnitudes of regression slopes at sites, known to receive high inputs of mercury from a combination of sewage sludge disposal and industrial discharges, were more than 3x higher in plaice, more than 4x higher in dab, and up to 30x higher in dogfish, than for the same species taken at sites where environmental mercury concentrations are low. Intra-specific differences were also apparent, as mercury concentrations in the dogfish were 5-10x higher than in plaice or dab from the same site (Leah *et al.*, 1991 a, b).

There was generally a lack of significant differences between sites during the study period, in the degree of mercury accumulation in eelpout muscle (as the slope of log mercury concentration regressed on length). Elliott and Griffiths (1986) indicated that, in 1983, the mercury concentration in skeletal muscle of eelpout from Longannet was significantly higher than that in eelpout from Port Edgar, when variability of mercury concentration due to fish size was controlled. The historical trend of decreasing mercury concentration with distance from the industrial mercury discharge at Grangemouth, as indicated by these workers, no longer appears to be significant, at least as manifested in fish tissues. It is possible that this is related to the overall large decrease in the input of mercury from the industrial source, as described in the Introduction. Such a pattern of decrease in eelpout muscle mercury concentrations over several years, following a decrease of major mercury inputs, was observed in the Ems estuary in the Netherlands (Essink, 1980, 1985, 1988). The relationship between inputs of mercury to the Forth Estuary and mercury concentrations in eelpout muscle tissue, is presented in Chapter 5.

2.4.3.4 Temporal variability of mercury concentrations of skeletal muscle

Temporal variability of skeletal muscle mercury concentrations was considered here on two time scales, seasonal (within years) and annual (between years). While there is some literature on annual variations of mercury concentrations in the literature, mostly as a result of obligatory monitoring programmes, only one other study of seasonal variability can be found. Kohler and co-workers (1986) reported seasonal differences in the skeletal muscle mercury concentrations of flounders (*Platichthys flesus*) from the Elbe Estuary in Germany. A sharp increase of mercury concentrations in summertime, rather than resulting from increases in concentrations in the sampled population, was ascribed to the influx of fish with higher concentrations from a more contaminated site, as they migrated away from a seasonally-deoxygenated locality. They concluded that seasonal distribution of this species in the Elbe interferes with the assumption that samples taken at various stations always represent the contaminant accumulation pattern at that station. It is unlikely that eelpout perform such migrations to escape deoxygenation events

in the Forth Estuary, as the region of the Estuary experiencing such oxygen sags is well upstream of the upper limit of eelpout's range (Elliott *et al.*, 1989). The pattern of seasonal variation observed in skeletal muscle in both years is discussed in the next section, in relation to fish condition.

With reference to comparisons between years, assessing the differences of mercury concentrations within single seasons, it appears that no significant annual variation is observed in skeletal muscle mercury concentrations during the study period. A maximum of three years could be compared, with the samples from Autumn 1989-1991. The statistically-significant differences between the elevations of regressions in Summer and Winter may well have no biological significance, as described in section 2.4.2, due to the risk of extrapolation of the regression beyond the range for which x-values were available (Zar, 1984). Even if significant differences were observed between years, the detection of temporal trends in mercury concentrations is unlikely over such a relatively short time-span. Hansen *et al.* (1982) and Jensen (1982) point to the need for longer time series of data, possibly in excess of 10 years, before temporal trends of mercury concentrations in fish tissues, related to changes in environmental levels, would be detected above the natural variation in such observations. Such a time series of data is now available for eelpout from the Forth Estuary, combining the results presented here with archival results of earlier studies by the Forth River Purification Board. These results are presented in Chapter 5.

2.4.3.5 Fish condition and skeletal muscle mercury concentration

Condition factors are used to compare the 'condition', 'fatness' or 'well being' of fish, and are based on the hypothesis that heavier fish of a given length are in better condition (Bagenal, 1978). The allometric condition factor, K' (see Materials and Methods), calculated for eelpout in this study, was selected rather than the commonly-used Fulton's condition factor, K. The calculation for K' is based on length raised to a power which has been calculated as the slope, b, of the regression of log (weight) on log (length). As a result this condition factor is reported as theoretically more appropriate than K, which is based on an approximation of this b value, of b=3 (Ricker, 1975). This latter is defined as: [100.(weight/length^3)], and is reportedly prone to misleading results when the fish sample involved has a large range of lengths, as in the seasonal samples under consideration here (Bagenal, 1978).

As a measure of 'fatness' of fish, the condition factor might reasonably be expected to reflect seasonal variability of factors which could affect the relative weight of the fish, such as the availability or nutritional quality of food, reproductive state or metabolic rate (as related to environmental variables such as water temperature: Braune, 1987). Some studies have shown such seasonal variability of condition factors in fish. Jangaard *et al.* (1967) showed that condition of cod (*Gadus morhua*) varied through an annual cycle, peaking in autumn, and

falling to a minimum value in spring. A similar pattern was observed in plaice (*Pleuronectes platessa*), with a maximum condition factor in September, decreasing to a minimum in the period March to May (White and Fletcher, 1985). The increase of condition from spring to an autumn maximum in plaice coincided with the period of active feeding. In five genera of tropical surgeonfish (Teleostei: Acanthuridae), seasonal changes in condition paralleled seasonal changes in fat deposits (Montgomery and Galzin, 1993).

This study has demonstrated that eelpout from the Forth Estuary also exhibit a repeating annual pattern of condition factor, similar in nature to the patterns observed in cod and plaice. Although the seasonal variability of condition factor in eelpout has not been causally related to any of the above-mentioned variables, the evidence of the above studies suggests that the increase in condition from spring to autumn in each year is related to the period of active feeding. Personal observations suggest that, from the presence of material in the gut of eelpout, active feeding took place from around early May to October or early November in both years. No prey items were found in the gut of any eelpout outwith this period.

The negative correlation and linear relationship demonstrated between condition factor and the mercury concentration of skeletal muscle (as the slope of the regression of log mercury concentration on length) implies that seasonal variability of muscle mercury may be linked with seasonal changes in condition. Similar observations have been made previously by Scott (1974), and Suns and Hitchin (1990). In both of these studies, the authors proposed that the inverse relationship between body condition and mercury levels might be the result of the adverse toxic effects of higher mercury levels leading to a reduced fitness of health of fish, reflected in lower condition factors. It is possible , however, given the stability of the binding of mercury in fish muscle tissue (Carty and Malone, 1979), that mercury concentrations at low levels in tissues do not actually exert such direct toxic effects. This would imply that either the amount of mercury in the muscle of the fish actually varies throughout the year, but in the opposite direction to variations in condition or, if not varying, is effectively "diluted" or "concentrated" in the tissue by seasonally-related increases or reductions in muscle mass respectively. Such a relationship has implications for the use of a fish species to monitor the bioaccumulation of mercury in an aquatic environment.

If seasonal fluctuations in mercury concentrations of skeletal muscle are related principally to dynamics of that tissue, then samples compared from different seasons between years could lead to misleading results, particularly if a species showing large seasonal variation of skeletal muscle mass is used. Results of studies of the seasonal variability of skeletal muscle mercury burden in relation to tissue dynamics are presented in Chapter 3.

2.4.4 Seasonal variation of liver mercury and liver somatic index

As the patterns of seasonal variability of mercury concentration in liver appear to occur in the

opposite direction to that of seasonal variations in relative liver size, or liver somatic index, it seems wise to consider possible causes of the latter variation when seeking an explanation for the former. The seasonal pattern of variation in liver somatic index (LSI) observed for eelpout in this study (at Longannet in 1990 and 1991, and Port Edgar in 1990), with significantly higher values of LSI in summer, has been observed in several studies of eelpout (Korsgaard and Petersen, 1979), and other fish in temperate waters. These include plaice (*Pleuronectes platessa* : Dawson and Grimm, 1980; White and Fletcher, 1985; George *et al.*, 1990), winter flounder (*Pleuronectes americanus*: Fletcher and King, 1978), and burbot (*Lota lota*: Pulliainen and Korhonen, 1990). The LSI of female rainbow trout (*Salmo gairdneri*), however, reached a maximum value in winter (December) in one study, while LSI of male fish showed no apparent seasonal variation (Olsson *et al.*, 1987).

The seasonal trend in eelpout LSI observed in this study seems to follow closely that of water temperature in the Forth Estuary, a relationship also indicated for plaice (*Pleuronectes platessa*) from north east Scottish coastal waters by George *et al.* (1990). The seasonal variation of water temperature indicated in the latter study, from 3-5°C in February to March, to 12-15°C in the period August to September, is similar to that found annually in the Forth Estuary (see Appendix 2.3).

White and Fletcher (1985) suggested that the seasonal increase in plaice LSI in their study was probably due to a combination of an increase in numbers of hepatocytes, and the deposition of lipid and glycogen in the liver during the period of intense feeding after spawning, declining again as the lipid and glycogen stores are mobilised during the winter period of poor feeding and maximum gonad development. From their results, it appears that the seasonal feeding cycle of the plaice has the most significant effect on liver size. In general, relatively inactive benthic fish tend to store lipids in the liver rather than in muscular tissue (Sargent, 1976). Seasonal variations in the amounts of lipid present in fish coincide generally with changes in environmental temperature but can also be related to changes in the availability of food (Sargent, 1976; Henderson and Tocher, 1987). An increase in the consumption of diet with increasing temperature is true of fish in general (Bagenal, 1978), and has been suggested by Henderson and Tocher (1987) as the reason for a linear increase in carcass lipid content of channel catfish with increasing temperature, as reported by Andrews and Stickney (1972).

In a study of lipid metabolism in female eelpout (*Zoarces viviparus*) from Danish waters, Korsgaard and Petersen (1979) observed a large increase in liver lipid content corresponding to a seasonal summer increase in relative liver size (liver somatic index, LSI). A similar peak in lipid content of liver was reported for both sexes in eelpout from the Gulf of Finland (Pekkarinen, 1980) The maximum value of LSI was reached in Danish fish in June, and remained fairly constant at approximately 3% of body weight until August. During the remainder of the year, the mean LSI was between 1 and 2%. These values are in the range

recorded in this study for eelpout from Longannet in both 1990 and 1991, and in Port Edgar in 1990, although LSI values were lower in Port Edgar fish in 1991.

The liver in female eelpout reaches its maximum size before the initiation of vitellogenesis, or production of yolk for developing oocytes in the ovary, and liver weight remains high during vitellogenic growth (Korsgaard and Petersen, 1979). A decrease in liver lipid content takes place simultaneously with a large decrease in the level of vitellogenin, and the level of serum lipid. This decline in lipid content also coincides with the decrease of relative liver size (LSI) over the autumn to winter, as recorded by Korsgaard and Petersen (1979), and in this study for Forth Estuary eelpout.

From the above, it is likely that the seasonal fluctuations in LSI observed for Forth Estuary eelpout are closely related to seasonal feeding patterns. These are, in turn, likely to be strongly influenced by seasonal variations in water temperature. Although accumulation of lipid in eelpout liver in summer months has been shown to be related to gonadal development processes such as vitellogenesis, the increase in lipid content and associated increase in LSI are presumably also simply a function of higher metabolic energy requirements, and greater prey availability in summer months. The significant difference in LSI between Longannet and Port Edgar in summer may be the result of differences in water temperature between the two sites, leading to different metabolic requirements for food. Similarly, a difference in the availability, or quality (from a nutritional standpoint), of prey species at different sites, could lead to differences in the amount of lipid accumulated in the liver. Differences of relative tissue weights between wild rainbow trout populations have been inferred to be a result of differences in available food supplies (Weatherley and Gill, 1981).

This study of seasonality in fish liver mercury concentrations and burdens appears to be the first of its kind. No other example can be found in the considerable literature on mercury in fish. The significant seasonal differences observed in mercury concentrations within each site are generally not reflected by seasonal variations in the total burdens of liver mercury (as the regressions of log mercury burden with log length). This implies that the seasonal variations observed in mercury concentrations are largely the result of the dilution and concentration of similar seasonal burdens by the demonstrated seasonal growth and shrinkage of the liver. This hypothesis is supported by the significant negative relationship between liver mercury concentration and LSI, at least for fish from Longannet. The range of LSI values observed in LO fish was much greater than in PE fish, with the mean LSI significantly higher at LO in both Summers. This difference may reflect real differences between the two sites in the environmental conditions which influence seasonal variation of LSI (see above), or may simply be an artifact of sample collection. If the former is true, then this highlights another potential source of natural variability which it might be necessary to consider if comparing liver mercury concentrations between sites. If differences in environmental conditions between seasons at a single site can significantly influence the variability of LSI, then presumably the same may be

true of differences between sites in a single season. It is also possible that apparent differences in concentration between years, even if controlling for season, may be due to differences in relative liver size between years, rather than to differences in the availability of environmental mercury. For this reason, some assessment of the actual mercury burden of the liver, as well as simple concentration, is perhaps advisable when comparing mercury in liver samples.

Significant seasonal variation of liver mercury concentrations also challenges the validity of the use of the muscle:liver mercury concentration ratio as an indicator of recent mercury contamination. The use of this ratio was proposed by Julshamn *et al.* (1982) who established that, on feeding either inorganic or methylated mercury to cod, *Gadus morhua*, an increase in liver mercury concentration preceded a longer term increase in muscle mercury concentration. They proposed that a muscle:liver ratio of 1 should indicate that mercury levels in the cod were in equilibrium with those in the environment, while ratios of less than 1 suggested that fish had recently been exposed to higher environmental mercury levels. The results presented here for eelpout show that mean mercury concentrations in liver can be up to 3.75 times higher in Winter than in Summer, a difference related more to variation in liver somatic index, than to changes in liver mercury burden. The liver somatic index of cod has been shown to vary between 2% and 4% of body weight from Winter to Summer, a similar range to that seen in eelpout. It might be expected, therefore, that mercury concentrations in cod liver might show seasonal variation similar to that observed for eelpout, even in an environment relatively uncontaminated by mercury. This highlights the danger of making assumptions about ratios of mercury concentrations in different tissues, and ascribing changes to differences in environmental mercury levels, without considering the underlying patterns of natural variability described here.

Like the seasonal variability observed in eelpout skeletal muscle, the results for liver have implications for the monitoring of levels of persistent pollutants in fish liver as a measure of bioavailability of these substances in the aquatic environment. The comparison of mercury levels between samples of fish livers may be fraught with difficulties as a result of seasonal fluctuations in concentrations. These results illustrate the necessity of quantifying natural sources of variation of liver mercury before embarking on a programme of monitoring for mercury bioaccumulation using this organ.

2.4.5 Variability of kidney mercury and kidney somatic index

Studies of mercury in fish kidney are very scarce in the literature, and tend to be simply a report of concentrations in one-off samples (Renzoni et al., 1973; Topping *et al.*, 1975;. The general lack of any significant differences between sites for kidney mercury concentrations and burdens reflects a similar lack of spatial variability of mercury in the other tissues studied. The significant correlations between mercury concentrations of kidney and most other tissues, including ovary tissues (but excluding broods), implies an increase in kidney mercury levels

with increasing mercury body burden. This may be related to the excretory function of kidney, or to longer term storage of mercury by this tissue.

Mercury concentrations were shown to increase with time in the kidney of flounder (*Platichthys flesus*) transferred from a mercury contaminated environment to clean seawater (Riisgaard and Famme, 1983). Subsequent excretion of mercury via the kidney was, however, not reported. The kidney has been proposed, by Olson *et al.* (1973) as site for the demethylation of methylmercury in rainbow trout (*Salmo gairdneri*). These workers reported an increase in kidney tissue, of the proportion of mercury as the inorganic form, with time following oral administration of radioactively labelled methylmercury. This increase was ascribed to the loss of carbon moities from demethylation of methylmercury, and retention of mercury in the inorganic form. Similar results were reported by Burrows and Krenkel (1973) for the bluegill (*Lepomus macrochirus*).

2.4.6 Variability of testes mercury and testes somatic index

The seasonal variability of mercury concentrations in testes may be a result of seasonal uptake and loss of mercury from this organ or may, as has been shown for both muscle and liver, be a function of seasonal variability in relative weight of the organ, perhaps without significant seasonal gain or loss of mercury. The latter explanation is certainly supported by the observed significant inverse relationship between TSI and mercury concentration, and the limited seasonal variability of testes total burdens of mercury, relative to body length. The seasonal variation of eelpout Testes Somatic Index (TSI) was first studied by Kristoffersson and Pekkarinen (1975), who showed that growth of testes in Summer is related to the process of spermatogenesis, which proceeds rapidly through Spring to a peak of testes condition in June, in synchrony with the process of oogenesis in females.

Statistically significant differences were observed in 1990 between seasons, in an overall test for coincidental regressions of $\log_{10}$(Hg burden) on length for eelpout testes, although the differences were significant only between elevations of the two regressions. As discussed in section 2.4.2, such a statistical difference between regressions may be meaningless in biological terms if , as here, the points of coincidence with the y-axis defined by the elevations are well below the range of values sampled for x. Given this interpretation, and as there was no significant difference between the regression slopes of Spring and Summer in 1990, the regressions of the two seasons may be considered as statistically similar, as were all seasonal regressions in the following year. Thus, while mercury concentrations in the testes tissue showed a similar pattern of seasonal variability in both years, this was not related to significant changes in the actual amount of mercury in the testes of a fish of standard size (length). This lack of significant seasonal differences in the mercury burden of eelpout testes implies that the Summer decrease of mean mercury concentration in testes is a seasonal effect, caused by the

increase in relative weight of that organ in relation to the reproductive cycle.

No other studies of seasonal variability of mercury in fish testes can be found in the literature. Some studies of seasonality of metal concentrations in bivalve molluscs have, however, considered the role of the gametes in the elimination of mercury from the body. The mercury concentrations in whole bodies of American Oyster (*Crassostrea virginica*) dropped sharply following spawning (Cunningham and Tripp, 1973). Similarly, a rapid decrease in the mercury content of mussels (*Mytilus edulis*) from west European coasts in Spring was thought to be related to spawning (De Wolf, 1975). Cossa *et al.* (1980) observed, however, that gonad maturation explained little of the variance (3.4%) in the relationship between cadmium content and body weight of *M. edulis*. .

The apparent lack of mercury loss from eelpout testes following mating may be a result of the viviparous mode of reproduction, whereby the male eelpout fertilises the female internally through the male genital papilla. The amount of sperm secreted by the testes may be relatively small compared to most other fish species which reproduce by external fertilisation and, as such, the amount of mercury eliminated may actually be very small.

2.4.7 Mercury in female reproductive tissues, in relation to reproductive cycle

The annual reproductive cycle of female eelpout has been described previously by Kristoffersson *et al.* (1973), and Korsgaard and Andersen (1985). Bretschneider and deWitt (1943) identified two periods of "pregnancy" in female eelpout from Dutch waters, although Kristoffersson *et al.* (1973) refuted this, reporting, as here, a single annual pregnancy for eelpout from the Baltic. The annual production of intraovarian broods by eelpout takes place 2-3 months earlier in the Baltic and the Gulf of Finland, than in the Forth Estuary (Kristoffersson *et al.*, 1973; Korsgaard and Andersen, 1985).

There is rapid increase of relative brood weight (Brood Somatic Index, BSI), from November/December to January, which has been demonstrated to be linear (Korsgaard and Andersen, 1985). This growth has been related to the change of the principal nutritional source, from the yolk sac, to the uptake of material from the embryotrophe. It has been hypothesised that the period of utilisation of the energy of the yolk sac in eelpout embryos is extended compared to non-viviparous fish, due to supplemental energy uptake from the embryotrophe during the yolk sac period (Korsgaard, 1986). Since the yolk sac is reportedly reabsorbed almost completely by 1-2 months after hatching (Kristoffersson *et al.*, 1973; Korsgaard, 1986), the energy required for growth of the embryos is presumably supplied entirely by the mother, through the embryotrophe.

Soin (1968) describes how the embryos of eelpout develop a mobile jaw and gill apparatus at an

early stage of development, when the yolk sac is still quite large. One effect of this is to supply embryotrophe fluid to the intestines, which are modified by extensive folding to form numerous lamellae, with a dense capillary network, ie modified in a way which is suitable for absorption of nutrients from the embryotrophe.

The presence of quantities of partially-digested cellular material in the intestines of intraovarian eelpout embryos has been recorded by both Stuhlmann (1887), and Kristoffersson *et al.* (1973). Both studies describe the cellular material as comprising both red blood cells, and other unidentified cellular material. Kristoffersson *et al.* (1973) hypothesised that the minute but variable amounts of protein in the embryotrophe may depend on the fragility of the inner ovarian wall, and its damage by the freely moving embryos. This would cause minor bleedings into the ovarian cavity, and the presence of red blood cells, both in the embryotrophe and in the alimentary tracts of the intestines, was presented as evidence that this may be considered as a normal phenomenon. The presence of this cellular material in the intestines of embryos suggests a mechanism for the transfer of mercury from mother to brood.

It is possible to hypothesise from this evidence that the transfer of mercury from the mother eelpout to the embryos, demonstrated to occur by the positive linear relationship between maternal and brood tissue concentrations, is related to the transfer of energy via the embryotrophe. It is possible that mercury would not be distributed evenly throughout the embryotrophe, as the cellular material and red blood cells would provide a much greater concentration of mercury-binding sites than the fluid component, with its low protein content (Kristoffersson *et al.*, 1973). If mercury is present in the fluid of the embryotrophe, then uptake of the fluid in the intestines, or its passage across the gill surface, provides a possible route for transfer to embryo tissues.

The rapid increase of total mercury content of the brood, as demonstrated for Year Class 4 female eelpout, coincides with the period of rapid increase of the brood weight. The rate of increase of mercury content with relative brood weight (as BSI) is non-linear, however, showing a good fit to an exponential model. It can be hypothesised that this is the result of the uptake, by the embryos, of increasingly larger amounts of the cellular material from the embryotrophe. Thus, as more of the energy requirements of growth were provided by cellular material, so the embryos would accumulate more mercury per unit of body weight gained. The presence and distribution of mercury in ovarian fluid will be studied in Chapter 3.

The loss of mercury from the ovarian tissues by this route may also provide an explanation for the lack of correlation between mercury concentrations of ovarian tissues and other tissues. The removal of cellular material from the inner ovary wall by the matrotrophic process of brood development might lead to a rapid turnover of ovarian sac tissue relative to other tissues. The ovarian sac tissues certainly had the lowest mean mercury concentrations of all tissues analysed, suggesting that mercury is indeed lost from this tissue during brood development.

Chapter 2 Appendices

Appendix 2.1 Details of sampling sites[1] for eelpout in the Forth Estuary and Firth of Forth

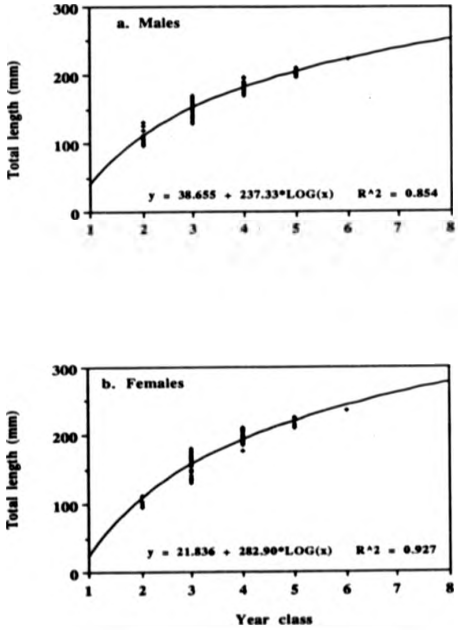
Site Name	Site Code	Distance of mid-point from ICI outfall (km)
Longannet	LO	1.7
Bo'ness	BO	5.6
Tancred	TC	9.3
Blackness	BN	12.8
Port Edgar	PE	16.0
Kingstone Hudds*	KH	35.3

Note. [1] Sites locations are shown in Figure 2.8

Appendix 2.2 Procedure for age determination of eelpout

The term 'year class' refers to the fish produced in a given year. Although there is not yet complete agreement on age designation or terminology, by common usage the age of fish is usually determined by reference to the annual marks on its hard parts (Bagenal, 1978). In this case, the number of annual rings on the otoliths, or ear bones, was determined by light microscopy, with transmitted light viewed from above. Eelpout captured during the six months, November 1989 to April 1990, were allocated to a particular year class according to the number of opaque rings laid down during the growing season (Bagenal, 1978). As no food was found in guts of eelpout over this period, it was assumed that no increase in length occurred over the Winter/ Spring period. The distribution of lengths for each year class are shown by individual sexes in the figures overleaf (Appendix 2.2 a, b) Year class 2 refers to a fish of 1-2 years of age, year class 3 to a fish of 2-3 years, and so on. As there was little overlap of lengths between year classes, later fish were allocated to a year class based on the maximum lengths recorded for each year class, as above.

Male and female fish were aged separately. The zero point for the 'growing year' was assumed to be the end of July, as this is the period when ripe eggs are present in females, and the male gonadosomatic index has a maximum value. Fish of year class 1 were taken as year class two fish from this point, and so on for each year class. Fish from year class 1, although present in female ovaries until late Winter, only appeared as free-swimming individuals in catches from June onwards, and the maximum length for Year class 1 fish in Summer (May-July) was taken as 113 mm.



(Appendix 2.2) Length at age for eelpout from the Forth Estuary, from otoliths taken between November 1989 and April 1990

Appendix 2.3 Calculation of Tissue Mercury Concentrations

The absorbance reading obtained for a particular sample is proportional to the mass of mercuric ions in the volume of sample assayed. Each initial absorbance value (A_I) must be corrected for background absorbance due to the solution matrix. The mean background absorbance (A_B) was subtracted from each initial absorbance reading to give the background-corrected absorbance (A_C).The regression equation for the calibration graph (Equation 2.1, Section 2.2.3.3.3) was rearranged to allow calculation of mercury masses from background-corrected absorbances:

$$\text{Mercury Mass in assayed aliquot, } M_A = (A_C - a)/b \qquad \text{(Equation 2.2)}$$

The aliquot mercury mass, M_A, was converted to the mass in the whole tissue sample, M_S, by the equation:

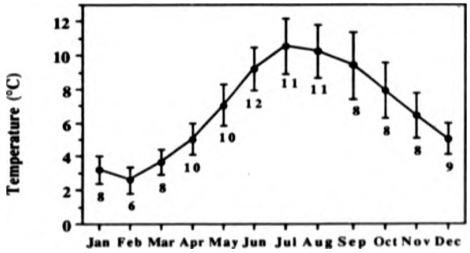
$$M_S = M_A \times (\text{Volume of tissue digest, ml}) / (\text{Volume of assayed aliquot, ml}) \qquad \text{(Equation 2.3)}$$

The mercury mass value, M_S, was converted to tissue concentrations (wet, ww, and dry, dw) by the following equations:

$$\text{Total mercury concentration (wet), } C_W = M_S / \text{Tissue Sample Weight (g, ww)} \qquad \text{(Equation 2.4)}$$

$$\text{Total mercury concentration (dry), } C_D = M_S / \text{Tissue Sample Weight (g, dw)} \qquad \text{(Equation 2.5)}$$

Units of concentration values are $ng.g^{-1}$, converted to $\mu g.g^{-1}$ by dividing by 1000. All calculated tissue mercury concentrations were corrected for recovery of the method (see Section 2.2.3.4) by multiplying each concentration by a factor of (100 / 92.8).



Appendix 2.4 Monthly variation of mean water temperature (mean+sem) in the middle Forth Estuary (site TC), from data collected routinely by the Forth River Purification Board, 1981-1992

Appendix 2.5 Calculation of Allometric Condition Factor (K')

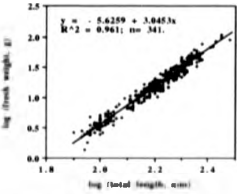
Condition factors are used to compare the 'condition', 'fatness' or 'well being' of fish, based on the hypothesis that heavier fish of a given length are in better condition (Bagenal, 1978). The allometric condition factor (or K') is calculated using the formula:

$$K' = 100^* \text{ weight} / \text{length}^b,$$

where b is determined as the regression coefficient (slope) of the regression of log weight on log length (Ricker, 1975). As K' was calculated to compare seasonal variation in body condition, and to examine the relationship between condition and degree of mercury contamination in muscle, the value of b was calculated using all fish to be included in the comparison (see Table 2.14). The relationship between log length and log weight for these fish is shown in the figure below, along with statistics for the regression. From these regression statistics, the value of b for this population is 3.0453. The value of K' was calculated, therefore, using the formula:

$$K' = 100^* \text{ weight} / \text{length}^{3.0453},$$

N.B. The presence of a brood in the ovary of female fish leads to a considerable increase in total body weight in the later stages of brood development. As it is the condition of the female carcass which was of interest, the weight of females carrying a brood was corrected by subtraction of the brood weight from fresh weight before inclusion in the above procedure.



(Appendix 2.5) Regression of log weight on log length for eelpout from Longanot and associated sites

Chapter 3

Whole Body and Tissue Mercury Burdens in Eelpout (*Zoarces viviparus* L.) from the Forth Estuary, Scotland

3.1 Introduction

3.1.1 Whole body and tissue burdens of mercury in fish

Although there is a considerable volume of literature regarding the accumulation of mercury in fish, especially in relation to the concentrations encountered in skeletal muscle samples from wild fish, only very rarely is the whole body mercury burden of fish considered. In only a few cases have studies been reported which relate the concentrations measured in (for example) the edible skeletal muscle, to the total amount of mercury in the fish. Such studies as have been reported are generally single, one-off measurements of tissue burdens in relation to whole body burdens, to provide a comparison with the distribution of mercury administered artificially to fish under controlled conditions. Two such examples are the distribution of whole body mercury in tissues of wild plaice, *Pleuronectes platessa*, and thornback ray, *Raja clavata* (Pentreath, 1976 a and d).

The most in-depth examination of fish whole body mercury burdens encountered in the literature was reported by Braune (1987) for Atlantic Herring (*Clupea harengus harengus*) from the Bay of Fundy, Canada. This study described both the whole body mercury burden, and that of the skeletal muscle, in relation to a number of biological parameters. These observations were ultimately used to test the validity of predictions of mercury accumulation by a bioenergetics-based mercury accumulation model for the herring. No consideration was given by Braune, however, to seasonal variability of either the whole body, or skeletal muscle mercury burdens, as the model was constructed to predict the burden at a standard time in each annual period.

A hypothesis was presented in Chapter 2, with regard to the seasonal variability of muscle mercury concentrations and their relation to fish condition. It was suggested that observed seasonal variations of mercury concentrations might be related to seasonal variability of skeletal muscle mass, an argument supported by evidence of a negative correlation between fish condition and mercury concentrations. To test this hypothesis, it is necessary to know how much of the body weight is comprised of skeletal muscle, how much mercury this muscle contains, and how both muscle weight and muscle mercury burden vary between seasons. Measurements of the whole body mercury burdens, and burdens of other tissues, of the same fish would entail only a little more work, but would provide some rarely collected information about the importance and potential functions of various tissues in relation to mercury.

Another hypothesis was presented in Chapter 2, in relation the the transfer of mercury between mother fish and broods during the viviparous reproductive cycle of eelpout. It was suggested that the observed consumption by later broods, of cellular material, including red blood cells, suspected to originate from the ovary wall of the mother fish, provided a mechanism for the

mercury transfer from mother to brood. Correlative evidence of a linear relationship between maternal and brood tissue concentrations supports the argument, which requires to be tested further by examination of the distribution of mercury in different components of the fluid. This distribution will be presented here in relation to the mercury burden of the whole ovary through the annual reproductive cycle.

3.1.2 Aims

1. To quantify the whole body burdens of total mercury in eelpout from sites in the Forth Estuary and Firth of Forth, and the variability of burdens in relation to a number of biological parameters.

2. To quantify whole body and tissue mercury burdens in relation to seasonal variability.

3. To study the seasonal variation of the mercury burden of the eelpout ovary, and the distribution of mercury in different ovarian components, including the brood and ovarian fluid.

3.2 Materials and Methods

3.2.1 Sampling Procedures

3.2.1.1 Sampling Sites, Dates and Fish Collection

In order to study the whole body and tissue total mercury burdens of eelpout, samples of fish were collected by trawling from several sites in the Forth Estuary and Firth of Forth between July 1991 and March 1992. The sites referred to hereafter have been fully-described in Chapter 2 (see Section 2.1.1). Fish were collected from Longannet (LO) and Port Edgar (PE) in July 1991, from Kingstone Hudds (KH) in the Firth in October 1991, from LO and PE in November 1991, and from Blackness (BN) in March 1992. Trawling procedures were described in Chapter 2.

Eelpout were taken in the different months to allow inter-season comparison of whole body and tissue mercury burdens. The division of the year into three-month seasons has been described in Chapter 2, and the same divisions are used here. Fish sacrifice and allometric measurements were performed as described in Chapter 2.

3.2.1.2 Whole Body Dissections and Tissue Collection

a) Procedures

Fish were placed in a freezer at -20°C on return to the laboratory. Tissue samples were collected from freshly frozen fish (within 24 hours of freezing), weighed, and then frozen until mercury analysis was performed. As no significant ice layer formed on the body surface, it is not likely that significant dehydration of the body resulted from the freezing (see Chapter 2, Materials and Methods).

All dissections were performed on a cork dissection board, on which had been pinned a sheet of "Cling Film" polythene, under a sheet of clean, white laboratory tissue. The sheets were replaced for each dissection. Disposable latex gloves were worn during all dissection work. All dissecting instruments were stainless steel. Disposable stainless steel scalpel blades were used, a new blade being used for each new dissection. There is a small risk of transfer of mercury between tissue samples collected from the same animal, but this was reduced by rinsing the blade regularly with DDW and dabbing on clean laboratory tissue. Other instruments were rinsed with DDW and wiped with clean tissue between dissections. They were also soaked overnight between daily uses in a solution of Decon-90 deterent (2%, v/v with DDW), and thoroughly rinsed with DDW before use. Scalpel blades were not cleaned before use but were taken directly from sealed packs.

To study the distribution of total mercury among the tissues of eelpout, the bodies of eelpout were comprehensively dissected into the component organs and tissues. Tissue samples were weighed to the nearest 0.01g, placed in clean, numbered polythene petri dishes, and frozen at -20°C for later mercury analysis.

b) Skeletal Muscle

Skeletal muscle was divided into three body regions for analysis: tail, abdomen and head, as shown in Fig. 3.1. The skin was removed from the muscle of each body region, and retained for inclusion with the carcass.

The skeletal muscle was dissected separately from each body region and weighed to the nearest 0.01g. A sample was taken of each body region's skeletal muscle for total mercury analysis. The left-side fillets of tail and abdominal muscle were retained from most fish. The whole tail muscle and abdominal muscle samples were collected from very small (Year Class I) fish in July. The entire head muscle sample was collected from each fish for mercury analysis.

It was not possible to dissect all of the muscle tissue from each fish, as tiny fragments of muscle were retained on the skeleton, for example between the vertebrae and around the base of fin rays. As, however, the mass left on the skeleton was relatively tiny compared to the mass of muscle dissected successfully off the skeleton, the dissected muscle has been assumed to represent the entire muscle mass.

c) Visceral Tissues

The liver and kidney were dissected whole from most fish and weighed to the nearest 0.01g. Kidney tissue proved impractical to collect from the very smallest fish and was included in the carcass in these specimens. The spleen was collected separately, only from fish taken in November 1991. All tissue samples were stored at -20°C for later mercury analysis.

The digestive tract was dissected out whole, opened, and any gut contents removed. Fish from October and November 1991 and March 1992 had no prey material in the digestive system, although some sedimentary material was usually present. Fish from July contained some sedimentary material, and prey items or their remains. *Crangon crangon*, *Carcinus maenas* and *Macoma balthica* were easily identifiable. Prey items were removed if present and all digestive tract samples were then rinsed with 5% nitric acid solution (v/v with DDW) to remove any sediment particles which might introduce mercury contamination to the tissue sample. This tissue was then included with the carcass for mercury analysis. The effectiveness of the rinsing procedure is unknown, but Lobel *et al.* (1991) point to the risk of sediment-bound metals in the digestive tract causing a variable and unquantifiable degree of contamination in whole-body metal studies.

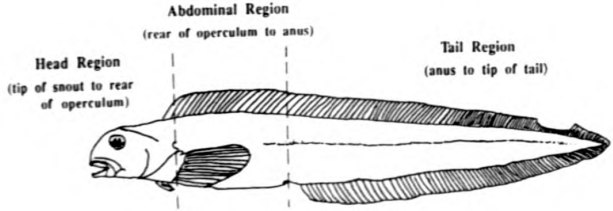


Fig. 3.1 Body divisions used for collection of eelpout skeletal muscle sub-samples are indicated by dotted lines.

d) Reproductive Tissues

The testes (in male fish) were dissected out whole from each fish and weighed to the nearest 0.01g. For female fish not carrying a brood, the ovary was dissected out whole and weighed to the nearest 0.01g. The ovarian sac was dissected out whole from gravid female eelpout while still frozen, and any ovarian fluid present was allowed to drain into a pre-weighed acid-cleaned dish as the sac thawed. The embryos were removed, and weighed to the nearest 0.01g.

The whole broods were retained for mercury analysis from fish taken in October 1991. In samples from later months (November 1991, March 1992), with larger individual embryonic fish, a sub-sample of 3-6 embryos was taken from each brood (enough to provide greater than 0.5g of tissue for mercury analysis if possible). In two fish from July, the ovary was dissected and the eggs removed from their follicles for weighing and mercury analysis. These ovaries contained no measurable ovarian fluid.

e) Further treatment of Ovarian Fluid

It was noticed that, on standing, a proportion of the material suspended in the ovarian fluid settled out in the bottom of the container. Examination by light microscopy (at magnification x400) revealed that the material consisted principally of unidentifiable organic material, with red blood cell fragments occasionally observed. As the possibility existed that mercury was not evenly distributed throughout the ovarian fluid, it was decided to study the distribution of mercury in the two components of the fluid.

If at least 0.5g of ovarian fluid was obtained from individual November and March fish, a sub-sample was processed further to separate the supernatant fluid from the pelletable fraction. Ovarian fluid, present in only small quantities in October, was assayed whole for mercury in female fish from these months. To separate the two fractions, the collected ovarian fluid sample was pippeted into an acid-cleaned, dry polypropylene centrifuge tube (of measured dry weight) on an electronic balance, and the weight of the whole sample was recorded to the nearest 0.01g. The sample was thoroughly mixed by shaking manually for one minute, and a sub-sample of whole fluid taken for mercury analysis to a cleaned, numbered polypropylene tube, which was stored at -20°C until analysed.

The centrifuge tube with remaining sample portion, was re-weighed and the weight of the sub-sample calculated by subtraction. The remaining sample portion was then centrifuged at 3000 revolutions per minute for 30 minutes. No organic material could be observed (at x400 magnification) in the fluid layer following this procedure, although the solution was usually coloured. The supernatant was then pippeted carefully (to avoid re-suspension of the pellet of material formed in the bottom of the tube) into a numbered, cleaned polypropylene storage tube,

on a tared electronic balance. The wet weight of the supernatant fluid sample was then recorded to the nearest 0.01g. The centrifuge tube was then re-weighed to allow calculation of the wet weight of the pellet. The pellet was resuspended in 1.0g (1ml) of DDW added to the centrifuge tube, and the resuspension was pippeted into a cleaned tube for storage as above. The wet weight of the pellet was then calculated by subtraction. Both supernatant and pellet fractions were then stored at -20°C for later mercury analysis.

f) Carcass

The remaining tissues, including gills, skin, skeleton, head (minus skeletal muscle), digestive tract, gall bladder, spleen and kidney (in smaller fish), and urinary bladder, were pooled as "carcass", weighed to the nearest 0.01g and frozen for later mercury analysis. The carcasses of July fish were not collected for analysis.

3.2.2 Mercury Analysis

3.2.2.1 Sample preparation and analytical methodology

The total mercury content of the tissue samples was measured by Cold Vapour Atomic Absorption Spectrophotometry (CVAAS), following pressure dissolution of the samples with nitric acid. The general methods, materials and apparatus used have been described in Chapter 2, and will not be redescribed in detail here. The calculation of tissue sample mercury concentrations from CVAAS absorbance values was also described earlier (see Chapter 2). Some features of the analytical procedures, however, regarding pressure dissolution of large tissue samples, and the treatment of certain tissue samples, were different from the methods used previously.

3.2.2.2 Treatment of Large Carcass Samples

Given the relatively large size of most carcasses (wet weight much greater than 2g in most cases), the previously-used method of digesting a whole tissue sample in a single vessel was impractical. Hence, large carcass samples were divided into approximately 2g (wet-weight) portions and these were dried and digested individually, then subsequently pooled for dilution and mercury analysis.

This procedure undoubtedly entails more work, and increases the chance of contamination from external sources, by increasing the number of items of apparatus encountered by the whole sample when compared with taking a sub-sample of a homogenised carcass sample for mercury analysis. Given, however, the extremely heterogeneous mixture of tissues in the carcass, with varying degrees of ease of homogenization, it was considered that a sub-sample of a

homogenised carcass was unlikey to be representative of the mixture of tissues present in the whole carcass sample. For this reason, the whole carcass was digested and sub-samples of the resulting solution assayed for mercury.

3.2.2.3 Treatment of Ovarian Fluid Samples

Ovarian fluid samples (whole, supernatant and pellet) were analysed only on a wet weight basis because of the potential risk of loss of volatile dissolved mercury compounds from solution during any drying process.

3.2.3 Data Treatment and Statistical Analysis

For comparison of whole body mercury burdens between months, the whole body burdens of female fish were calculated without the mercury content of the brood, and regressions were based on weight of the maternal fish without brood weight. Distribution of whole burden in tissues for females with broods is presented with the brood included and excluded. Apart from the above, data treatment procedures and statistical analysis were performed as outlined in Chapter 2.

3.3 Results

3.3.1 Whole body mercury burden

The whole body mercury burden of eelpout increased with both length and weight of eelpout, as shown in Fig. 3.2 for fish from the Forth Estuary in July 1991, November 1991, and March 1992, and the Firth of Forth in October 1991. The whole body burden of July fish is an estimate, calculated from the relationship between skeletal muscle mercury burden and whole body burden, presented in Fig. 3.5. In all four months, the increase with length gave a good fit to an exponential curve (Fig. 3.2, 1-4a). For eelpout from July 1991 and March 1992, whole body burden of mercury also increased in an exponential manner with weight (Fig. 3.2 1b and 4b). For fish from October 1991, the relationship with weight was linear (Fig 3.2, 2b), while the increase of burden with weight in the sample from November 1991 was best described by a second-order polynomial equation (Fig. 3.2, 3b).

A $\log_{10}$ transformation of both axes resulted in a highly significant linear relationship between $\log_{10}$ (burden) and $\log_{10}$ (weight) in all four months, as illustrated in Fig. 3.3. Linear regression statistics for the relationships are shown on each plot. The single-month regression equations of October, November and March fish were compared using analysis of covariance (ANCOVA: Zar, 1984). As the values for July fish were estimates, this month was excluded from the test.

An overall ANCOVA test for coincidental regressions revealed a significant difference between months ($F= 3.854$, degrees of freedom = 4, 31, $P< 0.025$). Although the slopes of the regressions were not significantly different ($F= 0.217$, $df= 2, 27$, $P> 0.25$), a significant difference was found between elevations ($F= 6.867$, $df= 2, 31$, $P< 0.025$). Tukey multiple comparison tests (October vs November, October vs March, and November vs March) revealed that all elevations were significantly different (Tukey tests: $q=4.963$, $P<0.005$; $q=3.352$, $P<0.025$; $q=4.036$, $P<0.025$ respectively, all degrees of freedom, 3 and 27).

3.3.2 Distribution of whole body mercury burden in tissues of eelpout

The relative weight of individual tissues (tissue somatic indices), the percentage of whole body mercury burden in each tissue, and the tissue/whole body mercury concentration factors are presented for individual months in Table 3.1 to 3.5. The greatest part of both the tissue mass and the whole body mercury burden was accounted for by the skeletal muscle, in both sexes in all months. Relative proportions of muscle on the different regions of the body were fairly constant between monthly samples, with >55-59% on the tail, 35-41% on the abdomen, and the remainder (<5%) on the head. There were differences between months in the percentage of mercury burden contained in the total skeletal muscle, which held 52-59% of the mercury burden in fish from July, October and March, while in fish from November, skeletal muscle

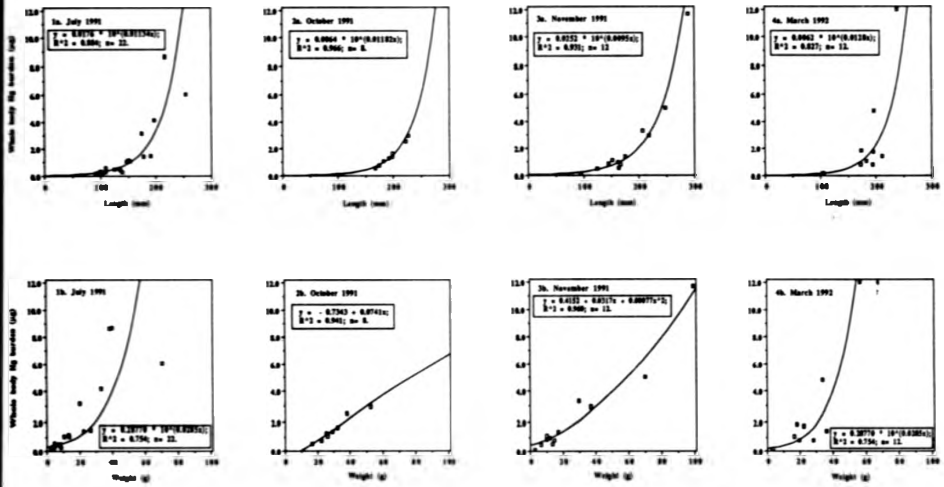


Fig. 3.2 Seasonal variation of whole body mercury with size parameters for eelpout from the Forth estuary (July, November and March) and Firth of Forth (October).

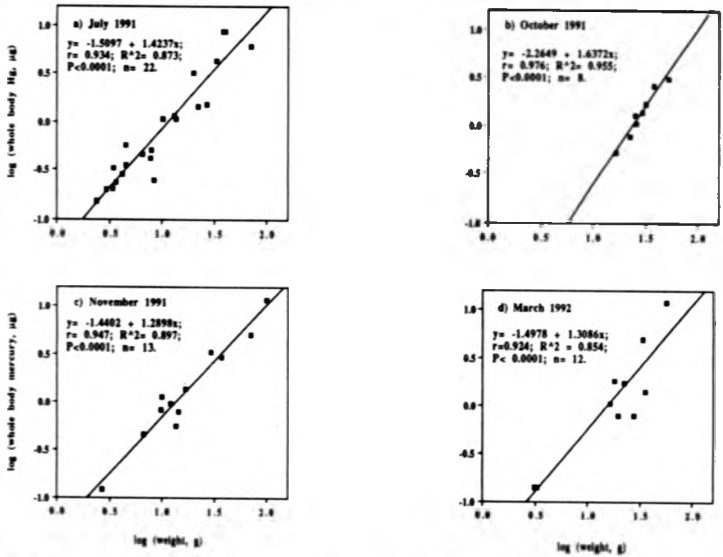


Fig. 3.3 Seasonal variation of regressions of $\log_{10}$(whole body Hg burden) on $\log_{10}$(fresh weight) for eelpout from the Forth Estuary (a, c, d) and Firth of Forth (b)

Table 3.1 Mean Tissue Somatic Indices, tissue distribution of estimated whole body mercury burden[1], and mean tissue Hg concentration factors[2] for adult female, male and Year-Class 1 eelpout from the Forth Estuary, July 1991.

Tissue	Tissue Somatic Index		% of Whole Body Hg Burden[1]		Tissue Hg Conc. Factor	
	x	sd	x	sd	x	sd
Adult Females (n=7)						
Liver	2.13	0.74	1.24	0.64	0.59	0.26
Kidney	1.48	0.25	0.11	0.20	0.22	0.40
Skeletal Muscle[3]	41.77	4.20	52.48	17.06	1.24	0.33
(Tail)	24.39	3.40	25.85	14.60	1.07	0.60
(Abdomen)	16.01	2.34	24.88	10.84	1.56	0.66
(Head)	1.36	0.72	1.75	1.21	1.46	1.06
Whole ovary	0.34	0.29	0.20	0.03	0.40	0.22
Adult Males (n=11)						
Liver	2.02	0.69	1.95	2.80	0.82	1.02
Kidney	0.08	0.13	0.04	0.10	0.12	0.28
Skeletal Muscle[3]	43.38	3.84	58.06	9.62	1.33	0.14
(Tail)	25.65	2.83	34.84	7.68	1.38	0.34
(Abdomen)	16.29	2.06	20.96	8.59	1.30	0.58
(Head)	1.44	1.02	2.25	1.72	1.51	0.61
Testes	1.57	1.14	0.98	0.47	0.78	0.50
Year Class 1 (n=4)						
Liver	1.49	0.24	0.70	0.13	0.49	0.15
Skeletal Muscle[3]	38.05	3.18	68.18	8.54	1.79	0.13
(Tail)	21.86	2.46	36.81	5.80	1.69	0.24
(Abdomen)	15.32	1.42	30.11	4.11	1.97	0.21
(Head)	0.87	0.26	1.25	0.44	1.43	0.37

Notes. [1]. Whole body mercury burden for July fish was estimated from relationship between total mercury burden in skeletal muscle and whole body mercury burden in Fig. 3.5.

[2]. Tissue Hg Concentration Factor is calculated as the ratio:
Hg concentration of tissue / Hg concentration of whole body

[3]. Skeletal Muscle comprises pooled Tail, Abdomen and Head Muscle sub-samples

Table 3.2 Mean tissue Hg concentration factors[1] for gravid female eelpout[2] from Kingstone Hudds, in the Firth of Forth, October 1991.

Tissue	Tissue Somatic Index		% of Whole Body Hg Burden		Tissue Hg Conc. Factor	
	x	sd	x	sd	x	sd
Females-Brood Included						
Liver	1.97	0.57	3.14	3.10	1.53	1.20
Kidney	0.27	0.10	0.30	0.10	1.12	0.23
Skeletal Muscle[3]	53.29	2.34	58.76	5.69	1.10	0.08
(Tail)	30.91	1.41	30.75	3.53	1.00	0.11
(Abdomen)	20.57	1.53	25.19	4.73	1.22	0.16
(Head)	1.80	0.82	2.82	1.59	1.54	0.31
Ovarian tissue	0.54	0.11	0.44	0.25	0.31	0.18
Ovarian Fluid	0.37	0.76	0.05	0.08	0.14	0.13
Whole Brood	0.62	0.57	0.52	0.39	0.95	0.48
Carcass	28.10	2.69	36.79	5.48	1.31	0.14
Females-Brood Excluded						
Liver	1.98	0.57	3.17	3.14	1.53	1.21
Kidney	0.27	0.11	0.30	0.10	1.12	0.23
Skeletal Muscle[3]	53.63	2.60	59.06	5.62	1.10	0.08
(Tail)	31.11	1.55	30.90	3.52	0.99	0.11
(Abdomen)	20.71	1.62	25.32	4.74	1.22	0.16
(Head)	1.81	0.82	2.84	1.59	1.54	0.32
Ovarian tissue	0.54	0.11	0.44	0.25	0.79	0.32
Ovarian Fluid	0.37	0.78	0.05	0.08	0.06	0.14
Carcass	28.27	2.89	36.98	5.51	1.31	0.16

Notes. [1]. Tissue Hg Concentration Factor is calculated as the ratio:
Hg concentration of tissue / Hg concentration of whole body
[2]. Sample size, n=7.
[3]. Skeletal Muscle comprises pooled Tail, Abdomen and Head Muscle sub-samples.

Table 3.3 Mean Tissue Somatic Indices[1], tissue distribution of whole body Hg burden and mean tissue Hg concentration factors[2] for male and gravid female eelpout from the Forth Estuary, November 1991. Values for females with broods included and excluded.

Tissue	Tissue Somatic Index		% of Whole Body Hg Burden		Tissue Hg Conc. Factor	
	x	sd	x	sd	x	sd
Females-Brood Included (n=2)						
Liver	1.24	0.05	2.30	0.61	1.86	0.57
Kidney	0.36	0.02	0.60	0.15	1.63	0.30
Spleen	0.03	0.003	0.04	0.06	1.19	1.69
Skeletal Muscle	39.23	3.66	76.06	4.24	1.94	0.07
(Tail)	21.62	0.39	38.76	2.05	1.79	0.13
(Abdomen)	16.22	3.78	33.56	7.72	2.07	0.01
(Head)	1.40	0.51	3.73	1.42	2.66	0.04
Ovarian tissue	0.82	0.02	0.54	0.42	0.66	0.50
Ovarian Fluid	11.92	0.59	0.96	1.14	0.08	0.09
Whole Brood	5.37	0.94	2.90	2.89	0.50	0.45
Carcass	27.62	3.00	16.59	6.79	0.59	0.18
Females-Brood Excluded (n=2)						
Liver	1.31	0.04	2.38	0.70	1.82	0.59
Kidney	0.38	0.02	0.61	0.13	1.59	0.26
Spleen	0.036	0.003	0.044	0.062	1.15	1.62
Skeletal Muscle	41.48	4.28	78.44	6.69	1.89	0.03
(Tail)	22.84	0.64	39.91	0.93	1.75	0.09
(Abdomen)	17.16	4.17	34.70	8.98	2.02	0.03
(Head)	1.48	0.53	3.82	1.35	2.59	0.01
Ovarian tissue	0.86	0.03	0.56	0.45	0.64	0.50
Ovarian Fluid	12.59	0.50	0.97	1.15	0.08	0.09
Carcass	29.17	2.89	16.99	6.50	0.57	0.17
Males (n=11)						
Liver	2.17	1.50	2.02	1.39	1.91	1.06
Kidney	0.45	0.30	0.42	0.28	1.53	0.91
Spleen	0.02	0.04	0.02	0.04	0.39	0.64
Skeletal Muscle[3]	46.96	7.62	72.07	10.11	1.57	0.34
(Tail)	26.20	5.38	39.38	5.56	1.57	0.41
(Abdomen)	19.00	3.14	30.22	5.98	1.62	0.38
(Head)	1.75	0.36	2.48	1.19	1.42	0.61
Testes	0.41	0.22	0.38	0.20	1.32	0.72
Carcass	24.87	10.16	24.92	10.18	0.80	0.33

Notes.[1]. Tissue Somatic Index is calculated as 100 x (wet weight of a tissue / fresh weight of fish)

[2]. Tissue Hg Concentration Factor is calculated as the ratio:
Hg concentration of tissue / Hg concentration of whole body.

[3]. Skeletal Muscle comprises pooled Tail, Abdomen and Head Muscle sub-samples.

Table 3.4 Comparison of tissue somatic indices (TSI, %) and tissue distribution of whole body mercury burden (%) for gravid[1] (including and excluding brood), and non-gravid[2] female eelpout from Blackness, Forth Estuary, March 1992.

Tissue	Tissue Somatic Index (%)						% of whole body Hg Burden					
	Gravid + Brood		Gravid - Brood		Non - gravid		Gravid + Brood		Gravid - Brood		Non - gravid	
	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd
Liver	1.09	0.23	1.36	0.30	1.60	0.23	1.77	1.00	2.01	1.21	1.99	0.94
Kidney	0.28	0.06	0.36	0.07	0.29	0.02	0.37	0.23	0.42	0.28	0.16	0.07
Skeletal Muscles	31.16	4.65	38.73	4.43	42.23	10.34	57.13	10.64	63.26	10.57	48.68	15.81
(Tail Muscle)	17.49	3.64	21.65	3.58	23.48	5.30	32.78	12.11	36.14	12.15	24.78	9.99
(Abd. Muscle)	12.29	1.18	15.34	1.53	16.76	5.79	21.51	2.90	23.93	3.89	20.79	7.83
(Head Muscle)	1.39	0.39	1.74	0.52	1.99	0.80	2.84	1.51	3.19	1.80	3.11	1.91
Ovarian Tissue	1.54	0.53	1.93	0.68	-	-	0.44	0.27	0.50	0.32	-	-
Ovarian Fluid	3.46	3.11	4.22	3.81	-	-	0.28	0.28	0.32	0.32	-	-
Whole Brood	19.97	5.77	-	-	-	-	9.76	4.42	-	-	-	-
Whole Ovary	-	-	-	-	1.11	0.76	-	-	-	-	0.36	0.31
Carcass	27.11	3.04	33.97	5.08	37.59	6.15	30.23	10.57	33.49	11.32	48.80	15.38

Notes: [1]. Sample size for gravid females: n=5.
[2]. Sample size for non-gravid females: n=3.
[3]. Skeletal Muscle comprises pooled Tail, Abdomen and Head Muscle sub-samples.

Table 3.5 Comparison of Tissue Hg Concentration Factors[1] for gravid[2] (including and excluding brood), and non-gravid[3] female eelpout from Blackness, Forth Estuary, March 1992.

Tissue	Tissue Hg Concentration Factor[1]					
	Gravid + Brood		Gravid - Brood		Non - gravid	
	x	sd	x	sd	x	sd
Liver	1.66	0.91	1.52	0.90	1.29	0.71
Kidney	1.22	0.53	1.10	0.52	0.57	0.29
Skeletal Muscle[4]	1.83	0.20	1.63	0.21	1.14	0.09
(Tail Muscle)	1.85	0.44	1.65	0.43	1.03	0.20
(Abd. Muscle)	1.76	0.23	1.56	0.17	1.24	0.08
(Head Muscle)	2.00	0.66	1.78	0.64	1.42	0.50
Ovarian Tissue	0.31	0.18	0.28	0.18	-	-
Ovarian Fluid	0.14	0.13	0.13	0.12	-	-
Whole Brood	0.50	0.22	-	-	-	-
Whole Ovary	-	-	-	-	0.32	0.22
Carcass	1.13	0.42	0.98	0.30	1.29	0.35

Notes: [1]. Tissue Hg Concentration Factor is calculated as the ratio: Hg concentration of tissue / Hg concentration of whole body.
[2]. Sample size for gravid females: n=5.
[3]. Sample size for non-gravid females: n=3.
[4]. Skeletal Muscle comprises pooled Tail, Abdomen and Head Muscle sub-samples.

held a higher percentage of the burden, from 72% in males, to 76% in females. For Year Class 1 eelpout from July 1991, skeletal muscle also held a relatively large part of the estimated whole body burden (68%). The distribution of mercury within the muscle of different body regions corresponds roughly to the distribution of muscle weight, although in females from July and October, the mercury content of abdominal muscle is proportionally greater than the percent of total muscle weight in that region.

The carcass was not analysed in fish from July. In fish from October, November, and March, the carcass accounted for 25-38% of the body weight, while accounting for 17-37% of the whole body mercury burden. The amount of the mercury burden contained in the brood varied greatly depending on the relative weight of the brood. In early development, in October, when the brood accounted for 0.6% of body weight, it contained 0.5% of the mercury burden. This value increased to 2.9% of the burden in broods accounting for 5.4% of total weight in November, and broods with a mean somatic index of around 20% accounting for almost 10% of the whole body mercury burden in March. Ovarian fluid accounted for a highly variable percentage of both total weight and whole body mercury burden. No measurable fluid was found in female ovaries in July, while the mean somatic index of fluid was low in October fish (0.54%), very high in November fish (12.59%) and lower again in March fish (3.5%). The fluid accounted for a very small part of the whole body mercury burden, from 0.05% in October, to 0.97% in November, and 0.8% in March.

Mercury concentration factors were generally highest in liver, kidney and skeletal muscle. In general, lower concentration factors were obtained for reproductive tissues, including broods. Values were particularly low for ovarian fluid.

3.3.3 Skeletal muscle: weight and total mercury burden

3.3.3.1 Skeletal muscle weight related to fish weight

The total weight of skeletal muscle increased in a linear manner with weight in eelpout from four months, as shown in Fig. 3.4, a-d. In all cases, the linear regression of muscle weight on total weight was statistically highly significant. Differences between the monthly regressions were tested using ANCOVA. An overall test for coincidental regressions revealed a significant difference ($F= 19.016$; $df= 6, 49$; $P< 0.001$). The rates of increase of muscle weight with body weight (ie the slopes of the regressions) were significantly different ($F= 13.102$; $df= 3, 49$; $P<0.001$). Significant differences between months were identified using Tukey Multiple Comparison Tests (for b in regressions, O>J, O>N, O>M, J>M, $P< 0.05$, all other combinations non-significant).

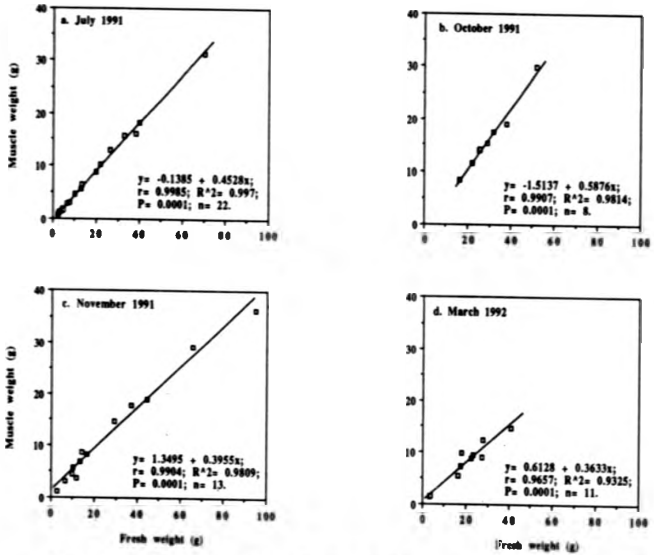


Fig. 3.4 Total muscle weight related to body fresh weight for eelpout from the Forth estuary (a, c, d) and Firth of Forth (b).

3.3.3.2 Skeletal muscle fraction related to water temperature and fish condition

The percentage of body weight as skeletal muscle, or skeletal muscle fraction was correlated with water temperature of the month of capture, the month prior to capture, and the mean of the two and three months prior to capture. The results of these correlations are shown in Table 3.6. Skeletal muscle fraction showed the highest correlation with mean water temperature of the three months prior to capture. The relationship is shown, along with statistics for the highly-significant linear regression, in Fig. 3.6. The skeletal muscle fraction also showed a significant positive correlation with the allometric condition factor for fish from all four months pooled (Pearson correlation coefficient, $r = 0.309$, $P = 0.023$, $n = 54$), although the linear regression of condition factor on muscle fraction, as shown in Fig. 3.7, is a very poor fit, with a considerable degree of scatter.

3.3.3.3 Skeletal muscle mercury burden related to fish length and weight

The total mercury burden of the whole skeletal muscle showed an exponential increase with length, as shown for fish from four months in Fig. 3.8, 1a-4a. The mercury burden also showed an exponential increase with weight in July and March, although the relations for fish from October and November both showed a good fit to a linear model.

Following a logarithmic transformation, the $\log_{10}$(total muscle mercury burden) varies in a linear fashion with $\log_{10}$(weight) in all four months, as illustrated in Fig. 3.9, a-d. In each case, as shown on the figure, the linear increase is described by a highly significant linear regression. The four monthly regressions were compared using ANCOVA. An overall ANCOVA test for coincidental regressions revealed a significant difference between months ($F = 4.704$; degrees of freedom= 46, $P<0.001$). Further testing revealed no significant difference between the slopes of the regressions ($F = 0.132$; $df = 46$; $P>0.25$). There were, however, significant differences between the regression elevations ($F = 9.796$; $df = 49$; $P<0.0005$), identified by Tukey multiple comparison tests as:

July vs October ($q = 6.602$, $df = 49, 4$; $P<0.05$) and,
March vs October ($q = 6.754$; $df = 49, 4$; $P<0.05$).

Elevations of both July and March samples were higher than that of October. All other comparisons were not significant (all $P>0.05$).

3.3.4 Distribution of mercury in the ovary of eelpout

The relative weight of the whole ovary, or ovary somatic index (OSI), showed seasonal variation, with maximum size in March, as shown in Fig. 3.10a. There was little difference in the value of ovary somatic index between post-partus, non-gravid females in March, and females with developing eggs in July. The whole ovary mercury burden showed a similar

Table 3.6 Correlation of skeletal muscle fraction with water temperature[1] at Tancred in the Forth Estuary.

Temperature period	Pearson correlation, r	P[2]
Month of capture	-0.007	0.956
Month prior to capture	0.3098	0.019
Mean 2 months prior to capture	0.4058	0.0017
Mean 3 months prior to capture	0.4957	0.0001

Notes. [1]. Water temperature at Tancred previously presented in Chapter 2, Appendix 2.3
[2]. Two-tailed probability

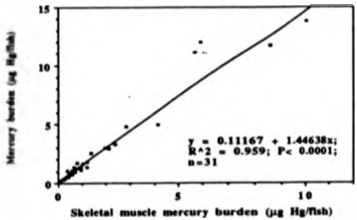


Fig. 3.5 Whole body mercury burden of eelpout in relation to skeletal muscle mercury burden.

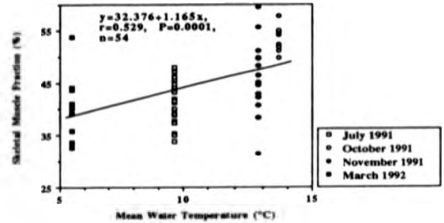


Fig. 3.6 Skeletal muscle (as a percentage of fresh weight) related to water temperature (mean of three months prior to capture date) for eelpout from the Estuary and Firth of Forth.

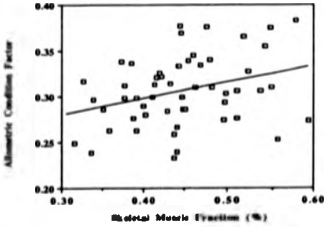


Fig. 3.7 Allometric condition factor related to skeletal muscle fraction of body weight for eelpout from the Forth Estuary and Firth of Forth

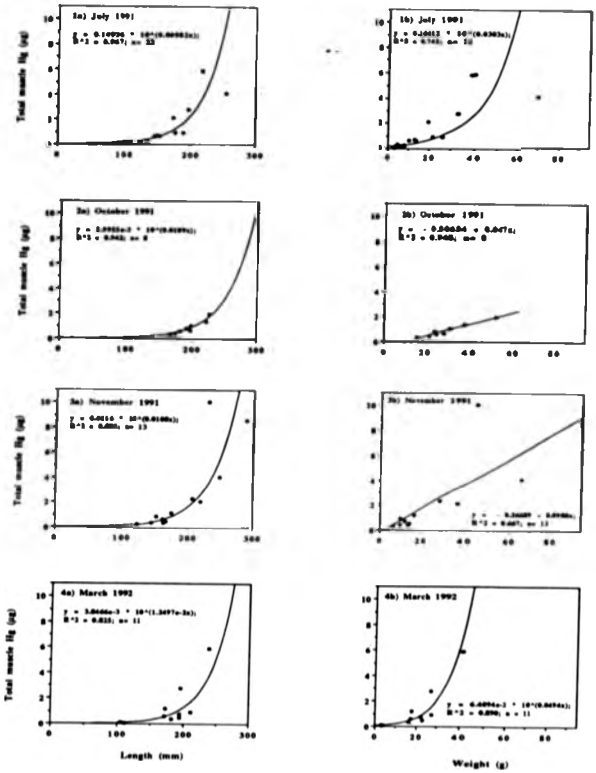


Fig. 3.8 Seasonal variation of skeletal muscle mercury burden with size parameters for eelpout from the Forth estuary (July, November and March) and Firth of Forth (October).

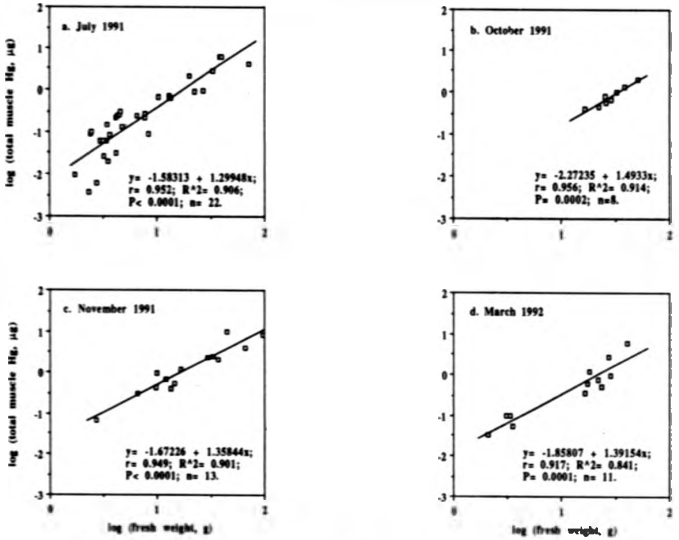


Fig. 3.9 Seasonal variation of regressions of $\log_{10}$(total muscle mercury) on $\log_{10}$(fresh weight) for eelpout from the Forth estuary (a, c, d) and Firth of Forth (b)

seasonal pattern to that observed for OSI, as shown in Fig. 3.10b.

The distribution of both the total ovary weight and whole ovary mercury burden in components of the ovary is shown in Fig. 3.11, for eelpout from four months. In July fish, the 'brood' refers to the eggs which were removed from the ovarian follicles for analysis. No collectable volume of ovarian fluid was found in the lumen of the ovaries in July fish. The relative volume of fluid varied in the other three months, and was responsible for the majority of the ovary weight in both fish analysed in November. The relative weight of the brood was very low in October and greatest in March.

The distribution of mercury in the ovary components followed the same general patterns as the distribution of weight. The proportion of total mercury in fluid was, however, considerably lower than the proportion of total weight in that component. In October, November and March, the proportion of total mercury in the brood was greater than the proportion of weight in that component.

The distribution of the total weight and total mercury of ovarian fluid is presented in Fig. 3.12 for eelpout from November and March. In both months, the great majority of the weight of the whole fluid was in the supernatant fluid produced by centrifugation, with the pellet of cellular material accounting for a mean of only 1% of ovarian fluid weight in November, and 6% in March. The pellet, by contrast, contained a mean of 23.8% of the total fluid mercury in November fish, and 28.0% in March fish. The mean ratios of mercury concentrations of pellet to supernatant were 76.96 (±37.7, sd) in November, and 9.3 (± 8.41, sd) in March.

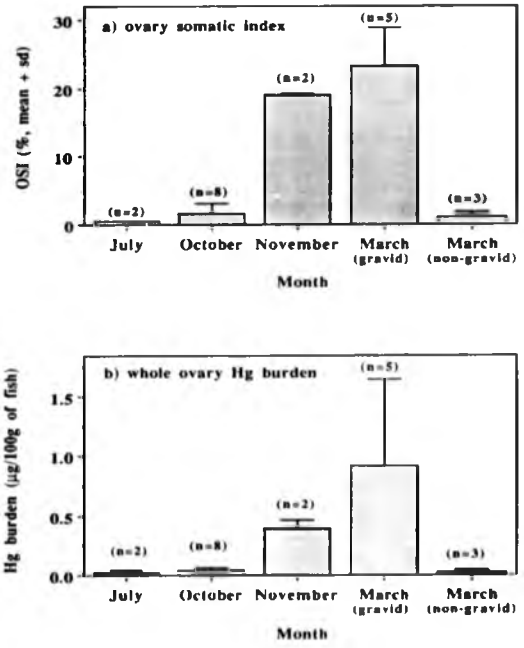


Fig. 3.10 Seasonal variation of: a) whole ovary somatic index and b) whole ovary Hg burden (including brood and ovarian fluid in gravid females)

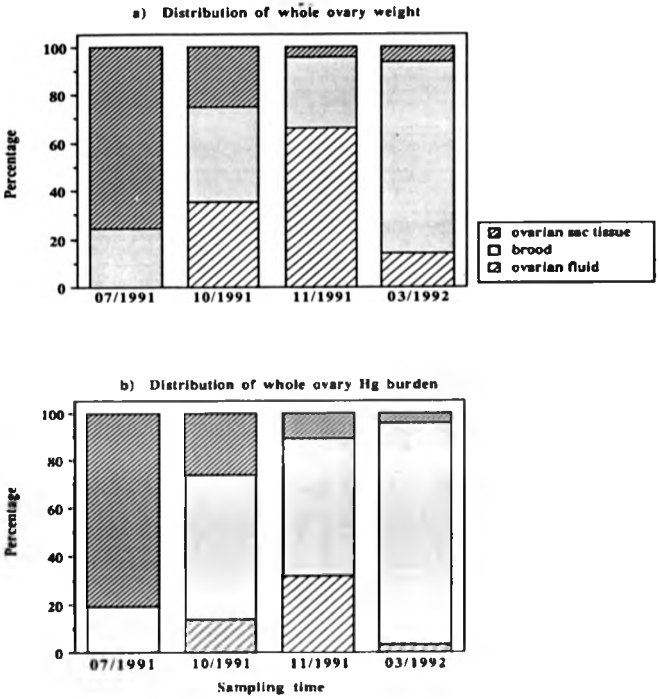


Fig. 3.11 Distribution of: a) Total weight, and b) Total mercury burden of eelpout ovary in tissue components

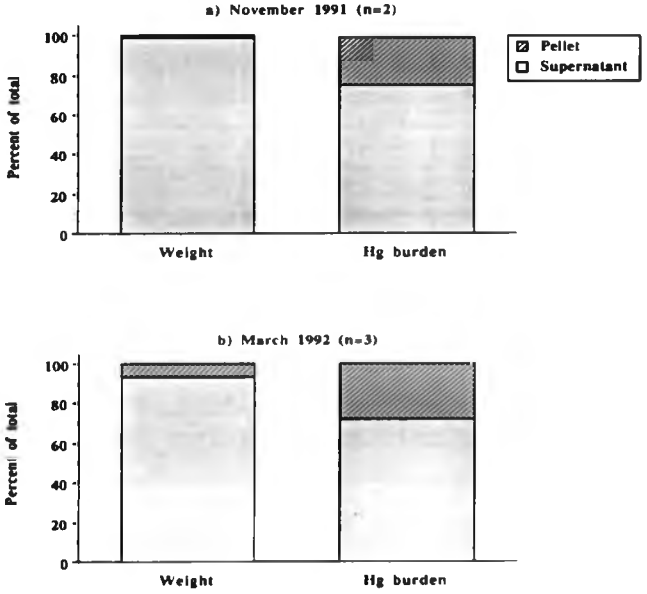


Fig. 3.12 Distribution of weight and mercury burden in components of ovarian fluid from eelpout ovaries in early and late pregnancy

3.4 Discussion

3.4.1 Whole Body Burdens of Mercury in Eelpout

Only one example of measurements of whole body mercury burden of fish can be found in the considerable published literature on mercury. Braune (1987) reported on the variation of whole body mercury with length, weight and age of Atlantic Herring (*Clupea harengus harengus*) from the Bay of Fundy, Canada. She found that, while there were significant negative linear correlations between whole body mercury burden and length or weight within samples of year class 1 and year class 2 herring, the general pattern of increase of burden with both length and weight for all fish pooled, followed an exponential curve.

A similar pattern was observed in this study, for the increase of whole body mercury burden of eelpout. The increase of skeletal muscle mercury burden with length and weight generally also followed a similar pattern. This was to be expected, considering the extremely high linear correlation between these two measurements. In Chapter 2, it was also shown that skeletal muscle mercury concentration showed a curvilinear increase with length, weight and age (as year class). Similar patterns have been reported frequently for mercury concentrations in marine fish (eg in Hake, *Merluccius merluccius*, and Poor Cod, *Trisopterus minutus capelanus* from the Tyrrenhian Sea: Barghigiani and De Ranieri, 1992). Braune (1987) suggested that this pattern of increase in herring might be the result of higher growth rates in juvenile fish, in combination with faster rates of clearance of methylmercury by smaller fish (as reported by Sharpe *et al*., 1977), appearing effectively to decrease whole body mercury concentration. This would result in an apparently slower accumulation of whole body mercury with length or weight, in smaller fish compared to larger fish.

The ANCOVA comparison of the monthly regressions of log mercury burden on log weight showed statistically significant differences only between the elevations of the regressions. On a logarithmic scale, this value refers to a point, the y-intercept, which lies well outwith the range for which values of x were obtained. As such, it is unclear how such statistical results should be interpreted, although it can be stated that the increase of burden with weight is statistically identical in all four months. The importance of regression elevations in biological terms has been discussed in Chpater 2.

3.4.2 Distribution of whole body mercury burden in tissues

Very few studies have considered the distribution of a naturally accumulated whole body mercury burden in the tissues of wild fish. Several studies have reported on the redistribution of an artificially administered dose of mercury. These studies are discussed more fully in Chapter 4 of this thesis. It is notable, however, that some of these studies report that in the long-term, perhaps after 100 days following dosing, the tissue distribution of mercury

(especially if administered as methylmercury, the most common form in wild fish) is very similar to that in wild fish (eg Giblin and Massaro, 1973).

The distribution of a large part of both the weight and whole body mercury burden in skeletal muscle, as presented for eelpout in this study, has also been reported for a small number of other species in the literature. Pentreath (1976a) reported that skeletal muscle, while accounting for 55.5% of body weight in plaice (*Pleuronectes platessa*), held around 82.8% of the whole body mercury burden. Both values were lower in the thornback ray (*Raja clavata*), with 43.9% of body weight, and 59.5% of mercury burden in the skeletal muscle (Pentreath, 1976d). These values are in the same range as those for eelpout in this study, although the skeletal muscle of November fish contained a much higher percentage of the burden compared with other months. As there was no clear significant difference between whole body burdens of November fish and those from the other months sampled, it is unclear if there is some seasonal effect in operation. The increased mercury in muscle appears to be principally from the burden in carcass tissues, compared to the relative proportions in muscle and carcass in other months.

The two studies mentioned above also found, as in this study, that very small amounts of the whole body burden were retained in other organs, such as liver, kidney and gonads, which are relatively very small compared to skeletal muscle. It is possible, as suggested in several studies, that turnover of mercury in certain tissues is very high, related perhaps to accumulation, detoxification or excretion processes. Observations, such as those above, give no measure of the dynamics of mercury in the tissues, and it is in response to such failings of field observations that the artificial administration of mercury under controlled conditions can be used to investigate such processes. Chapter 4 of this thesis presents results of such work on eelpout.

3.4.3 Variability of skeletal muscle mass and mercury burden

There were significant differences between the slopes of the regressions of eelpout skeletal muscle weight with body weight for the monthly samples, but not between the slopes of the regressions of total muscle mercury burden with body weight. Thus, in a standard fish from, for example, July 1991 and March 1992, the same total burden of mercury is present in different amounts of skeletal muscle. In Chapter 2, seasonal variability was reported in the value of the linear regression slope of $\log_{10}$(Hg concentration) on length, and the slope was also shown to be significantly negatively correlated with allometric condition factor. The above observations, combined with the significant positive correlation, presented here, between skeletal muscle fraction and allometric condition factor, suggests a hypothesis to explain the seasonal variability of skeletal muscle mercury concentrations, and its apparent relation to fish condition.

Skeletal muscle mass of eelpout varies seasonally, while the total amount of mercury in the skeletal muscle remains fairly constant. It was hypothesised in Chapter 2 (section 2.4.3.5), to explain the seasonal variation of muscle mercury concentrations, that the mercury mass in the

muscle of a fish of given size might effectively be diluted or made more concentrated by increases or reductions of muscle mass relative to body weight. This hypothesis is supported by the results presented here, the concentration of mercury in skeletal muscle of eelpout increasing or decreasing as the muscle mass of the fish shrinks or grows respectively in different seasons. Skeletal muscle mass, as a fraction of total body weight, is also shown here to have a significant positive correlation with allometric condition factor.

The seasonal changes of skeletal muscle mass may be the result of seasonal differences in factors such as feeding activity, efficiency of utilisation of food, and tissue turnover rates, which have been shown to be directly related to water temperature (Winberg, 1956; Weatherley and Gill, 1981). This argument is supported by the significant relationship between skeletal muscle fraction and mean water temperature of the previous three months. Such a result also suggests a mechanism to explain some of the variability of mercury concentrations between years observed in this study. Research on temporal trends of concentrations of mercury and other metals in fish tissues shows that, even when environmental levels of the metal appear relatively stable, concentrations in tissues can show considerable variation between years (Hansen *et al*., 1982; Jensen and Cheng, 1987). It is suggested that differences in the value of environmental factors, such as water temperature, between years may result in annual differences in tissue anabolic and catabolic processes. Thus, even if fish were exposed to similar degrees of environmental mercury contamination in both years, in prey and in water, the actual concentrations in tissues could show considerable variation, perhaps greater than could be explained by differences in accumulation alone. Presumably, though, such variability would be less than that observed with an annual cycle between seasons, where the water temperature differential is far greater than that between years in the same season.

The above argument does, of course take a very simplified view of what must be an immensely complex interaction of variables, both environmental and internal to the fish. Nevertheless, the relationship between water temperature and skeletal muscle fraction, while not necessarily causal, does indicate a possible way in which environmental factors may influence apparent tissue mercury concentrations. Successful attempts have been made to relate in a much simplified way, the principal environmental variables affecting mercury uptake and excretion processes, to the degree of mercury accumulation, using bioenergetics-based bioaccumulation models (Fagerstrom and Asell, 1973; Fagerstrom *et al*., 1974, 1975; Norstrom *et al*., 1976; Braune, 1987). It is suggested that the data presented here, on whole body mercury burdens of eelpout in different seasons, would provide suitable observed results against which to test such a model for prediction of mercury accumulation by eelpout. It is proposed, therefore, that such a model should be constructed using available literature values to provide the required physiological parameters, and growth data collected during this study, in conjunction with the considerable environmental data from the Forth Estuary, held by the Forth River Purification Board. No published examples of such a model yet exist for an estuarine fish species, previous models considering only freshwater or fully marine species.

3.4.4 Distribution of mercury in the ovary of eelpout

Following the complete reabsortion of their yolk sacs after several weeks of growth, the larval eelpout developing in the ovary depend, for nutrition during the remainder of their growth period, entirely on energy supplied from the mother via the embryotrophe fluid. It is logical to suppose, therefore, that as the mercury burden of the brood continues to increase during the latter part of the development, the mercury is supplied entirely via this embryotrophe fluid. It was hypothesised in Chapter 2, based on observations by Stuhlmann (1887), a.id later by Kristoffersson *et al*. (1973), that the practice of matrotrophy used to supply nutrition to the embryos in later development, was a possible mechanism for the transfer of mercury between the mother and brood. More specifically, the presence of cellular fragments from the inner wall of the ovary, including both red blood cells and unidentified cellular material, was noted in the above studies in both the ovarian fluid, and partially digested in the intestine of the embryos. Ovarian fluid was found in this study to contain small amounts of such cellular material, which also included some red blood cells and their fragments. The concentration of mercury in this cellular material, after collection as a centrifuge pellet, was nearly 80 times higher than in the supernatant fluid in period of early development (November), and more than 9 times higher in later development (March). This provides good evidence for the cellular fragments as a source of mercury to broods.

It is also possible that, at least during early development, the mercury content of the cellular material, which is apparently removed from the ovary wall by the movements of the brood within the ovary (Kristoffersson *et al*., 1973) might be correlated with that of the other maternal tissues. This might explain how the broods develop a mercury concentration in a linear correlation with that of maternal muscle, as demonstrated first by Elliott and Griffiths (1986), and also in this study. Too small a sample size, however, prevented the testing of this hypothesis.

There was a substantial decrease of the ratio of mercury concentrations in pellet to supernatant fluid between November and March. This may have been the result of the removal of material from the inner wall of the ovary, which in early development had been accumulating mercury for several months. As the brood developed, however, the ovarian wall tissue might be replaced such that it was progressively more newly produced, with a resulting lower mercury concentration. If the brood is supplied with increasingly lower concentrations of mercury in the cellular material from the ovary wall, it might be expected that the rate of mercury accumulation by the brood would decrease in later development. As, however, the growth rate of the brood slows as the development period reaches the point of *partus* (see patterns of increase of brood somatic index in Chapter 2), it would be difficult to separate the reduced mercury uptake from a reduced ration, from that resulting from reduced availability in the cellular material of the embryotrophe.

Chapter 4

Laboratory studies of methylmercury dynamics in the Eelpout (*Zoarces viviparus* L.): Internal redistribution, retention and clearance of ^{203}Hg-labelled methylmercuric chloride.

4.1 Introduction

4.1.1 Accumulation of methylmercury by fish

The accumulation of mercury by fish is the balance of uptake and elimination processes (Hannerz, 1968). It has been well established that fish can accumulate methylmercury, the principal form in which mercury occurs in fish tissue (Westoo, 1973; Bloom, 1992), both by direct uptake from solution and by trophic uptake from food (Pentreath, 1976 a,b,c,d; deFreitas et al., 1977; Boudou and Ribeyre, 1983, 1985, Ribeyre and Boudou, 1984 a,b). The principal processes leading to mercury accumulation in fish are summarised in Fig. 4.1 and the body burden of mercury at any time is the net of the mercury accumulated minus the mercury excreted. As the rate at which fish are able to eliminate accumulated methylmercury is much slower than the rate of uptake by either route, methylmercury concentration generally increases with size and age of fish (Jarvenpaa *et al.*, 1970; Ruohtula and Miettinen, 1975; Pentreath, 1976 a,b,c; Sharpe et al., 1977; Boudou and Ribeyre, 1983).

4.1.2 Methylmercury Uptake

4.1.2.1 Direct uptake from water

Accumulation of inorganic mercury and methylmercury from seawater was studied in the marine flatfish, plaice, *Pleuronecies platessa* by Pentreath (1976 a,b). Both forms of mercury were accumulated rapidly, although uptake rates were much greater for methylmercury than for inorganic mercury. The largest fraction of the accumulated methylmercury was taken up by skeletal muscle tissue. It would appear that the direct accumulation of inorganic mercury from sea water may account for a large fraction of the inorganic mercury burden (Pentreath, 1976a). Direct accumulation of both inorganic mercury and methylmercury from water was shown to occur principally across the gills for rainbow trout, *Salmo gairdneri*, with methylmercury accumulated in ten times greater quantities than inorganic mercury (Olson *et al.*, 1973).

4.1.2.2 Trophic uptake from food

Pentreath (1976c) followed work on the accumulation of mercury direct from seawater by plaice, *P. platessa* , by studying the accumulation of mercury from food. Fish were either force-fed starch pellets labelled with ^{203}Hg or prey items which had naturally taken up $^{203}HgCl_2$ or $CH_3{}^{203}HgCl$. Only 10% of the inorganic mercury was retained from either prey species of pellets, and this was almost entirely associated with the gut wall. The relatively short biological half-life ($B_{1/2}$) of 3-28 days for ^{203}Hg assimilated by plaice from the polychaete, *Nereis*, labelled with $^{203}HgCl_2$, may represent the rate of sloughing and renewal of the epithelial cells of the gut wall. In contrast to the inorganic form,

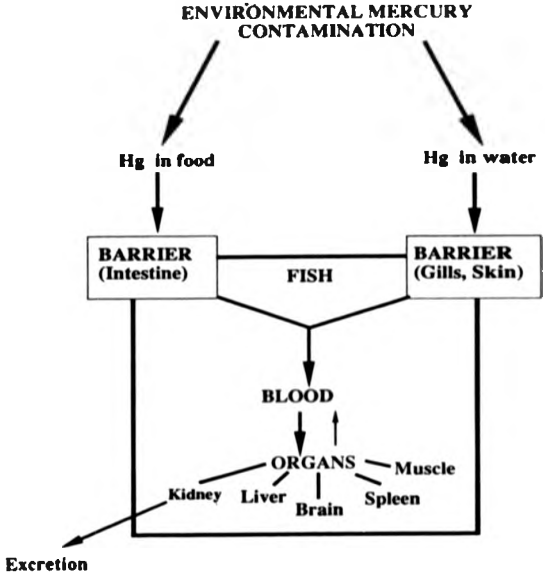


Fig. 4. 1 Principal processes leading to mercury accumulation in fish (after Boudou *et al.*, 1983) .

methylmercury is readily absorbed and only slowly eliminated. The short $B_{1/2}$'s for inorganic mercury compare with 100-250 days for ^{203}Hg taken up from the polychaete *Nereis*, labelled with $CH_3{}^{203}HgCl$. There were differences, notably, in the retention of methylmercury from different prey species, the percentage retention ranging from 98-100% of the ^{203}Hg eaten in the polychaete, *Arenicola marina*, to 7-42% for the bivalve mollusc, *Mytilus edulis*.. Unfortunately, inert tracers were not used to assess 'digestibility' of the different animals. These differences highlight one problem of extrapolation from uptake experiments in the laboratory, to predictive modelling of the more complex position with wild fish.

The efficiency of assimilation of methylmercury by fish from food is much higher than that from water, in the order of 70-100% of the ingested dose, as shown below in a summary of several studies of methylmercury retention:

Name	Species	Retention of dose (%)	Reference
Plaice	*Pleuronectes platessa*	89[1]	Pentreath, 1976c
Thornback Ray	*Raja clavata*	97	Pentreath, 1976d
Pike	*Esox lucius*	95	deFreitas et al., 1977
Goldfish	*Carassius auratus*	95	deFreitas et al., 1977
Rainbow trout	*Salmo gairdneri*	68	Phillips & Buhler, 1978

Note. [1] value for retention from *Nereis* plychaetes, much lower retention (7-42%) from *Mytilus*.

While there is a clear picture of the differences in uptake patterns of different mercury species by fish, there are conflicting reports regarding the relative importance of food and water as sources of mercury to fish. Early work by Hannerz (1968), who exposed Swedish lake communities, including the predatory fish, pike (*Esox lucius*), to methylmercury, found that tissue concentrations of mercury in the organisms were not related to trophic level. This suggests that uptake from water was the major route of methylmercury accumulation. By contrast, of the mercury burden of pike from three Swedish rivers, some 60% was estimated to have been consumed with food (Jernelov and Lann, 1971).

This apparent contradiction was addressed by Phillips and Buhler (1978), who exposed rainbow trout (*Salmo gairdneri*) to methylmercury in water, in food, or both. Methylmercury accumulated from both sources was quantitatively additive. They found that food consumption rate and, therefore, growth rate had no influence on the accumulation of mercury from water. Estimates of the efficiency with which the experimental fish extracted methylmercury from food and water showed that uptake from food (at 68%) was much more efficient than from water (at 10%). From these results, and the extremely low concentrations of methylmercury in most

natural waters, the authors postulated that it may be possible to estimate the proportion of methylmercury assimilated from food and water from a knowledge of the food alone. Pentreath (1976d) also observed the uptake of inorganic and organic mercury from sea water and food by the thornback ray, *Raja clavata* . He proved that the ray is capable of obtaining both forms of mercury from both food and sea water. Only some 14% of the intake of inorganic mercury from food is retained. This is approximately the same as that from sea water, although distribution is quite different, with 60% of the retained inorganic mercury from food associated with the gut wall.

The accumulation of methylmercury from seawater was greater than that of the inorganic form, and no excretion was observed, while methylmercury was readily absorbed from food and only slowly eliminated. The importance of the input from seawater could not be evaluated for wild fish, due to the lack of knowledge, at that time, about its presence in seawater. It was implied, however, that the observed burden of mercury in wild rays could be explained by the accumulation of methylmercury, at a low daily uptake rate from food sources with relatively low mercury concentrations, such as those observed in wild *Nereis* polychaetes.

4.1.3 Tissue dynamics, redistribution and elimination of methylmercury by fish

Following uptake of methylmercury via direct or trophic routes, there is considerable redistribution between tissues occurring with time after uptake (Hannerz, 1968; Giblin and Massaro, 1973, 1975 ; Olson *et al*., 1973, 1978; Fang, 1974; Boudou and Ribeyre, 1983, 1985; Ribeyre and Boudou, 1984, a,b). The route of uptake affects the pattern of distribution in the tissues (Pentreath, 1976 a,b,c). Tissues which are important in methylmercury uptake processes are, invariably, different from those which are important for longer-term storage.

For the viviparous fish species, eelpout (*Zoarces viviparus* L.), where a trophic relationship exists between the mother and the brood of larval fish developing in her ovarian cavity (Korsgaard and Andersen, 1985), the transfer of mercury between generations is implied by the positive correlations between their mercury concentrations observed in wild fish (Elliott *et al*., 1988). This relationship, between maternal and brood mercury content, and the division of the total ovarian mercury burden between the various compartments, has already been described in Chapters 2 and 3 of this thesis. A mechanism for the transfer of mercury to the growing larval fish has also been proposed in Chapter 3. Such a transfer, which has not been described previously under controlled laboratory conditions, is essentially a special case of tissue redistribution of an accumulated mercury burden, and will be investigated further here.

A number of studies have shown that, in contrast to the rapid uptake of mercury compounds from solution or food, the clearance or elimination of these compounds from

the body occurs at a much slower rate. Differences exist in the rate of clearance of different mercurial compounds. Following exposure of plaice (*P. platessa*) to inorganic or methylmercury in solution, the time for clearance of half the accumulated body burden (biological half-life, $B_{1/2}$), was calculated for both forms. The $B_{1/2}$ for methylmercury taken up from solution (231-347 days) was approximately double that for inorganic mercury, of 103–162 days (Pentreath, 1976 a,b). Other studies of methylmercury elimination have calculated values of $B_{1/2}$ as high as 500 - 1200 days (Jarvenpaa *et al.*, 1970; Ruohtula and Miettinen, 1975). Following oral dosing of rainbow trout (*Salmo gairdneri*), no difference was observed in the biological half-lives calculated for methylmercury, whether administered in ionic or protein-bound form (Ruohtula and Miettinen, 1975).

Laboratory measurements of biological half-life, such as those discussed above, do seem to bear some similarity to the behaviour of methylmercury in wild fish. The elimination of methylmercury has also been shown to be very slow in wild fish kept under natural conditions. Lockhart *et al.* (1972) captured pike (*Esox lucius*) which had been heavily contaminated with methylmercury under natural conditions and released them, after tagging, in another lake which was relatively free of mercury contamination. Mercury levels in muscle biopsy samples at the time of transfer and at subsequent recaptures indicated that only 30 % was eliminated in one year. Distribution among various body tissues was essentially unchanged. There is, however, insufficient evidence to determine if estimates of biological half-life for methylmercury derived from laboratory studies are truly comparable with those from field studies (Niimi, 1983).

Most dynamic models of contaminant accumulation (eg Norstrom *et al.*, 1976; deFreitas *et al.*, 1977; Braune, 1987) usually assume a single compartment (or first order kinetic relationship) of elimination (Niimi, 1983). There is, however, some limited evidence to suggest a two compartment clearance of methylmercury which includes a short first-order elimination followed by a longer second order clearance. Such a pattern of elimination has been demonstrated experimentally by Burrows and Krenkel (1973), and Ruohtula and Miettinen (1975). The pattern of whole-body methylmercury clearance by eelpout following ingestion of an oral dose of ^{203}Hg-labeled methylmercury will be studied and discussed later in relation to internal dynamics of methylmercury.

4.1.4 Aims

This study was designed to investigate the dynamics of methylmercury in the eelpout (*Zoarces viviparus* L.), following administration of ^{203}Hg-labelled methylmercuric chloride. The aims of the study were as follows:

1. To establish the relative affinities for methylmercury of eelpout tissues, and to quantify the

distribution of administered body burdens in the various tissue compartments;
2. To study the short-term and long-term tissue redistribution of an administered dose of ^{203}Hg-labelled methylmercuric chloride, including the transfer of methylmercury between generations;

3. To establish the retention/elimination dynamics and biological half-life of an orally-administered whole-body ^{203}Hg burden.

4. To compare the tissue distributions of ^{203}Hg resulting from intraperitoneal and intragastric administration of ^{203}Hg-labelled methylmercuric chloride. This will permit an assessment of the representiveness of the more artificial intraperitoneal administration in relation to administration by a more natural oral route.

These aims will be met by administration of methylmercuric chloride, labelled with radioactive ^{203}Hg, to eelpout. Administration will be made by intraperitoneal (i.p.) injection, or by an orally administered dose.

4.2 Material and Methods

4.2.1 Fish collection, holding, husbandry, and transport procedures

Eelpout were collected for intraperitoneal (ip) dosing on 31-08-91 from Kingstone Hudds in the Firth of Forth by trawling (see Chapter 2 for site and sampling details). Fish were transferred live in barrels of seawater to a recirculating seawater aquarium system at the University of Stirling. Water temperature in the system was 14 ±1° C, and salinity was a relatively constant 34.0 ‰ during the period. Fish were fed lightly with commercial trout pellets five times per week. Constant aeration was supplied. The daily light regime was 14 h light/10 h dark during the period.

Fish were transported to the Ministry of Agriculture, Fisheries and Food (MAFF) Fisheries Laboratory, Pakeham Road, Lowestoft, Suffolk, on 01-09-91, in heavy-duty polythene bags containing approximately 30 litres of seawater, with up to 15 fish per bag. The bags were tied loosely with string before aerating with oxygen. Once the air space in a bag was pressurised (bulging walls), the top was tightly tied. The bags were transported in plastic dustbins with the bases packed with ice. The fish were transported for nine hours in this way, with no mortalities incurred, either during transport, or in the following 72 hours. On arrival at Lowestoft, fish were transferred to tanks in a running seawater aquarium system. Water temperature was approximately 17°C, salinity was 34.0‰, the light regime was 14h light:10 h dark, and tanks were constantly aerated. Fish were maintained in this system unfed for 72 hours before methylmercury administration.

Eelpout for oral dosing, and for the study of intra-uterine mercury transfer, were collected at Kingstone Hudds on 14-10-91 by trawling and transferred, as above, to the MAFF Laboratory, where they were held in running seawater aquaria. Fish were fed daily *ad libitum* on chopped squid. By the time the oral dosing experiment was performed (early December 1991), the temperature of seawater supplied to the tanks had fallen to 12±1°C.

4.2.2 Procedures for Anaesthetising Fish

During the following experimental procedures, fish were anaesthetized with phenoxyethanol prior to handling for measurement, methylmercury administration, or whole body activity counting (see section 4.2). A solution of phenoxyethanol (0.001% volume/volume with seawater: Sigma Chemicals) was prepared in a plastic bucket (5ml phenoxyethanol in 5 litres of seawater) and thoroughly mixed. Fish were placed singly into the solution and removed once anaesthetized. Fish were sacrificed by a lethal dose of anaesthetic (0.004% volume/volume solution of phenoxyethanol in seawater) administered as above.

4.2.3 Experimental Apparatus and Tank System

Methylmercury is very volatile and also extremely toxic to humans (GESAMP, 1986a). A static tank system was constructed, therefore, as shown in Figure 4.2, to allow for aeration of the seawater in the tank, while preventing the escape to the laboratory environment of any methylmercury venting at the air/water interface.

Transparent polythene tanks of 15 litres volume were used (Hyware, England). Perspex lids (5mm thickness) was cut to fit each tank individually. Two holes were drilled in the lid to pass an aeration line and a venting pipe to and from the tank. An airtight silicone gasket was prepared on the tank rim and left to cure for 24 hours. The seal was made more airtight with a layer of silicone lubricant gel which was renewed each time the lid was removed. A number of small clips around the rim helped to improve the seal still further. Sealant was also used around the pipe holes to make them airtight. Exhalant air was scrubbed to remove methylmercury by bubbling through an acidified potassium permanganate solution (1 g of potassium permanganate ($KMnO_4$) dissolved in 40 ml of concentrated sulphuric acid (c.H_2SO_4) and diluted to two litres with distilled water).

Tanks were half-filled with seawater and left aerating for 24 hours in the constant temperature (CT) radioisotope laboratory where the intraperitoneal dosing experimental work was to be carried out. Air temperature in the experimental laboratory was set at 17° C. Once fish had been dosed with ^{203}Hg, the following procedure was followed when opening the tank lids for any reason. The aeration was turned off five minutes before opening to allow contaminated aerosol to settle. The lid was removed carefully, and any condensation allowed to drip back into the tank. Tank water was not changed, and fish were not fed during the 72 hour experimental period.

The same tank system was used in the oral dosing experiment. The air temperature in the constant temperature laboratory was set at 12°C, the ambient temperature of the running seawater in which the fish had been maintained. Fish were not fed during the 16-day experimental period. After each whole-body activity counting session, tank water was replaced with clean seawater which had been cooled to the ambient temperature.

4.2.4 Radioisotope Solution Preparation

4.2.4.1 Intraperitoneal Dosing

All radioisotope solution preparation was carried out within the MAFF Laboratory's Radioactive Preparation Room, in a radioisotope-designated fume cupboard. Methylmercuric chloride, labelled with ^{203}Hg, was obtained from Amersham International plc (Amersham Laboratories,

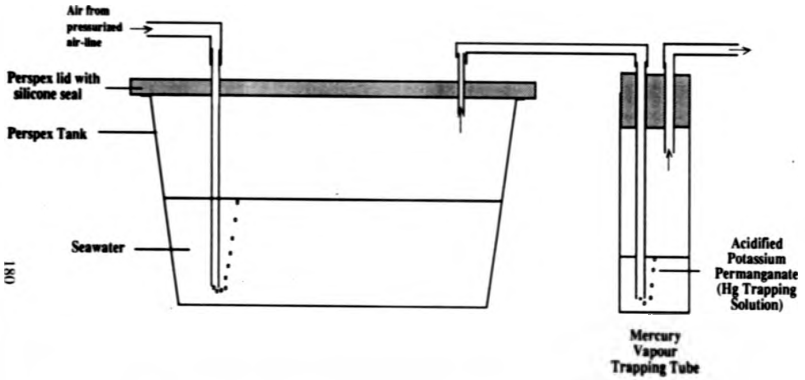


Fig. 4.2 Schematic figure of airtight tank system used for holding eelpout dosed with ^{203}Hg-labelled methylmercuric chloride.

Arrows indicate direction of movement of aeration and vented air.

White Lion Road, Amersham, Bucks. HP7 9LL, UK). The isotope was delivered, in solid form, directly to the MAFF Laboratory. Total activity of the sample was 1.0 milliCurie (mCi), with a specific activity of 49.16 mCi g^{-1} Hg at 1200 hr on 16-08-91. The total activity of the sample was 0.76 mCi at 1200 hr on 03-09-91. For administration of the methylmercury to the fish, a solution of sterile saline containing the ^{203}Hg activity was produced. The entire stock was dissolved completely in 1 ml of methanol. The activity of this stock solution was 0.76 mCi ml^{-1}. An aliquot of 0.25 ml was then diluted with sterile saline solution (0.9% sodium chloride in distilled water), to a final volume of 9.58 ml. The activity of this solution (the "working solution") was:

$$(0.766 \times 0.25) \times (1/9.58) = 0.0199 \text{ mCi ml}^{-1} = 19.9 \text{ µCi ml}^{-1}.$$

Fish were injected i.p. with a dose of 0.1 µCi ^{203}Hg (2.1 µg Hg) g^{-1} body weight, i.e. a volume of 0.005 ml working solution.g^{-1} body weight. A dose of 0.1 µCi.g^{-1} body weight is equivalent to 2.124 µg Hg.g^{-1} body weight, calculated as shown in Appendix 4.1.

4.2.4.2 Oral Dosing

The stock solution (0.76 mCi ml^{-1}) prepared on 03-09-91 (see Section 4.2.4.1) was used to prepare a working solution for oral dosing of eelpout. Using the radioactive decay equation, as described above, the activity of the remaining stock solution was calculated as 0.260 mCi ml^{-1} on 04-12-91. An aliquot of this stock was diluted with sterile saline solution, to produce a solution of activity 2.0 µCi.ml^{-1}, for oral administration of a dose.

4.2.5 Experimental Design and Isotope Administration

4.2.5.1 Intraperitoneal Dosing

Four fish were dosed by intraperitoneal (i.p.) injection for each of seven sampling times, a total of 28 individuals. Prior to intraperitoneal administration of ^{203}Hg-labelled methylmercuric chloride solution, fish were anaesthetised as above, weighed to the nearest 0.01g, and laid ventral side up on a damp tissue in an aluminium foil tray. Each fish was then injected i.p.with a dose of 0.1 µCi ^{203}Hg.g^{-1} body weight. Care was taken to insert the minimum possible length of syringe needle into the body cavity, at a shallow angle, to avoid accidental injection into internal organs.

After dosing, each fish was placed directly into an aerated static seawater tank, four fish per tank, for each of seven treatment groups. Once all four fish in a group were injected, the tank was sealed as described in section 4.2.3. The fish were left undisturbed until time of sampling. Four fish (the contents of a single tank) were sacrificed at each of seven time intervals of 1, 2, 5, 10, 24, 48 and 72 hours post injection, and tissue samples taken as described in section 4.2.6.

To study the transfer of methylmercury between the mother fish and a developing brood, four pregnant female eelpout were dosed i.p. on 04-12-91, with a volume of sterile saline containing 10 μCi per 100 g body weight (equivalent to 8.346 μg Hg.g^{-1} body weight). They were maintained unfed in the tank system described in section 4.2.3, for 72 hours and then sacrificed as above. A number of tissue samples were then taken, as described in section 4.2.6.

4.2.5.2 Oral Dosing

Eight fish were dosed by oral administration. Fish were anaesthetised prior to dosing, and weighed to the nearest 0.01 g. Fish were given an oral dose of 1.0 μCi per 100g of body weight (i.e. 0.834 μg Hg.g^{-1}), administered by syringe, through silicone tubing into the oesophagus. The volume of the dose was 0.5 ml per 100g of body weight. The tubing was inserted to a distance of at least 30 mm into the the oral cavity before administration, to reduce the potential risk of loss of solution by coughing or vomiting.

Fish were placed into clean sea water immediately after dosing, four per tank, and the whole body activity of each fish was counted, within 15 minutes of dosing, by the procedure in Section 4.2.8. Whole body activity of all 8 fish was counted again 2 days after dosing. Four of the fish were sacrificed at 2 days (48 hours) after dosing, following measurement of live fish whole-body ^{203}Hg activity; tissue samples were taken from each (see section 4.2.6). The whole body activity of the remaining four fish was counted at 2, 5, 9 and 16 days after dosing, to study the whole-body elimination of methylmercury, and these fish were then sacrificed for tissue sampling at 16 days after dosing.

4.2.6 Tissue Collection

4.2.6.1 Intraperitoneal Dosing

After sacrifice, each fish was weighed to the nearest 0.01g, length was measured to the nearest 1 mm and several tissue samples were collected from each fish. Length and weights of fish in each group are given in Appendix 4.2. Each tissue sample was placed directly into individual, pre-weighed and numbered polypropylene counting vials, which were capped immediately.

Blood samples, taken by syringe from the caudal vein, proved difficult to collect satisfactorily, and only 14 samples of blood were obtained. The blood was stored whole. A fillet of skeletal muscle (≥ 1g in weight) was dissected from the front portion of the tail, on the left side of the animal on each occasion, and the skin removed. The liver, kidney, spleen, heart and gall bladder (with fluid contents) were dissected out whole from each fish. Testes were dissected out whole from male fish. The ovary was dissected whole from female fish, the eggs dissected out from the ovarian follicles, and the ovarian tissue and eggs stored in separate vials. The intestine, from behind the stomach to the rectum, was dissected out of each fish.

Samples of skeletal muscle (tail) were collected from the four pregnant female fish as above

The ovarian fluid was collected from the ovarian sac of two fish by ovarian puncture with a syringe, the other two ovaries containing insignificant volumes of fluid. The whole brood was removed from each ovary, and the ovarian sac tissue dissected out separately.

The weight of each tube (dry and with tissue sample) was measured and recorded, and the tissue sample wet weights were then calculated by subtraction. One ml of formalin solution (10% volume/ volume with distilled water) was then pipetted into each sample tube to prevent decomposition of tissues, and a build-up of gaseous pressure in the tube. Tubes were stored, with caps re-fitted, in a radioactives' containment room until they could be counted for ^{203}Hg activity.

4.2.6.2 Oral Dosing

After sacrifice, the length of each fish was measured to the nearest 1 mm., and wet weight recorded to the nearest 0.01g. Samples of a number of tissues were collected by dissection from each fish. Tissues were collected as described above, with the following differences. A blood sample was obtained successfully from each fish. The skull was split longitudinally and a sub-sample of the brain tissue removed from each fish. The hearts of two of the Day 2 fish were inadvertantly not collected. The gall bladder of one of the Day 2 fish was ruptured on dissection and the contents lost, and the sample was thus was not retained for counting. One of the Day 2 fish was a female, the ovary of which was removed and stored whole for counting.

4.2.7 Tissue Activity Counting Procedure and Counting Geometry

4.2.7.1 Tissue Activity Counting Procedure

The same procedure was followed for counting the ^{203}Hg activity of tissues collected from both ip and orally dosed fish. The total ^{203}Hg gamma activity of each tissue sample was counted using a Wallac Decem-GTL 300-500 Gamma Sample Counter (Wallac, Turku, Finland), connected to a Canberra Series 35 Plus 2048-channel Multiple Channel Analyzer (Canberra Industries Inc., Meriden, Conn., USA). The window for counting ^{203}Hg (i.e. the upper and lower limits defining the range of radiation energies about the γ peak for ^{203}Hg which are accepted for counting, was established with the ^{203}Hg activity from a spot of the 20 μCi.ml^{-1} working solution (see section 4.2.4). The same upper and lower channel numbers were used to set up the same counting conditions each time activity was counted. A number of background counts were recorded with an empty counting tube in the counter. The counting procedure was automated, each tissue sample was counted for 10 minutes, and total counts recorded for each sample.

4.2.7.2 Tissue Sample Counting Geometry

Due to the differences in physical sizes of the different organs and tissues collected for counting, and the addition of 1.0 ml of formalin solution to each sample, the volume of material

was not equal in all tubes. The effect of different volumes of solution (containing the same activity) on the efficiency of counting was, therefore, investigated. As the total volume of most samples+formalin was approximately 1.5 ml, a spot of working solution was placed in the bottom of a counting tube and 1.5 ml of 10 % formalin solution was added. The activity, in counts per minute, was measured as described above. The volume of water in the tube was then increased to 2 ml by addition of another 0.5 ml of DW, the activity measured, and the process continued, up to a total volume of 4 ml, the maximum volume of any tissue sample counted.

The effects of increasing volume on counting efficiency are shown, in Table 4.1, as a percentage of the activity measured in 1.5 ml. The largest volume (4ml) results in a loss of less than 3% of the count in 1.5 ml. Since the loss is minor, and as only one sample had a total volume of 4 ml, and seven had a volume of approximately 3 ml, compared with over 200 samples of approximately 1.5 ml, no correction was made for the small reduction of counting efficiency due to differences in the volume of counted solution.

4.2.8 Whole Body Counting Apparatus and Procedure

4.2.8.1 Whole Body Counting Apparatus

The whole body ^{203}Hg gamma activity of live fish was measured using a whole-body counter. This consists of two 127 mm sodium iodide (NaI) crystal assemblies connected to a Northern NS633 multi-channel (256-channel) pulse-height analyzer (Northern, Middleton, Wisconsin, USA). The NaI crystal assemblies are contained inside a lead-brick box (1200 x 1000 x 1000 mm) to minimise the activity counted from external sources, and are accessed via a pneumatically-driven lead door.

4.2.8.2 Whole Body Counting Procedure

The procedure for whole-body activity counting was as follows. To calibrate the whole-body counts, a spot source of known ^{203}Hg activity was prepared by adding 0.1 ml of a 2μCi.ml^{-1} solution of ^{203}Hg-labelled methylmercuric chloride (ie 0.2 μCi of activity) to a piece of tissue paper in a glass vial which was subsequently sealed. The window for counting ^{203}Hg activity, was established with the ^{203}Hg activity from this spot source. Replicate counts were made of the spot source activity prior to each counting session. A number of background counts were also recorded for each whole-body counting session, with the counting chamber empty and the door sealed.

In order to correct for differences in counting geometry resulting from the physical differences between the ^{203}Hg spot source and the fish being counted, the activity of a standard "phantom" was determined in replicate at each counting session. The phantom was prepared to simulate the length and weight of the fish by heat-sealing 50 ml of seawater, containing the same ^{203}Hg

Table 4.1 Counts per minute (cpm) from a spot source of ^{203}Hg for different volumes of isotope solution

Volume of Isotope Solution (ml)	Activity (cpm)	Percentage[1]
1.5	1,270,000	100.00
2.0	1,264,300	99.55
2.5	1,259,064	99.14
3.0	1,246,349	98.14
4.0	1,235,796	97.31

Note. [1] Cpm in x ml as a percentage of that in 1.5 ml. solution

activity as the spot source (0.2 μCi), inside a polythene tube of appropriate dimensions. Use of the phantom to correct geometry is described in Appendix 4.3.

Fish for counting were anaesthetised individually prior to further handling (see section 4.2.2). The total body length was measured to the nearest mm, and weight was measured to the nearest 0.01 g. The fish was then placed immediately into a polythene bag containing 50 ml of clean seawater at 12° C and the top of the bag was sealed with tape. The bag was then placed in a small plastic dish which held it upright. The bag and dish were placed in between the crystal assemblies in the whole-body counter, and the lead door was closed and sealed. The ^{203}Hg gamma activity of the fish in the bag was then recorded as triplicate one minute counts. The fish was then removed and immediately placed back into a tank of seawater, except where fish were sacrificed for tissue sampling (see section 4.2.5). The water in the tanks was replaced with clean seawater while fish were being bagged and counted.

4.2.9 Data Treatment and Statistical Analysis

Tissue Somatic Index: Where a tissue could not be sampled whole, and sub-samples were collected, an estimate was made of the tissue somatic index (TSI), based on previous observations of eelpout, or literature sources. Following intraperitoneal dosing, only a sub-sample of the post-stomach digestive tract tissue was collected to represent the whole digestive tract. The TSI for digestive tract tissue was assumed to be 1.7% of body weight, which was the value of TSI for eelpout digestive tract measured in orally-dosed fish (See Results: Table 4.4). Similarly, only sub-samples of blood and brain were collected. No literature value could be found for the TSI of either tissue for eelpout. A TSI value of 1.7% of body weight for blood, and 0.31 for brain, was assumed, therefore, after the values estimated for plaice, *Pleuronectes platessa* (Pentreath, 1976 a).

All data sets were tested for normality, as decribed in Chapter 2, before any statistical comparisons or testing was carried out. Normalisation was usually achieved, when required, by $\log_{10}$ transformation. Parametric statistical methods were used (after Zar, 1984), except where data sets could not be normalised, when non-parametric methods were applied (after Sokal and Rohlf, 1981). The particular test applied in each case is given in the text.

4.3 Results

4.3.1 Changes in concentrations of Hg (as methylmercuric chloride) in eelpout tissues for 72 hours following intraperitoneal injection

The tissue concentrations of Hg (as methylmercuric chloride) for eleven tissues, taken from eelpout at seven sample times between 1 and 72 hours, are shown in Fig. 4.3. Three general patterns of Hg variability in tissues were observed with time during the 72 hour period following intraperitoneal (ip) injection. These were:

1. An overall decrease of Hg concentration with time from initial high concentrations (Fig. 4.3 a, c, d, e, h, i);

2. No significant gains or losses over the period (Fig. 4.3 b, j, k);

3. An overall increase in Hg concentration with time (Fig. 4.3 f, g).

The loss of Hg from the digestive tract tissues (Fig 4.3 a) is described by the log-linear equation:

$$\text{Log}_{10}(\text{Hg concentration, } \mu g.g^{-1}) = 1.5099\text{-}0.00903 \text{ x (Time, hours).}$$

This linear model explained approximately 54% of the variability (r^2= 0.538) and the regression was highly significant (P = 0.0000, n=26).

The loss of Hg from the whole gall bladder tissues with time is described by the log-linear equation (Fig 4.3 c):

$$\text{Log}_{10}(\text{Hg concentration, } \mu g.g^{-1}) = 0.3432\text{-}0.00647 \text{ x (Time, hours).}$$

This model was a poorer fit than that for the digestive tract (r^2= 0.226), although the regression was still highly significant (P= 0.0141, n= 26).

A log-linear relationship also described the loss of Hg from the spleen with time (Fig 4.3 e):

$$\text{Log}_{10}(\text{Hg concentration, } \mu g\ g^{-1}) = 1.4407\text{-}0.00729 \text{ x (Time, hours),}$$

with approximately 45% of the variation in the data explained by this highly-significant linear model (r^2= 0.456; P=0.0002; n=25).

The $\log_{10}$ of kidney tissue Hg concentration increased in an approximately linear fashion to a maximum mean value of 12.95 $\mu g.g^{-1}$ at 10 hours, followed by an approximate linear decrease over the remaining time (Fig. 4.3 d).

There is a suggestion of a parabola in the distribution of $\log_{10}$(Hg concentrations) of whole blood with time (Fig. 4.3 h). The data show a reasonable fit to a parabola described by a second-order polynomial equation (Curve Fitter, Shareware computer package):

$$\text{Log}_{10}(\text{Hg concentration, } \mu g.g^{-1}) = 0.60746 + 0.00721(\text{time}) - 0.00013(\text{time})^2.$$

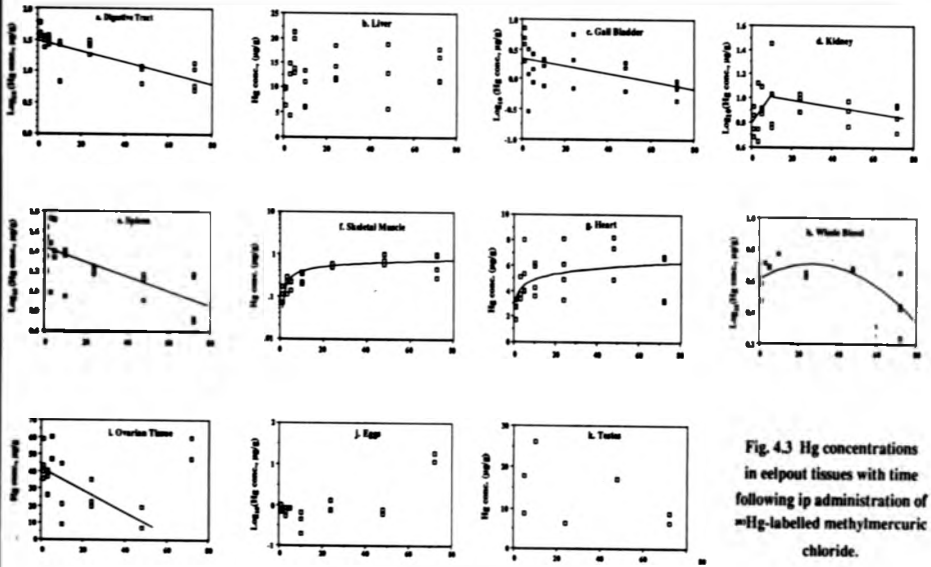


Fig. 4.3 Hg concentrations in eelpout tissues with time following ip administration of ^{203}Hg-labelled methylmercuric chloride.

This model explains approximately 50% of the variability of the data (r^2= 0.494). The model shows a peak in blood concentrations of Hg around 24 hours after injection.

The Hg concentration of liver tissue was found to display a highly-significant negative correlation with liver weight (Pearson correlation r = -0.51; P= 0.0078; n= 26), the only tissue to show an organ-weight dependence for Hg concentration. The Hg concentration of liver tissue was, therefore, corrected for the effect of liver weight by normalising to the mean liver weight, using the significant linear regression between liver weight and Hg concentration. Details of the normalisation procedure are given in Appendix 4.4. The Hg concentrations for liver tissue shown in Fig. 4.3 b are corrected for liver weight.

Three tissues (Liver, concentrations corrected for the effects of liver weight, Fig. 4.3 b; Eggs, Fig. 4.3 j; Testes, Fig. 4.3 k) showed no significant changes from the 1-hour Hg concentration over the remainder of the experimental period. No significant Pearson correlations between Hg concentration and time were observed for any of the three tissues (all P values >0.10).

Two tissues (skeletal muscle, Fig. 4.3 f and heart, Fig. 4.3 g) showed an increase in Hg concentration over the 72-hour period. The rate of increase was initially steep in both tissues, although a reduction in the rate was observed in both after 10 hours. Both tissues showed a good fit to logarithmic equations (Curve Fitter, Shareware computer package). The best-fit equation describing accumulation of Hg by skeletal muscle was :

$$\text{Hg concentration } (\mu g \text{ Hg} \cdot g^{-1}) = 0.0986 \times (\text{Time, hours})^{0.4746}$$

The model explained > 76% of the variability in the data ($r^2 = 0.766$, n=26).

Accumulation in heart was described best by the logarithmic equation:

$$\text{Hg concentration } (\mu g \text{ Hg} \cdot g^{-1}) = 3.2295 \times (\text{Time, hours})^{0.1482},$$

which explained approximately 30% of the variability in the data ($r^2 = 0.301$, n=26).

4.3.2 Transfer of methylmercury between generations

A transfer of methylmercury between generations was observed in female fish, with a significant correlation (Spearman rank correlation coefficient, r_s = 0.684; P=0.001; n=19) between the Hg concentrations of ovarian tissue and the intraovarian eggs, which were removed for separate weighing and ^{203}Hg activity counting. A significant positive linear relation between the $\log_{10}$ values of ovarian tissue and egg Hg concentrations is shown in Fig. 4.4. The linear regression equation describing the relationship is:

$$\text{Log}_{10}(\text{Hg conc.}) \text{ in eggs} = -1.7815 + 1.1892 \times (\log_{10}(\text{Hg conc.}) \text{ in ovarian tissue}).$$

The transfer of administered methylmercury between generations was also observed when female fish carrying a developing brood were injected intraperitoneally with ^{203}Hg-labelled methylmercuric chloride (Table 4.2). The broods of four female fish contained a mean of approximately 5% of the administered dose 72 hours after dosing, although individual values showed considerable variation (range = 1.25 to 10.65%). Relatively high concentrations of Hg

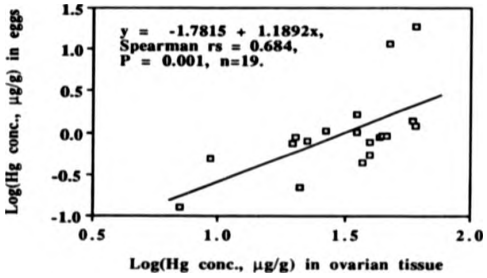


Fig. 4.4 **Maternal tissue/egg Hg concentrations in eelpout injected intraperitoneally with 203Hg-labelled methylmercuric chloride solution.**

Table 4.2 Concentrations ([Hg]) and retention of Hg in maternal and brood tissues of pregnant female eelpout, 72 hours after intraperitoneal administration of ^{203}Hg-labelled methylmercuric chloride

	Maternal Tissues										Whole Brood Tissues			
	Skeletal Muscle		Ovarian Sac Tissue				Ovarian Fluid							
	[Hg]	CF	[Hg]	CF	tsi	% of dose	[Hg]	CF	tsi	% of dose	[Hg]	CF	tsi	% of dose
mean	1.95	0.23	33.86	4.06	1.26	4.55	0.69	0.09	1.17	0.10	3.79	0.43	10.41	5.04
sd	0.53	0.06	3.68	0.44	0.26	1.50	0.73	0.09	0.03	0.10	2.91	0.35	0.89	4.06

Notes. Sample size, n = 4, except for ovarian fluid where n=2. Hg concentrations are in μg Hg.g-1 wet weight; CF is Hg Concentration Factor: the ratio of tissue Hg concentration / administered dose (0.834 μg.g^{-1}); tsi is tissue somatic index, the weight of tissue/organ expressed as a % of total body weight. % of dose is the % of the administered dose in the tissue/organ.

were measured in the ovarian sac tissue. The intra-ovarian fluid was sampled from two ovaries, and found to contain small but measurable amounts of Hg. Due to the small sample size, no sensible exploration could be made, in this case, of the relationships between maternal and brood tissue Hg content.

4.3.3 Distribution of whole body Hg burdens among tissues, and Hg concentration factors in tissues following intraperitoneal (ip) injection.

The mean tissue somatic indices, tissue distribution of whole body Hg burden, and mean tissue Hg concentration factors are shown in Table 4.3 for eelpout at 10 and 48 hours after ip injection of ^{203}Hg-labelled methylmercuric chloride. As no measurement was made of whole-body loss folowing ip dosing, the whole body Hg burden was taken as the injected dose, and tissue Hg concentration factors and tissue burdens were calculated using this value.

Since only subsamples of skeletal muscle were collected for counting, a value of 53.3 % was assumed for the TSI of this tissue. This is based on a measurement of mean skeletal muscle TSI for a sub-sample of eelpout from the Firth of Forth, collected at the same time (14-10-91) as the fish used in this work (see Chapter 3).

The tissues analysed accounted for similar amounts of the total body weight: 61.3% ± 0.88 (sd) in the 10-hour sample and 61.7% ± 0.92 (sd) in the 48 hour sample. The largest proportion of the weight (greatest value of TSI) was accounted for by skeletal muscle, with liver, testes, digestive tract and blood accounting for more than 1% each in both groups of fish. The three most important tissues, in terms of the fraction of the administered dose retained, are digestive tract, liver and skeletal muscle. Redistribution of the Hg burden in these tissues with time is shown in Fig. 4.5, which shows a general pattern of decreasing Hg burden in digestive tract tissue with time, and the opposite pattern in skeletal muscle.

4.3.4 Comparison of Hg concentrations in eelpout tissues two days and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride

All Hg concentrations were calculated on a wet weight basis. The mean concentrations (with standard deviations) of Hg in a number of eelpout tissues, two days and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride, are shown in Fig. 4.6.

Only one of the tissues collected, testes, showed no effective change in Hg concentration during the study period (Day 2 conc.: 0.528±0.124 $\mu g.g^{-1}$, Day 16 conc.: 0.490±0.261 $\mu g.g^{-1}$). As only three of the Day 2 fish were male, a statistical test for similarity of Day 2 and Day 16 Hg concentrations in testes tissue is not possible.

A number of tissues exhibited a decrease in Hg concentration between 2 days and 16 days after administration. Digestive tract tissue showed both the highest tissue concentrations of Hg (Day 2: 10.562 ± 5.666 $\mu g.g^{-1}$), and the greatest loss of Hg between Day 2 and Day 16 (Day 16

Table 4.3 Tissue Somatic Indices, tissue distribution of Hg burden, tissue Hg concentrations and concentration factors in eelpout 10 and 48 hours after intra-peritoneal injection of ^{203}Hg-labelled methylmercuric chloride

Time	Tissue	Mean Tissue Somatic Index[1] ± sd	Mean % of whole body Hg burden ± sd	Mean Tissue Concentration (µg Hg.g^{-1})	Mean Tissue Hg Concentration Factor[2] ± sd
10 Hours	Digestive Tract	1.70 (4)	14.16 ± 10.16	17.73 ± 12.72	9.52 ± 7.48
	Liver	2.20 ± 0.30 (4)	9.03 ± 4.48	8.62 ± 3.80	4.06 ± 1.79
	Gall Bladder	0.13 ± 0.09 (4)	0.08 ± 0.04	1.65 ± 0.63	0.78 ± 0.30
	Kidney	0.13 ± 0.04 (4)	0.78 ± 0.75	12.95 ± 11.00	6.10 ± 5.18
	Spleen	0.09 ± 0.01 (4)	0.89 ± 0.35	20.49 ± 7.79	9.65 ± 3.67
	Skeletal Muscle	53.3 (4)	7.18 ± 2.18	0.29 ± 0.09	0.14 ± 0.04
	Heart	0.10 ± 0.03 (4)	0.24 ± 0.08	5.01 ± 1.28	2.36 ± 0.60
	Blood	1.60 (1)	4.41	5.85	2.76
	Testes	3.28 (1)	40.47	26.19	12.33
	Ovary Tissue	0.56 ± 0.25 (3)	6.01 ± 3.46	25.00 ± 17.98	11.77 ± 8.46
	Eggs	0.99 ± 0.32 (3)	0.22 ± 0.08	0.54 ± 0.35	0.27 ± 0.23
48 Hours	Digestive Tract	1.70 (3)	7.7 ± 2.34	9.64 ± 2.91	4.51 ± 1.37
	Liver	1.88 ± 0.47 (3)	9.66 ± 4.61	10.69 ± 3.35	5.03 ± 1.58
	Gall Bladder	0.18 ± 0.03 (3)	0.11 ± 0.04	1.35 ± 0.65	0.64 ± 0.30
	Kidney	0.17 ± 0.06 (3)	0.63 ± 0.33	7.81 ± 1.85	3.68 ± 0.87
	Spleen	0.07 ± 0.01 (3)	0.42 ± 0.18	12.15 ± 3.47	5.72 ± 1.64
	Skeletal Muscle	53.3 (3)	19.64 ± 5.67	0.78 ± 0.23	0.37 ± 0.10
	Heart	0.08 ± 0.01 (3)	0.27 ± 0.10	6.85 ± 1.72	3.22 ± 0.81
	Blood	1.60 (2)	3.55 ± 0.04	4.70 ± 0.06	2.22 ± 0.03
	Testes	3.66 (1)	29.73	17.22	8.11
	Ovary	1.03 ± 0.71 (2)	5.00 ± 0.20	13.14 ± 8.49	6.27 ± 4.12
	Eggs	1.24 ± 0.05 (2)	0.26 ± 0.25	0.44 ± 0.44	0.21 ± 0.21

Notes. Values in parentheses are number of fish in sample

[1] Tissue Somatic Index is calculated as 100* (wet weight of a tissue / wet weight of fish)

[2] Tissue Hg Concentration Factor is calculated as the ratio: Hg concentration of tissue / Hg concentration of whole body

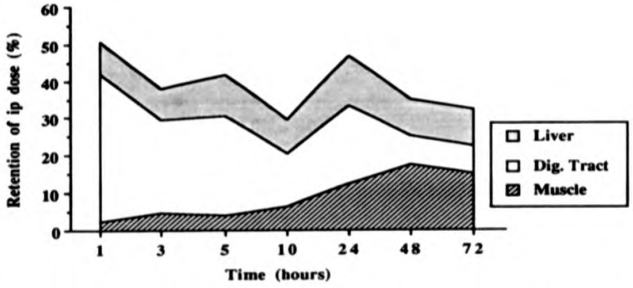


Fig. 4.5 Redistribution of i.p. injected methylmercury with time

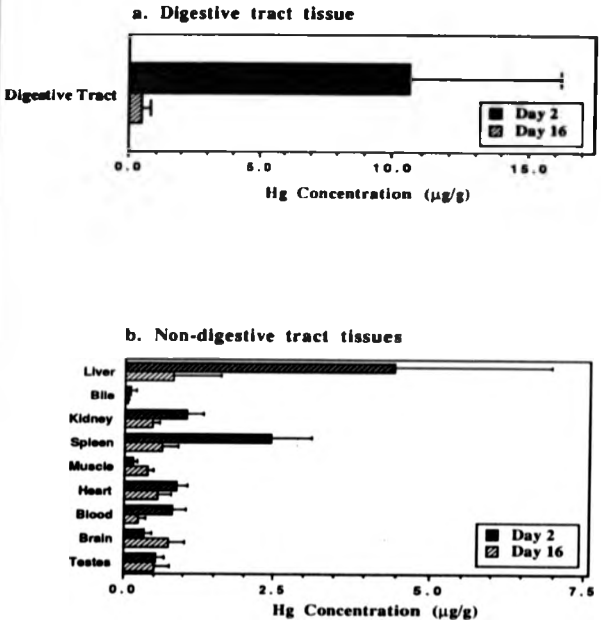


Fig. 4.6 Mean Hg concentrations (+ standard deviation) in eelpout tissues after 2 days and 16 days following oral administration of methylmercuric chloride (0.834 ug.g body weight). Sample sizes are as in Table 4.4.

whole digestive tissue: 0.490 ± 0.321 $\mu g.g^{-1}$).The mean Hg concentration of the whole digestive tissue on Day 2 is significantly higher than the Day 16 value (Unpaired t-test: t=3.674, two-tailed P=0.0104). Liver tissue also exhibited a relatively high mean Hg concentration on Day 2 (4.452 ± 2.503 $\mu g.g^{-1}$, n=4), with a significant reduction to 0.796 ± 0.800 $\mu g.g^{-1}$ (n=4) by Day 16 (Unpaired t-test: t=2.782, two-tailed P=0.0319).

Kidney tissue, with a Day 2 mean Hg concentration of 0.729 ± 0.606 $\mu g.g^{-1}$ (n=3) exhibited a decrease by Day 16 to 0.430 ± 0.143 $\mu g.g^{-1}$ (n=4), although the small sample size on Day 2 prevents the difference from being tested statistically. The mean Hg concentration of heart tissue also fell between Day 2 (0.875 ± 0.188 $\mu g.g^{-1}$, n=2) and Day 16 (0.552 ± 0.221 $\mu g.g^{-1}$, n=4) although, again, this difference cannot be tested statistically. Similarly, the reduction of mean Hg concentration of the whole gall bladder (incl. fluid contents) from Day 2 (0.099 ± 0.093 $\mu g.g^{-1}$, n=3) to Day 16 (0.039 ± 0.029 $\mu g.g^{-1}$, n=4) cannot be tested for statistical significance.

The mean whole spleen Hg concentration was relatively high on Day 2 (2.437±0.687 $\mu g.g^{-1}$, n=4) but exhibited a statistically-significant loss (Unpaired t-test: t=3.674, two-tailed P=0.010) by Day 16 (0.613 ± 0.286 $\mu g.g^{-1}$, n= 4). Whole blood showed a significant decrease (Unpaired t-test: t=3.445, two-tailed P=0.0137) in mean Hg concentration between Day 2 (0.731±0.256 $\mu g.g^{-1}$, n=4) and Day 16 (0.231 ± 0.137 $\mu g.g^{-1}$, n=4).

Only two tissues, skeletal muscle and brain, exhibited an increase in concentration of Hg between Day 2 and Day 16. The mean Day 2 concentration in muscle tissue (0.152 ± 0.052 $\mu g.g^{-1}$) was relatively low compared to most of the other tissues examined. The Hg concentration in muscle had, however, increased significantly by Day 16 (Unpaired t-test: t = -3.659, two-tailed P=0.0106) to 0.366 ± 0.105 $\mu g.g^{-1}$ (n=4). The mean Hg concentration in brain tissue increased, although not significantly (Unpaired t-test: t =-2.409, two-tailed P = 0.0527), from Day 2 (0.337 ± 0.117 $\mu g.g^{-1}$, n=4) to a Day 16 mean of 0.722 ± 0.298 $\mu g.g^{-1}$ (n=4).

The tissues are ranked on each day in order of mean Hg concentrations as follows (from highest to lowest values) :

Day 2:

Digestive Tract > Liver > Spleen > Kidney > Heart > Blood > Testes > Brain > Muscle > Bile

Day 16:

Liver > Brain > Spleen > Heart > Testes = Digestive Tract > Kidney > Muscle > Blood > Bile

Comparison of the two lists above show some changes in the rank of tissue Hg concentrations between Day 2 and Day 16. Notable changes were the loss of rank by digestive tract, blood, and kidney tissues, and an increase in the ranks of brain, heart, and muscle tissues. The positions of liver as one of the tissues with highest concentrations, and bile as the tissue with lowest concentrations, remained unchanged over the period.

4.3.5 Distribution of whole body Hg burdens in tissues and organs of eelpout two and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride.

The mean percentages of body weight constituted by each tissue or organ (Tissue Somatic Index, TSI) collected for Hg determination and that of the remainder of unanalysed tissues (carcass), two and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride, are given in Table 4.4, along with the percentage of the whole body Hg burden contained in each tissue.

A mean of 44.75 % of the total wet weight was accounted by the tissues collected for analysis on Day 2. These tissues contained a mean of 49.65 % of the whole body Hg burden. The largest mean percentage (21.605 % ± 4.853 (sd), n=4) of the body burden in the tissues analysed was contained in the digestive tissues. The next largest percentage of the total Hg burden was contained in the skeletal muscle (12.77 ± 2.74 %), with a further 9.43 ± 6.98 % in the liver. Apart from a mean estimated value of 2.89 ± 1.9 % in the blood, the remaining tissues each contained less than 1 % of the whole body Hg burden .

The collected tissues on Day 16 accounted for 43.85 % of the total wet weight, a similar value to the Day 2 figure. These tissues contained a lower percentage (43.47%) of the whole body Hg burden than was observed in the Day 2 fish. Differences were also observed between the Day 2 and Day 16 distribution of the Hg burden among the tissues.

On Day 16, the largest percentage of the burden was contained in skeletal muscle (mean of 37.09±13.41%), more than triple the Day 2 value. The liver contained 3.09 ± 2.66% of the burden (approximately one-third of the Day 2 value). The digestive tissues, containing a mean of 21.6 % of the Hg burden on Day 2, held a mean of only 0.62 % of the burden on Day 16. Each of the other tissues analysed on Day 16 held no more than 1% of the total burden.

The estimated percentage in blood in Day 16 fish was one-third of the Day 2 percentage. The estimated percentage contained in the brain more than doubled between Day 2 (0.227 ± 0.064 %) and Day 16 (0.580 ± 0.275%). The kidney, on Day 16, contained approximately one-fifth of the burden held on Day 2, while the percentage of the burden in the heart fell from Day 2 to Day 16 by about 30%.The spleen burden on Day 16 was approximately one-fifth of the Day 2 burden. The percentages of the whole body Hg burden in both testes and gall bladder remained relatively unchanged between Day 2 and Day 16.

4.3.6 A comparison of tissue concentration factors (ratio of tissue concentration to whole body concentration) of Hg, at two and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride.

The tissue Concentration Factor (CF) of a substance was calculated as the ratio of the concentration of Hg in a tissue to that in the whole body. The mean tissue concentration factors of Hg are given in Table 4.4 for each tissue two days and sixteen days after oral administration

Table 4.4 Tissue Somatic Indices, tissue distribution of Hg burden, tissue Hg concentrations and concentration factors in eelpout two and sixteen days after oral administration of ^{203}Hg-labelled methylmercuric chloride

Day	Tissue	Mean Tissue Somatic Index[1] (%)	Mean % of whole body Hg burden	Mean Tissue Concentration (μg Hg.g^{-1})	Mean Concentration Factor[2]
Day 2	Digestive Tract	1.696 ±0.588 (4)	21.605 ± 4.583	10.562 ± 5.666	22.501 ± 14.208
	Liver	0.938 ± 0.131 (4)	9.427 ± 6.979	4.452 ± 2.503	10.036 ± 7.402
	Gall Bladder	0.182 ±0.250 (3)	0.032 ± 0.013	0.099 ± 0.093	0.119 ± 0.076
	Kidney	0.117 ± 0.030 (3)	0.370 ± 0.241	0.729 ± 0.606	2.921 ± 1.392
	Spleen	0.122 ± 0.087 (4)	0.778 ± 0.188	2.437 ± 0.687	5.431 ± 1.858
	Skeletal Muscle	39.220 ± 1.201 (4)	12.766 ± 2.743	0.152 ± 0.052	0.325 ± 0.070
	Heart	0.108 ± 0.011 (2)	0.226 ± 0.064	0.875 ± 0.188	2.082 ± 0.376
	Blood	1.60* (4)	2.888 ± 1.900	0.731 ± 0.256	1.799 ± 1.179
	Brain	0.31* (4)	0.227 ± 0.064	0.337 ± 0.117	0.731 ± 0.215
	Testes	0.455 ± 0.110 (3)	0.631 ± 0.220	0.528 ± 0.124	1.375 ± 0.301
	Ovary	0.562 (1)	0.685	0.911	0.973
	Carcass[3]	35.31 ± 0.33 (4)	51.05 ± 4.55	0.490 ± 0.192	1.026 ± 0.063
Day 16	Digestive Tract	0.527 ± 0.090 (4)	0.624 ± 0.342	0.490 ± 0.321	1.345 ± 0.983
	Liver	0.935 ± 0.194 (4)	3.091 ± 2.656	0.796 ± 0.800	2.194 ± 2.405
	Gall Bladder	0.324 ± 0.094 (4)	0.037 ± 0.038	0.039 ± 0.029	0.107 ± 0.091
	Kidney	0.059 ± 0.013 (4)	0.072 ± 0.019	0.430 ± 0.143	1.228 ± 0.478
	Spleen	0.367 ± 0.120 (4)	0.176 ± 0.064	0.613 ± 0.286	1.670 ± 0.887
	Skeletal Muscle	38.977 ± 0.303 (4)	37.086 ± 13.411	0.366 ± 0.105	0.960 ± 0.372
	Heart	0.113 ± 0.011 (4)	0.160 ± 0.070	0.552 ± 0.221	1.449 ± 0.742
	Blood	1.60* (4)	0.968 ± 0.653	0.231 ± 0.137	0.615 ± 0.435
	Brain	0.31* (4)	0.580 ± 0.275	0.722 ± 0.298	1.882 ± 0.959
	Testes	0.624 ± 0.129 (4)	0.678 ± 0.468	0.490 ± 0.261	1.340 ± 0.809
	Carcass[3]	56.32 ± 0.33 (4)	56.28 ± 16.48	0.381 ± 0.066	0.996 ± 0.289

Notes. Values after means are (±) standard deviations; * estimated value for tissue somatic index - see Materials and Methods for details;

[1]. Tissue Somatic Index is calculated as 100* (wet weight of tissue / wet weight of whole body);

[2]. Tissue Hg Concentration Factor is calculated as the ratio: Hg concentration of tissue/Hg concentration of whole body;

[3]. Carcass weights calculated as (Fresh weight - total weight of other tissues). Mass of Hg in carcass calculated as: (Whole-body Hg mass - total Hg mass in other tissues).

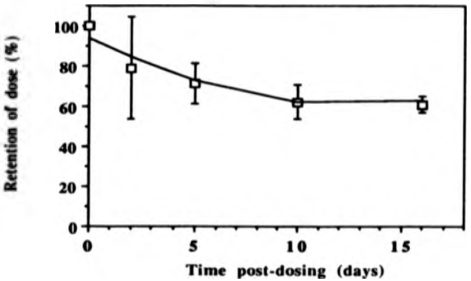


Fig. 4.7 Whole body retention of Hg by eelpout (mean ± sd, n=4 on each day), following oral administration of labelled methylmercuric chloride

of ^{203}Hg-labelled methylmercuric chloride.

Concentration Factors (CF) for Hg were very high (>10) in digestive tract and liver two days after oral administration of a dose of 0.834 µg Hg.g^{-1} body weight. Relatively high values of CF (>2) were also observed in spleen, kidney and heart. Concentrations higher than whole body concentration (CF>1) were observed in blood and testes. CF values of <1 (indicating tissue Hg concentration lower than whole body concentration) were observed in skeletal muscle, brain and gall bladder), and a single ovary sample on Day 2 had a CF value close to 1.

By sixteen days after administration only one tissue, liver, had a mean CF > 2, this with a large standard deviation. The mean CF of digestive tract tissue was much lower on Day 16, having fallen to a little over 1. Mean CF values of kidney and spleen, while still greater than 1, were considerably lower than on Day 2. The mean CF values of both testes and gall bladder on Day 16 were virtually the same as the Day 2 values. Only skeletal muscle and brain tissue showed an increase in the value of CF between Day 2 and Day 16. Brain CF was more than twice as high, whilst muscle was virtually three times as high by Day 16. By Day 16, the mean skeletal muscle CF was almost equal to 1, and in brain tissue was approaching CF=2.

4.3.7 Retention and excretion of whole-body Hg burden by eelpout following oral administration of ^{203}Hg-labelled methylmercuric chloride

The whole body Hg activity of each fish was counted within 30 minutes of oral administration of ^{203}Hg-labelled methylmercuric chloride. The calculated whole body burden at (effectively) time = 0 days was compared with the known administered dose for each fish. It was found that fish had retained between 94.4 and 46.7% of the administered dose (mean = 69.6%, sd =14.54, n=8), the remainder presumably having been lost by vomiting or coughing. As this loss of Hg activity occurred before its uptake from solution by the fish, the whole body burden of Hg measured at 'time=0' (i.e. first count), rather than the administered dose, was taken as the 100% value for studying excretion/retention.

The percentage retention of whole body Hg activity for each counting session is shown in Fig. 4.7 for the four individual fish in the 16 Day group. A retention of 112% was measured for one fish on Day 2, accounting for the large standard deviation of the mean on that day. The mean retention fell over the 16 days of the experimental period, from 100% at time = 0 days, to 60.8% (± 4.23, sd) at time = 16 days. The mean retention of the four fish sacrificed for tissue samples on Day 2 was 87.13% ± 30.86. The rate of loss of whole body mercury was not linear over the 16 day downrun period. Approximately 20% of the ingested burden was lost over the first two days, and another 20% over the next 14 days. The data gave a poor fit to a second order polynomial model: $y = 93.834 - 5.296\,(x) + 0.209\,(x^2)$,
which explained a little over 30% of the variability of the retention values (r^2 =0.321; r =0.566). The regression was statistically significant (ANOVA: regression df = 2, residual df = 17, total df = 19, F= 4.0125, P= 0.037).

4.4 Discussion

4.4.1 Internal dynamics of methylmercury in eelpout

The patterns of Hg redistribution with time shown by a number of eelpout tissues (Fig. 4.4) suggest that the dynamics of intraperitoneally (i.p.) administered methylmercury are related closely to the structure and biological functions of the tissues. In general, the transfer of Hg from the peritoneum to the tissues is rapid, high or maximum concentrations being observed in several tissues within one hour of dosing (i.e. digestive tract, liver, gall bladder, spleen, blood and ovarian tissue). A similar short timescale for transfer to the tissues was observed in rainbow trout (*Salmo gairdneri*), which had received an intragastric dose of ^{203}Hg-labelled methylmercuric chloride (Giblin and Massaro, 1973).

Extensive investigation of the accumulation of both inorganic and methylmercury by rainbow trout (*Salmo gairdneri*), via both direct and trophic uptake, was reported by Boudou and Ribeyre (1983). Their results led them to hypothesize that the blood-irrigation characteristics of each organ or tissue are very important in mercury transfer processes which depend on the density of the capillary network, the volume of blood per unit weight of tissue, and the blood flow.

The individual structural and metabolic features of the tissues can be predicted to determine the accumulation patterns of methylmercury in these tissues, depending on the properties of the membrane barriers, the number of available mercury binding sites and their accessibility, and the rate of cell renewal (Boudou and Ribeyre, 1983).

Digestive tract: The initial high uptake, and rapid loss, of Hg by the digestive tract is consistent with the role of this tissue in the uptake of the breakdown products of digestion. The digestive tract is extremely well-supplied with blood vessels, providing a rapid route into the blood for methylmercury crossing the outer intestinal wall. Such a route of uptake, from the peritoneum to the digestive tract, is an artifact of the i.p. administration, although it does facilitate a rapid accumulation of Hg. The loss of methylmercury from the digestive tract is probably a combination of the transfer of methylmercury away from tissue in the bloodstream, and the excretion of non-absorbed methylmercury from the digestive tract into the water column.

The observed behaviour of methylmercury in this study, *viz.* the rapid accumulation of methylmercury by the digestive tract, supports the results of Farmanfarmaian (1985), who investigated the distribution of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the intestine of the toadfish, *Opsanus tau*, under *in vitro* conditions. An initial, rapid binding of both mercury compounds to the mucosal surface of the intestine was followed by a very slow tissue permeation of $HgCl_2$, but a rapid permeation of CH_3HgCl. A similar, rapid accumulation of methylmercury from

food was reported by Pentreath (1976c) for plaice (*Pleuronectes plaessa*), which had been force-fed with gelatin or starch pellets, or with a prey species, *Nereis* polychaetes, labelled with $CH_3{}^{203}HgCl$. Evidence from both i.p. and oral dosing (Tables 4.2 and 4.4) shows that a highly significant loss of Hg (as $CH_3{}^{203}HgCl$) from the digestive tract occurs with time. It is not clear, however, from the results of this work whether the significant loss observed by 16 days following oral administration is the result of a continuing trend of decreasing concentration similar to that observed in the digestive tract following i.p. administration.

Liver: The liver is the largest single organ in most vertebrates, with a principal function as a site of intermediary metabolism; it receives the absorbed products of digestion from the digestive tract via the bloodstream (Martin, 1976). The structure of the liver is directly related to this and the other important functions. It has an extremely good blood supply, receiving more blood per unit time than any other organ (Roberts, 1977), and the liver cells are in intimate association with blood vessels and bile channels. This may explain the rapid uptake of Hg by this organ, reaching the maximum observed level within one hour of i.p. administration (Fig. 4.4b).

The liver retained an almost identical portion of the administered dose (= 9%) after 48 hours, regardless of whether the dose was administered intraperitoneally or orally. This suggests that the initial accumulation of Hg by the liver, within one hour of i.p. injection, was via the bloodstream, rather than as an artifact of direct uptake from the peritoneum.

The large volume of blood supplied to the liver suggests that the concentration of Hg in this organ, following i.p. administration, could be a function of that in the blood contained in the vascular space. The decrease of Hg levels in the blood after 24 hours was, however, not reflected by a similar fall in the liver. This implies that methylmercury supplied initially to the liver becomes bound to some liver cellular components, rather than to blood cells in the blood space of the tissue. Blood-bound methylmercury accounted for only 10% of the total amount in the liver of rainbow trout, *S. gairdneri*, 24 hours after direct exposure to methylmercury in water. The subcellular distribution of methylmercury in fish liver cells was investigated by Olson *et al*. (1978). In liver cytosol of *S. gairdneri*, a low molecular weight methylmercury-binding thionein-like protein accounted for up to 40% of the total methylmercury bound (Olson *et al*., 1978). The majority of the remainder was associated with high molecular weight fractions.

The short-term (3-day) lack of any significant change of Hg concentration in the liver following i.p. injection was not reflected over the sixteen days following oral administration, where a significant loss of Hg was observed. It is notable that following exposure of bluegills (*Lepomis macrochirus*) to methylmercury in water, Burrows and Krenkel (1973) also observed very high mercury levels in the liver (up to seven times higher than whole body concentration). There was, however, no discernible change in the liver concentration over the following 94

days, a much longer period of study than was possible in this work.

Gall Bladder and Bile: The role of bile secreted by the liver, and stored in the gall bladder, in the excretion of methylmercury from the body via the intestine, has been described for a mammalian system, the rat (Norseth and Clarkson, 1971; Ballatori and Clarkson, 1982). Faecal excretion, the main route of elimination, appears to be determined mainly by biliary secretion of methylmercury bound to the sulphydryl groups of glutathione. The importance of faecal excretion of methylmercury was confirmed tentatively for fish by Giblin and Massaro (1973) in a study using rainbow trout, *Salmo gairdneri*. These workers showed that, while the intestinal contents contained only 0.05% of an administered dose of methylmercury, 95% of this mercury was in the posterior intestine.

The loss of mercury from the gall bladder and bile of eelpout after i.p. administration (Fig. 4.4c), regardless of whether the initial high levels were accumulated from the peritoneum or from the blood, may indicate an important pathway of excretion for methylmercury in fish. The relatively similar levels of Hg in the gall bladder, however, two and sixteen days after oral dosing, both much lower than those resulting from i.p. administration, may be more typical of the pattern observed following the less artificial uptake of methylmercury from the digestive tract.

Kidney: Several studies have demonstrated a high affinity of the kidney tissue of fish for mercury, both after experimental exposure (Lockhart *et al.*, 1972; Burrows and Krenkel, 1973; Weisbart, 1973; Bishop and Neary, 1975; Boudou and Ribeyre, 1983), and in wild fish (Renzoni *et al.*, 1973; Pentreath, 1976a; Riisgard and Famme, 1988). The role of the kidney in excretion of mercury compounds is not clear, although Boudou *et al.* (1983) state, unsupported by data, that fish excrete mercury via the kidney. The experimental evidence for this is contradictory.

In goldfish, *Carassius auratus*, dosed i.p. with inorganic mercuric nitrate, the mercury concentration of kidney tissue increased in a linear fashion throughout a 670 hour experimental period (Weisbart, 1973). It was suggested that kidney in this species may lose mercury at a rate slower than that of the body as a whole. Burrows and Krenkel (1973) reported no loss, over a 94 day period, of methylmercury from the kidney of bluegill (*Lepomis macrochirus*) following exposure to the substance in water. Ribeyre and Boudou (1984), by contrast, reported that, following exposure of rainbow trout, *S. gairdneri*, to methylmercury in either water (direct) or food (trophic), and subsequent transfer to clean water, the kidney showed an immediate reduction of the accumulated mercury.

Spleen: The intimate association of the spleen with the blood as a site of red blood cell degradation might be explain the early peak in mercury concentrations of this organ. The effect

of methylmercury on the degradation rate of red blood cells is unknown, however. It is possible that the loss of methylmercury from the spleen simply reflects the reduction in blood mercury concentrations as mercury is excreted or transferred to longer term storage tissues such as skeletal muscle.

Blood: The blood plays a fundamental part in the bioaccumulation processes of methylmercury in fish. Giblin and Massaro (1973, 1975) showed that methylmercury is taken up rapidly, and to high concentrations, by red blood cells of rainbow trout (*S. gairdneri*), both *in vitro* and *in vivo*. These workers also showed (Giblin and Massaro, 1975) that the binding of methylmercury within the red blood cell is freely reversible, again both *in vitro* and *in vivo*.

Haemoglobin (Hb) was shown to be the most important methylmercury transport protein in the blood of rainbow trout (*S. gairdneri*), with the relatively large number of available sulphydryl (-SH) groups per molecule (4) responsible for the high concentrations attained (Giblin and Massaro, 1975). This protein bound up to 90% of the whole blood burden following intragastric dosing. Hb was also shown to be able to compete for and bind methylmercury bound to other -SH groups, and to transfer methylmercury readily to -SH groups outside the cell. Thus, the red blood cells do not sequester and consequently decrease methylmercury toxicity but, rather, they increase its potential for toxic influence by facilitating its distribution to tissue or organ proteins (Giblin and Massaro, 1975). Notably, in contrast to these observations, Olson and co-workers found that both inorganic and organic mercury bound strongly to red blood cells, such that, even when in excess, sulphydryl-containing compounds (e.g. albumin, cysteine, and reduced glutathione) failed to remove bound mercury (Olson *et al.*, 1973).

In this study, following a rapid accumulation of Hg within 1 hour of i.p. administration, concentrations peaked in eelpout whole blood samples around 24 hours after intraperitoneal administration of ^{203}Hg-labeled methylmercuric chloride (Fig. 4.4h). The parabolic distribution of Hg concentrations with time may reflect the redistribution of methylmercury, via the bloodstream, from tissues important in uptake which lost Hg rapidly, to tissues important for methylmercury storage which accumulated methylmercury at a relatively lower rate.

Skeletal Muscle: Skeletal muscle has been shown in several studies of wild fish to be the tissue to which the majority of the body burden of mercury is found (see Chapter 3, section 3.4). In Chapter 3, it was shown that up to 78% of the whole body burden of total mercury in wild eelpout is found in the skeletal muscle. Unlike the majority of the other tissues examined, the accumulation of methylmercury in skeletal muscle following i.p. dosing continued throughout the experimental period. The relatively low concentrations of Hg in muscle immediately after dosing were also in contrast to the rapid accumulation observed in most other tissues. Although skeletal muscle has a greater capacity for storage of the total methylmercury burden, the slow

accumulation may be due to a combination of factors.

Skeletal muscle of rainbow trout was shown, by Olson *et al.* (1978) to have a much lower volume of blood per unit weight of tissue than a number of other tissues (spleen, liver, gill, kidney). This suggests that there is a lower transfer of blood, and hence of methylmercury, to the skeletal muscle.
Another important factor may be the position of skeletal muscle in relation to the tissues important in uptake and blood circulation processes. The digestive tract may be the most important tissue, following both i.p. and oral dosing, for the initial transfer of methylmercury to the bloodstream (see above). Thus, methylmercury would not reach skeletal muscle in the bloodstream until after its passage into and through the principal enterohepatic tissues (digestive tract, liver, gall bladder) and circulatory tissues (heart, spleen). Initially high concentrations in these tissues would reduce the amount of methylmercury available to be supplied to the muscle. Only with the later release of methylmercury from these other tissues, and its subsequent sequestering and transport by the red blood cells, would mercury be supplied in greater quantities to the skeletal muscle.

Possible explanations for the observed asymptotic accumulation of Hg in muscle following i.p. administration could be:
1. The saturation of available methylmercury binding sites in muscle, or
2. A reduction of available methylmercury in the bloodstream as the amount released by other tissues, and sequestered by the red blood cells, and then skeletal muscle, decreases with time.

There are many available methylmercury binding sites in the skeletal muscle, especially the sulphydryl groups and disulphide linkages of the muscle proteins (Carty and Malone, 1979). This, in addition to the continuous, long-term (100 day) increase of mercury concentrations in fish skeletal muscle following a single exposure to methylmercury (e.g. Giblin and Massaro, 1973; Olson *et al.*, 1978), suggests the second explanation is more likely. Skeletal muscle contained a larger part of the body burden of methylmercury at sixteen days after oral dosing than at three days after i.p. dosing. This may reflect differences in mercury dynamics due to dosing methods, i.e. i.p. dosed fish held more of the dose in muscle than oral dosed fish two days after dosing. It is also possible, however, that this reflects a continuing transfer with time of the remaining dose to skeletal muscle, as described by the other workers mentioned above.

Heart: Comparing i.p. and oral dosed fish, heart tissue contained very similar fractions of the body burden of methylmercury, with similar concentration factors, at 48 hours after dosing. The implication of this is that the heart receives methylmercury via the bloodstream following intraperitoneal administration. This may not be unexpected as the heart lies in the pericardial cavity, separated from the peritoneum by the fibrous pericardium (Romer and Parsons, 1986). This presumably prevents immediate direct contamination by the injected dose. The

accumulation of Hg to the relatively high concentrations in heart tissue may reflect the Hg content of the relatively high blood volume passing through the heart. As, however, the pattern of change with time did not reflect the decreasing concentration showed by blood after 24 hours, the increase may also be related to methylmercury binding processes in heart tissue.

Like skeletal muscle, the heart shows an asymptotic increase of Hg concentrations with time after i.p. dosing (Fig. 4.3g). In skeletal muscle, this pattern was suggested to be the result of reduced availability of methylmercury, rather than the saturation of methylmercury binding sites. In contrast, although the muscular nature of heart tissue implies that there are a relatively large number of binding sites per unit weight of tissue (Carty and Malone, 1979), the role of the heart in blood circulation and its small size relative to blood volume suggests that the asymptote may be the result of the saturation of such sites.

Brain: Methylmercury has been shown here and in previous studies to cross the blood-brain barrier in fish, probably by rapid diffusion across the lipid bilayers of membranes (Giblin and Massaro, 1973; Pentreath, 1976 b,d; Lakowicz and Anderson, 1980). The brain of trout has been shown to accumulate a smaller portion of an administered dose of methylmercury than the brain of rat (Giblin and Massaro, 1973). In this study, the brain tissue was only one of two (cf skeletal muscle) to show a clear increase in concentration and total burden of methylmercury over 16 days. This suggests that the brain may be an organ which will be at risk from long-term methylmercury exposure. This is supported by the evidence from human studies which show that neurological effects only occur after prolonged exposure to methylmercury (Swedish Expert Group, 1971). The effect of methylmercury accumulated in the brain tissue of fish, in very small amounts over a lifetime of natural exposure, has not been addressed in the literature. It is possible, however, that storage of methylmercury in the skeletal muscle provides a natural protection mechanism against neurotoxic effects, particularly in older fish where such effects might be more likely due to increased duration of exposure.

4.4.2 Transfer of administered methylmercury between generations

This study appears to be the first to demonstrate, under controlled conditions, the transfer of mercury between generations in a viviparous teleost species, the eelpout (*Zoarces viviparus*). Such a transfer, between maternal and brood tissues of eelpout,has been studied for another pollutant heavy metal, cadmium (Joensen and Korsgaard, 1986). These workers showed that, following intraovarian injection with cadmium, different compartments of the ovary, including the embryos, are able to eliminate an acute toxic loading of cadmium. The injected cadmium was quickly eliminated from the ovarian fluid to the serum via the ovarian sac tissue. It was suggested that the structure of the ovary of pregnant eelpout, a thin sac with numerous follicles,with a dense capillary network, facilitates the rapid clearance of the injected dose, although the mechanism of transport is unclear. The transfer of mercury between generations in other fish species can be inferred from the presence of mercury in eggs of Winter Flounder

(*Pleuronectes americanus*). Another study revealed the presence of mercury in intra-ovarian larval sharks (or 'pups'), embryos, or embryonic yolk sacs of several species from the North Atlantic, although no further examination was made of the relation with maternal mercury levels (Windom *et al.*, 1973). Evidence from mammalian studies has shown a placental transfer of methylmercury to the developing litters of rats, although the placenta seems to act as a barrier to mercuric chloride and phenylmercuric acetate (Suzuki *et al.*, 1967). The placental transfer of methylmercury has also been shown in humans, in the Minimata disease incident (Takizawa, 1979).

Significant correlations have observed between the total mercury concentrations of maternal skeletal muscle and whole brood tissues of wild eelpout from the Forth Estuary (Elliott and Griffiths, 1986; this study, Chapters 2 and 3). Such relationships are, however, purely correlative and no causality can be implied. The results presented here confirm that, following administration of a single dose to the mother fish, the concentration of methylmercury in the developing eggs is significantly correlated with that in the maternal tissues (ovarian sac).

The mechanism of accumulation of methylmercury by eggs was not investigated here, and is not known. It is possible that methylmercury only binds to the outside of the egg, and does not accumulate internally. A number of studies have, however, shown toxic effects of both inorganic and organic mercury compounds on the eggs and unhatched embryos of several oviparous fish species, which suggests that fish eggs do accumulate mercury internally (Kihlstrom *et al.*, 1971; Heisinger and Green, 1975; Weis and Weis, 1977; Khan and Weis, 1987).

Unlike the work described above for cadmium in the ovary of eelpout, there was no evidence of elimination of the accumulated methylmercury from the eggs during the course of the study. It is possible that the high protein content of eggs provides a high concentration of methylmercury binding sites, in the same way as skeletal muscle. This might ensure a long half-life for elimination related to protein synthesis and turnover, as suggested above for skeletal muscle. A growing embryonic fish in the egg, producing new tissues, and an increasing mass of skeletal muscle, would also provide an increasing number of binding sites for any methylmercury taken up across the egg membrane. It is also possible that the rapid accumulation of methylmercury by the eggs is the result of partitioning of this non-polar, lipophilic compound in the high concentration of lipids found within the egg, although it has been suggested from solubility studies by Lacowicz and Anderson (1980) that partitioning of methylmercuric chloride into lipid bilayers is small.

The transfer of methylmercury between mother fish and a developing brood has also been demonstrated here, as has the transfer of methylmercury from outside the ovary to the intra-ovarian fluid which surrounds the brood in the ovary. The limited availability of experimental animals (i.e. gravid female eelpout) prevented the testing of any time-course, or dose-related variability of the relationship between mother and brood, and the study was very limited in

nature. This demonstration of the accumulation of a Hg burden by the brood following dosing of the mother is notable, however, as the first example of this transfer under laboratory conditions. The transfer of methylmercury from an intraperitoneal dose to the intraovarian fluid provides some general evidence to support the possible mechanism, suggested in Chapter 3 of this thesis, for the accumulation of mercury from intraovarian fluid by developing broods. The distribution of the methylmercury in different components of the ovarian fluid was not investigated, however, and would be worthy of further investigation. Intra-ovarian loading with methylmercury, as was carried out previously for cadmium (Joensen and Korsgaard, 1986), is required to study the elimination of methylmercury from the ovary and brood. Such a study was not possible here, however, and no information is known about the clearance of methylmercury from the ovary.

4.4.3 Retention/Elimination of methylmercury by eelpout

The inital loss of Hg activity prior to the first measurement of whole body counting probably occurred due to vomiting or coughing action by the fish. A similar loss of an orally administered dose of methylmercury was reported by Hartman (1978), when rainbow trout (*Salmo gairdneri*) vomited 20-50 % of the administered dose within 1 hour of oral dosing.

The loss of whole-body methylmercury was described by a single polynomial regression model, with an initial rapid loss and longer-term slow loss of whole body Hg. Other workers have described an exponential loss of a whole-body methylmercury burden by fish (Jarvenpaa *et al.*, 1970; Burrows and Krenkel, 1973; Ruohtula and Miettinen, 1975; Sharpe *et al.*, 1977). A two-compartment, bimodal elimination of methylmercury was reported for several fish species, by Burrows and Krenkel (1973), Ruohtula and Miettinen (1975), and deFreitas *et al.* (1977). The loss of methylmercury by goldfish (*Carassius auratus*), however, as reported by Sharpe *et al.* (1977), occurred as a first order, single-compartment loss. Similarly, the elimination, by the same species, of a whole-body Hg burden administered as mercuric nitrate, also occurred as a single-compartment, linear loss (Weisbart, 1973).

The loss, in this study, of around 20% of the whole body burden in the first two days is similar to the initial rapid loss reported by Burrows and Krenkel (1973), and deFreitas *et al.* (1977), of 25% of the burden. Given the short duration of the experiment and the poor fit to the descriptive model, it is inadvisable to attempt a calculation of biological half-life from these results. The long half-lives reported in the literature for methylmercury are highly dependent on observations of a long period of elimination, often in excess of 100 days. It is probable that any estimate based on the data presented here would seriously understimate the true value of the $B_{1/2}$. Burrows and Krenkel (1973) reported a $B_{1/2}$ of 38.5 days for the rapid loss of 40 % of an administered dose, and a $B_{1/2}$ of 130 days for the slow loss of the remainder. By contrast, the $B_{1/2}$ for the rapid component (12 % of the dose) was only 2 (± 0.5) days for rainbow trout, with a $B_{1/2}$ of 320 (± 46) days for the slow elimination of the remainder (Ruohtula and Miettinen,

1975). Size of fish may be highly important in determining the values of $B_{1/2}$, as demonstrated by Sharpe *et al.* (1977), who found that the $B_{1/2}$ for elimination of accumulated methylmercury was twice as long (114-118 days) for 10g goldfish (*C. auratus*), as for 5g fish (53 days).

The pattern of elimination of a whole-body burden of methylmercury has been related to the internal dynamics of this compound by Giblin and Massaro (1973), who reported a two-phase clearance following oral dosing. They reported that the slow elimination period was due to a slow elimination of mercury from muscle, relative to the other tissues. The inital, rapid loss of methylmercury was reported, by deFreitas *et al.* (1977), to coincide with the voidance time of the gastrointestinal tract, and to represent the clearance of that portion of the ingested dose of mercury not absorbed by the intestinal system. The results reported here support this hypothesis, where there is a rapid loss of methylmercury from the digestive tract tissues following i.p. dosing, which coincides approximately to the observed period of rapid loss of whole-body activity following oral dosing.

4.4.4 Comparison of results obtained by i.p. and oral administration of Hg

If the purpose of dosing fish is to study changes in specific activity with time within tissues and organs, or to study excretion of the administered substance, then administration by an intraperitoneal injection has the advantage of producing a high level of activity rapidly in the fish (Pentreath, 1975). The principal disadvantages of this method are, however:

1. The risk of accidentally discharging the dose directly into a specific organ (e.g. liver, gonad, digestive tract) or,
2. The possibility of creating experimental artifacts, i.e. producing high concentrations in tissues where, under natural uptake conditions, these would not occur.

In relation to point 1 above, in this study two fish were not considered in the results because the majority of the dose was inadvertently administered to the ovarian sac. With regard to point 2, the initial high Hg concentrations in digestive tract tissues were probably an artifact, created by the relatively very large surface area available for uptake to these tissues from the peritoneum. This is not thought to interfere seriously with the validity of the study. High concentrations could be expected initially in these tissues if the dose was orally administered via a stomach tube, as was observed in this study (and also by Giblin and Massaro, 1973), or in labelled food material (Pentreath, 1976c).

It is likely that the high percentage of the total dose in the testes of one male fish at each of 10 hours and 48 hours after dosing (see Table 4.2) is also an artifact of the administration method. The testes of these individuals were relatively very large, and provided considerable surface areas for uptake of the methylmercuric chloride. A comparison with the amount of total burden in the testes of fish receiving an oral dose (Table 4.4), or in wild fish (Chapter 3) show these values to be extremely high.

Appendix 4.1

Calculation of Tissue Mercury Concentrations from Activity Counts

To allow calculation of accurate tissue mercury concentrations, the counts recorded by the Wallac counter were related to a known activity using a calibrated machine with a ^{203}Hg internal standard. A spot of working solution was diluted to 50 ml with DW and the total activity counted on the calibrated gamma counter used to calibrate all other gamma counting machines at the MAFF Laboratory. Total activity of this solution on 19th September 1991 was 0.33703 μCi. A count on the Wallac counter of 1 ml of this solution gave 46, 269 counts in five minutes, i.e. a count of 154.23 counts.second^{-1}.ml^{-1}. This is equal to 7712 counts second^{-1} in 50 ml of solution. A factor to convert counts recorded on the Wallac to μCi activity can, therefore, be calculated:

Conversion Factor = Activity from calibrated machine/counts.second^{-1}.ml^{-1} from Wallac counter

$$= 0.33703/\ 7712 = 4.3705 \times 10^{-5}$$

Using this factor, the total activity of a tissue sample (in μCi):

$$= \text{Activity in counts (from Wallac)} \times 4.3705 \times 10^{-5}.$$

To convert the calculated activity of each sample to a ^{203}Hg concentration.(μg $^{203}Hg.g^{-1}$ of tissue), it was first necessary to correct the activity of counted samples for radioactive decay. The activity of each sample was corrected back to the date of injection, 03-09-91, using the following equation:

$$A_0 = A_t.(2^{t/t_{1/2}}),$$

where A_0 = activity in tissue at time, t=0 (3-9-91), A_t = activity at time of counting, t days after injection, and $t_{1/2}$ is the half-life of the isotope in days (46.59).

It is known from the supplier's details that the whole mass supplied contained 20.4 mg of methylmercuric chloride (CH_3HgCl), with a specific activity on 16-08-91, of 49.16 mCi.g^{-1}. By the date of injection, eighteen days later, on 03-09-91, this specific activity had decayed to $[49.16 \times (2^{18/46.6})] = 37.613$ mCi.g^{-1}. To calculate the mass (M, μg) of CH_3HgCl in a tissue sample, using the activity in μCi corrected for decay back to activity on 03-09-91:

$$M = (X / 37.613) \times 20400$$

where X is the counted activity of tissue sample in μCi (20400 is the mass of Hg in original sample, in μg). The mass of Hg in the sample is then calculated by:

Hg mass.(μg) =CH_3HgCl mass x (atomic wt. of ^{203}Hg/molecular wt. of $CH_3{}^{203}HgCl$)

$$= CH_3HgCl \text{ mass} \times (203/254.08).$$

A tissue concentration of Hg (μg $^{203}Hg.g^{-1}$ wet weight) may be calculated by dividing the mass of Hg in the sample by the wet weight of the tissue sample (in grammes).

Note: Not all of the mercury in the 20.4 mg sample of CH_3HgCl is ^{203}Hg. Therefore, the tissue concentrations calculated as described below are for Hg, not ^{203}Hg. The assumption is made that radioactive and inactive Hg behave in the same way in the physiological system, and that the ratio of stable to active isotope remains constant.

Appendix 4.2

Mean lengths and weights of eelpout dosed intraperitoneally (i.p.) with ^{203}Hg-labelled methylmercuric chloride, for each treatment group and whole experimental population

Treatment[1] Group (Hours)	Sample Size	Mean Length (mm)	standard deviation	Mean Weight (g)	standard deviation
1	4	215.8	10.01	34.40	7.760
3	3	220.0	15.62	43.67	14.123
5	4	214.2	25.49	39.58	18.483
10	4	217.5	20.63	38.36	13.260
24	4	220.0	5.66	37.39	6.711
48	3	226.3	19.55	51.38	28.104
72	4	223.2	12.82	39.12	10.988
All groups pooled	26	219.3	15.16	40.02	13.86

Note [1] Groups by sample time, hours after i.p. injection

Appendix 4.3

Calculation of Whole Body Hg Burdens from Activity Counts

The calculation described here is based on activity counts (counts per minute, cpm), obtained as described in Section 4.2.8. The following procedure was used to calculate the whole-body Hg burden of orally-dosed fish.

1. The mean value of counts per minute was calculated for each set of triplicate whole body activity measurements, and corrected for background activity by subtraction of the mean background counts.

2. A correction factor for counting geometry was applied:
Geometry correction factor, G = Activity of spot source, cpm (d)/Activity of phantom, cpm (d), where d is the day of counting.

3. The geometrically-corrected whole-body count for a fish was calculated as:

Fish Activity, cpm (geometrically-corrected, d) = Fish Activity, cpm (d) x G

4. The Fish Activity (whole-body, geometrically-corrected, d) is then normalised back to day 0 by correcting for changes in position of the spot by:
Fish Activity (geom-corr., d) x [spot activity (0) / spot activity (d, corrected for decay to day 0 using $x2^{\Delta t/t_{1/2}}$)].

5. This activity is corrected for half-life decay back to a count on day 0 using $x2^{\Delta t/t_{1/2}}$, using the appropriate values for t.

6. The day 0 Fish whole-body activity is converted to μCi using the known day 0 value of the spot source (0.2 μCi):

Fish Activity, μCi = 0.2 x (Fish activity, cpm / Spot Activity, cpm).

7. The mass of Hg in the whole body was calculated by the procedures outlined in Appendix 4.1, with appropriate values for t substituted in the half-life decay correction.

Appendix 4.4 Correction of liver mercury concentrations for liver size effects

Liver mercury concentrations were significantly and negatively correlated with liver weight in eelpout administered i.p. with ^{203}Hg-labelled methylmercuric chloride. In order, therefore, to examine the pattern of concentrations with time after administration, it was necessary to correct for the effects of liver weight. The statistically significant linear regression of mercury concentration on liver weight is described by the regression statistics:

Liver Hg concentration ($\mu g.g^{-1}$) = 17.386 - 6.481 (liver weight, g);
Pearson correlation coefficient, r = -0.51;
Coefficient of variation, r^2 = 0.26;
Two-tailed significance, P = 0.0078; n = 26;
Mean liver weight = 0.767g.

A correction factor for the liver mercury concentration was calculated:

Correction factor = (liver weight - mean liver weight) x 6.481.
Corrected liver mercury concentration = (original concentration + correction factor)

The corrected concentration showed no correlation with liver weight (r = 0.204, P = 0.317).

Chapter 5

Temporal trend monitoring of mercury in Eelpout (*Zoarces viviparus* L.), and a calculation of Environmental Capacity for mercury in the Forth Estuary

5.1 Introduction

5.1.1 Aims

This Chapter presents an analysis of temporal trends in mercury contamination of the eelpout, *Zoarces viviparus*, in the Forth Estuary, followed by a calculation of the Environmental Capacity of two sites in the Forth Estuary for mercury inputs, with regard to mercury concentrations in muscle of eelpout. Use is made of previously collected data on mercury concentrations, in combination with results presented earlier in Chapter 2.

5.1.2 Trend monitoring of marine pollutant concentrations in biota

The assessment of pollution of marine waters has been accepted as a primary interest of the International Council for Exploration of the Seas (ICES) since 1967 (Misra *et al.*, 1988). The development of baseline contaminant levels in species of fish eaten as food was selected as the highest priority task, and a Coordinated Monitoring Programme was established in 1974. The necessity of establishing whether or not contaminant levels are changing with time was identified, and has been stated to be the single-most important question regarding chemical contamination levels in fish populations (Misra *et al.*, 1988).

A large number of studies have been carried out under the auspices of ICES to determine sources of variability which might interfere with interpretation of temporal trends in contaminant levels (e.g. Lassen, 1982; Hansen *et al.*, 1982; Jensen, 1982a,b; Jensen and Cheng, 1987; Misra *et al.*, 1988; Rees and Nicholson, 1989; Nicholson *et al.*, 1991; Misra *et al.*, 1993). The ultimate aim of these studies was the development of a standard monitoring strategy which would allow comparison of contaminant levels, both geographically and temporally.

The ICES organisation planned monitoring strategies to meet three requirements:

1. To provide a continuing assurance of quality of fish and shellfish for human consumption.
2. To survey wide geographical areas on an intermittent basis.
3. To provide analysis of trends for selected pollutants in selected species from selected areas (Hansen *et al.*, 1982).

Several different strategies for contaminant trend monitoring have been explored under the direction of ICES. Monitoring of contaminant metals, including mercury, in both fish and shellfish, have been carried out successfully by statistical comparisons between years with multilinear regression (MLR) models, relating logarithms of metal levels to various biological parameters (Hansen, 1982; Hansen *et al.*, 1982; Jensen, 1982, 1983; Lassen, 1982; Jensen, 1985; Rees and Nicholson, 1985; Jensen and Cheng, 1987; Nicholson *et al.*, 1991). Analysis

of covariance (ANCOVA), and latterly multivariate analysis of covariance (MANCOVA), has also been used in the statistical analysis of time trends in contaminant levels in Canadian Atlantic Cod, *Gadus morhua* (Misra and Uthe, 1987; Misra *et al.*, 1988, Misra *et al.*, 1993). These models have been criticised as inconvenient and difficult to interpret in comparison to the MLR approach (Jensen and Cheng, 1987). A simple ANCOVA was used successfully to compare both seasonal and annual samples in this work (see Chapter 2), and was also useful in the investigation of spatial trends.

Contaminant levels in fish may vary with sex, size, age, season, physiological condition, and degree of contaminant exposure. It was shown in Chapter 2, for example, that mercury concentrations in skeletal muscle of eelpout varies with length, weight and age, and that seasonal variability also occurs, although differences between seasons were not statistically significant. In order to allow valid comparisons (e.g. geographical or spatial) of muscle mercury concentrations between samples, therefore, it is necessary to minimise or quantify variability due to all but the degree of exposure, improving the so-called "signal-to-noise ratio" (Jensen, 1982, 1983; Elliott *et al.*, 1988). As has been demonstrated previously in this study (see Chapters 2 and 3), mercury concentration in eelpout skeletal muscle, in common with most fish, increases with size (as length and weight) of the fish. This size-dependency of mercury concentrations can be minimised by adopting a length-stratification procedure, as recommended by ICES (1984), and reported in practice by Jensen and Cheng (1987), and Elliott *et al.* (1988).

A length-stratification procedure, first presented in the ICES protocol for monitoring contaminants in marine fish (ICES, 1984), has been used by the Forth River Purification Board (FRPB) since 1982 in its programme to monitor mercury levels in Forth Estuary flounder. The results of this monitoring programme are also submitted to the Joint Monitoring Programme of the Oslo and Paris Commissions (see Chapter 1). This length-stratification procedure is based on the division of fish samples by length, into equal, constant logarithmic intervals.

Elliott *et al.* (1988) suggested such a procedure, representing the length range 0 mm to 300 mm with six equal logarithmic intervals of 0.09 log units, for mercury contamination work with the flounder (*Platichthys flesus*). Elliott and co-workers demonstrated that, for comparative purposes, selection of a single length class significantly minimised size-related variability of mercury concentrations in this species, when monitoring temporal changes of mercury levels. The length-intervals into which samples were divided are as follows:

1. < 100mm
2. 100-124 mm
3. 125-154 mm
4. 155-194 mm
5. 195-239 mm
6. 240-300 mm

It was recommended that, where possible, a length class of older (i.e. longer) fish was selected, as these fish would have a mercury burden integrated over a longer time period than younger, shorter fish. This is, of course, tempered by the requirement to obtain sufficient numbers of fish to permit sensible comparisons between years. In actuality, the length classes used by the FRPB in their length stratified sampling procedure are larger than those recommended above, as the requirements for the reduction of size-related variability and sufficient sample sizes are both satisfied by selection of fish in the length class 180-230 mm (FRPB, 1987).

The evaluation of results from contaminant monitoring programmes has been aided by reference to standards or guidelines which have been developed for various contaminants by organisations like the European Community, the Joint Monitoring Group of the Oslo and Paris Commissions (JMG), and the Food and Agriculture Organisation of the United Nations (Franklin, 1987).

A series of arbitrary, purely descriptive guidelines has also been adopted by the JMG of the Oslo and Paris Commissions, to refer to the ranges of contaminant concentrations which would be expected to occur. These are based on the results submitted by all participating countries in the Joint Monitoring Programme of the JMG. The guidelines recommended by the JMG for mercury in fish, crustaceans and molluscs are described below:

Contamination Level	Fish Muscle and Crustaceans ($mg.kg^{-1}$ wet weight)	Molluscs ($mg.kg^{-1}$ dry weight)
Lower	< 0.1	< 0.6
Medium	0.1-0.3	0.6-1.0
Upper	>0.3	>1.0

The levels above do not necessarily imply any risk to human health, or to the environment, but are used purely to indicate a possible need for action by the regulatory authorities, e.g. mercury levels in the "upper" category would require a much higher priority to establish the source(s) of contamination than if levels were in the "lower" range (Franklin, 1987).

The development of the EQS of 0.3 mg/kg wet weight for mercury in skeletal muscle of fish was first described by Preston and Portmann (1981) from MAFF, who established the EQS for commercially-fished flatfish from Liverpool Bay. They selected this value because it was the lowest value, accurately detectable by analysis, above an ambient background value of 0.2 mg/kg in uncontaminated fish. There was also a public health element involved in the establishment of the EQS value, as it is significantly lower than the 1 mg/kg estimated to cause physical symptoms in 5% of the consumer population. The 0.3 mg/kg limit was later to be confirmed as acceptable based on the World Health Organisation recommended provisional

tolerable weekly intake (PTWI) of 0.3 mg mercury, in combination with a local dietary study of people with a high consumption rate of the fish in question, and estimates of mercury uptake rates to blood of consumers (GESAMP, 1986b).

5.1.3 The Environmental Capacity concept

The Environmental Capacity of an environment (also known as receiving, absorptive or assimilative capacity) is defined as a property of the environment, a measurement of its ability to accommodate a particular activity, or rate of activity, such as the discharge of contaminants, without unacceptable impact (GESAMP, 1986b).

The Environmental Capacity concept is based on three premises, listed by Pravdic (1985) as:

1. A certain level of certain contaminants may not produce any undesirable effect on the marine environment and its various uses.
2. Each environment has a finite capacity to accommodate some wastes without unacceptable consequences.
3. Such capacity can be quantified, apportioned to a certain activity, and utilised.

The question as to what constitutes an unacceptable or undesirable impact or effect of pollutants on the marine biosphere must be considered separately for each individual case (GESAMP, 1986b; Stebbing, 1992). The vital question for any individual body of water receiving waste materials is: which contaminants can be assimilated and in what quantities ? (Stebbing, 1992). It is important to note that some proponents of the concept accept that the capacity of a body of water could be zero for the most toxic substances (Portmann and Lloyd, 1986).

Krom (1986) identified the initial attempts to calculate values of Environmental Capacity in attempts in the 1960's to estimate the transport of radio-isotopes, discharged to the marine environment, along so-called "critical pathways" to man. The concept of Environmental Capacity for non-radioactive contaminants was first applied in the UK in a study of the control of mercury inputs to UK coastal waters (Preston and Portmann, 1981). More recently, the Environmental Capacity of Haifa Bay, on the Mediterranean coast of Israel was estimated with respect to inputs of mercury from a chloralkali plant (Krom *et al.*, 1990).

A three-stage methodology was suggested by GESAMP (1986b) for the assessment of Environmental Capacity of the marine environment, involving a planning stage, a preliminary scientific assessment stage, and a monitoring and adaptation stage (see Figure 5.1). The GESAMP scheme recognises the inputs from the scientific and socioeconomic fields as two "interactive and complementary activities in decision making in integral, environmentally-compatible, development planning" (GESAMP, 1986b).

Initial socio-economic goals are the trigger for the desired activity. Scientific assessment is

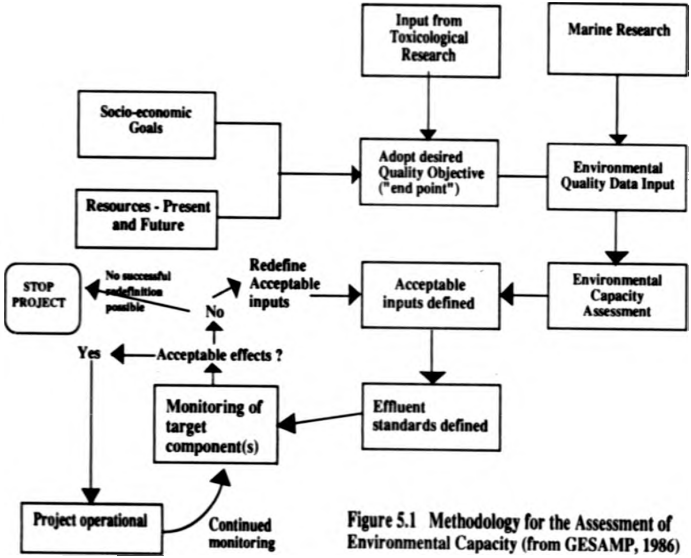


Figure 5.1 Methodology for the Assessment of Environmental Capacity (from GESAMP, 1986)

required to identify the present and future resources. In the final and most important stage, monitoring and adaptation, the defined environmental quality objective (EQO) ,the so-called "end-point" of the exercise (Krom *et al.*, 1990)) is translated into a maximum-allowable input of the contaminant in question (GESAMP, 1986b). Using environmental data and the defined end-points, based on some water quality or other criteria, the assessment of Environmental Capacity, and apportionment of a fraction of it for the development in question, enables the establishment of maximum-allowable inputs (Preston and Portmann, 1981; GESAMP, 1986b).

A monitoring stage tests whether the Environmental Capacity is balanced, allowing the operation to proceed. Should monitoring reveal that the Environmental Capacity has been exceeded, then a revision of the project would be required, with a tightening of, for example, effluent treatment processes. In the absence of an economically or technologically acceptable improvement or alternative, environmental considerations would be likely to close down the project (see Fig. 5.1).

5.1.4 Environmental Capacity of the Forth Estuary with regard to mercury inputs

As described in Chapter 2, following the discharge of mercury into the Forth Estuary for several decades by ICI at Grangemouth, by December 1992, the mercury input from this source had fallen to close to zero. In this chapter, the Environmental Capacity of the Forth Estuary is calculated *a posteriori*, with respect to mercury inputs. Acceptability of the impact of the mercury inputs has been defined as the meeting of the conditions of the Environmental Quality Standard (EQS) for mercury in fish, set by the European Community Directive 84/156/EEC (European Communities, 1984), of a mean mercury concentration in muscle of a representative sample of fish caught locally not in excess of 0.3 mg.kg^{-1} wet weight.

The eelpout, *Zoarces viviparus* (L.), a resident estuarine fish species found commonly in the Forth Estuary, has many of the characteristics defined by Phillips (1980) as important for an indicator species for metals in the aquatic environment. These were described in Chapter 2. Eelpout also had historical importance as a food fish in the Forth Estuary (Day, 1884), and has been caught more recently as a food fish in the Baltic region (Soin, 1968). It thus provides the potential for a risk to human health as a pathway for mercury to man, and can be regarded as a representative fish species for the Forth Estuary. In Chapter 2, the spatial, temporal and biological variability of mercury concentrations in tissues of eelpout from the Forth Estuary and Firth of Forth were explored and quantified. This allows the elimination of variation in muscle mercury due to factors other than the degree of environmental exposure, to permit the selection, for comparison, of fish representing similar environmental and biological conditions.

5.2 Materials and Methods

As has been shown in Chapter 2, there is generally no significant size-related difference of muscle mercury concentrations between the sexes for the eelpout, *Zoarces viviparus*. This potential source of variation can be ignored, therefore, and samples of mixed sex were used. Although no significant difference was found in the rate of increase of mercury concentration of muscle with length between seasons, there was an annually repeated pattern of variation, with highest value in Spring of each year. It was decided, therefore, to select fish from Spring in each year, in order to allow comparisons between years on a "worst case" basis. This was possible for fish collected from 1990 to 1992. Fish from other years were not selected on the basis of month.

It was decided to select a single length interval, of fish of total length 155-194 mm, to compare mercury concentrations between sites and between years. This length-class of eelpout was chosen for four reasons:

1. There was no significant correlation between length and mercury concentration in the restricted-length samples (Pearson correlations, results of tests not reproduced).

2. This length class provided enough individuals on most occasions to permit sensible comparisons between samples. As a study of a "worst-possible scenario" with regard to mercury contamination, it might be more appropriate to select fish of greater lengths, with higher mercury concentrations. Insufficient numbers of larger fish were obtained on many sampling occasions, however, meaning that most years would be under-represented or unrepresented if a study was based on such fish. Use of this length class can be viewed, therefore, as a compromise between using the fish with the highest muscle mercury levels, and obtaining sufficient individuals to permit the study to be undertaken.

3. This length class is approximately equivalent to fish of year-class 3 to year-class 4. Fish of this age may be expected to reflect the differences between ambient environmental mercury levels of sites to a greater degree than younger fish, which have experienced shorter exposure times.

4. Fish of this size would probably be at the lower end of the size range exploited by a commercial fishery, or eaten by amateur anglers. This provides any comparison made, between the mercury concentration of eelpout muscle and the European Community EQS for mercury in fish muscle, with greater relevance for contaminant monitoring as it is based on a hypothetical threat to human health.

Archival records were kindly made available by the Forth River Purification Board of analyses

of eelpout skeletal muscle samples for total mercury concentrations during the years 1978 to 1988 inclusive. Some of the results, for 1983, have been published previously (Elliott and Griffiths, 1986), but the majority are unpublished (Dr. S. Hull, FRPB, pers. comm.). The values of eelpout skeletal muscle mercury concentrations in this chapter representing the years 1989 to 1992 have been presented previously in this thesis (see Chapters 2 and 3). The dry weight mercury concentrations from this study, covering the years 1989 to 1992, were converted to a wet weight basis using the mean water content of eelpout skeletal muscle given in Chapter 2 of this study. This permitted the comparison of these results with those from the earlier studies, and with the EQS value for mercury in fish muscle. Values of mercury inputs to the estuary were taken from the data already presented in Chapter 1.

Statistical significance of the variability of mercury concentrations between-sites within-years, and within-sites between-years, was tested by analysis of variance (ANOVA: Zar, 1984). A difference between samples was accepted as significant at the 5% level ($P< 0.05$). Where a significant difference was observed between more than two sites, the significant difference(s) was identified using a Scheffe Multiple Comparison (Zar, 1984), with significance accepted at the 5% level ($P<0.05$). Correlation coefficients were compared using the chi-squared (χ^2) statistic (Zar, 1984).

5.3 Results

5.3.1 Temporal trends of total mercury concentrations in eelpout tissues in the Estuary and Firth of Forth, 1978-1992.

Fig. 5.2 shows the annual variation, from 1978 to 1992, of the mean mercury concentration (with standard error) of eelpout skeletal muscle at the sites Longannet and Port Edgar in the Forth Estuary, and at Kingstone Hudds in the Firth of Forth. The dotted line in each figure indicates the European Community Environmental Quality Standard (EQS) for mercury in fish skeletal muscle. The mean total lengths and fresh weights, and sample sizes, of the fish in each annual sample are given for each site in Table 5.1.

The annual mean mercury concentration peaked in samples from LO in 1983 (Fig. 5.2a), in which year the mean value exceeded the EQS value. The mean mercury concentration fell sharply thereafter until 1988, when a small increase was again observed over the following three years. The mean mercury concentration in samples from PE also peaked in 1983, although the EQS value was not exceeded in any year sampled during the period (Fig. 5.2b). As with samples from LO, a small increase in mean mercury concentration was observed at PE from 1988 to 1991, although the mean value at PE fell again between 1991 and 1992.

Results from the Firth of Forth were only available for the years 1978, 1987,1988,1990 and 1991 (Fig. 5.2c). As no results are available for 1983, it is not possible to say if there was a peak in mercury levels, as observed at the other two sites. It can be seen, however, that in all years where results were available, the mean mercury concentration in skeletal muscle of eelpout from the Firth of Forth was always lower than in the other two sites, and never exceeded the EQS value.

In summary, after a peak in 1983, an overall decreasing trend with time was observed for mean mercury concentrations in eelpout skeletal muscle in samples collected from two sites within the Forth Estuary. The degree of contamination was greater in all years at LO, the upstream site, compared to PE at the downstream end of the estuary. At only one site in one year, LO in 1983, did the mean mercury concentration exceed the European Community EQS for mercury in fish muscle (see above). No clear trend was observed with time, in a limited number of samples from the Firth of Forth, although mean mercury concentrations were always lower than those recorded in the samples from the Forth Estuary sites.

(a) Temporal Variability Within Sites

Annual mean mercury concentrations were compared within-sites by analysis of variance (ANOVA: Zar, 1984). Temporal variability between-years was observed in mean muscle mercury concentrations at all three sites. Between-year ANOVA results are summarised for all

Table 5.1 Annual mean length (mm) and weight (g) of eelpout in the size class 155-194 mm from the Forth Estuary and Firth of Forth

Site	Year	Sample Size	Mean Length	std error	Mean Weight	std error
Longannet	1978	7	177.1	3.59	nr	-
	1983	7	177.1	1.84	27.8	1.84
	1984	6	172.0	3.82	17.8	1.65
	1985	7	171.4	4.61	18.1	2.69
	1988	1	180.0	-	20.6	-
	1989	2	177.5	1.41	25.6	6.44
	1990	35	170.1	0.25	17.3	0.68
	1991	3	183.7	1.33	20.6	0.32
Port Edgar	1978	2	190.0	0.00	nr	-
	1981	1	190.0	-	nr	-
	1983	14	177.6	2.59	24.3	1.97
	1985	6	172.7	2.27	19.4	1.69
	1988	3	161.7	1.67	17.4	0.98
	1989	4	177.5	0.75	27.4	3.77
	1990	22	171.5	2.49	18.4	0.78
	1991	1	193.0	-	24.2	-
	1992	4	180.2	5.10	19.0	3.2
Kingstone Huddle	1978	2	185.0	5.00	nr	-
	1987	11	176.8	3.46	22.7	1.40
	1988	4	168.8	7.74	20.1	4.17
	1990	12	173.2	3.30	22.7	0.91
	1991	4	180.5	6.36	16.8	1.59

Notes. nr, value not recorded

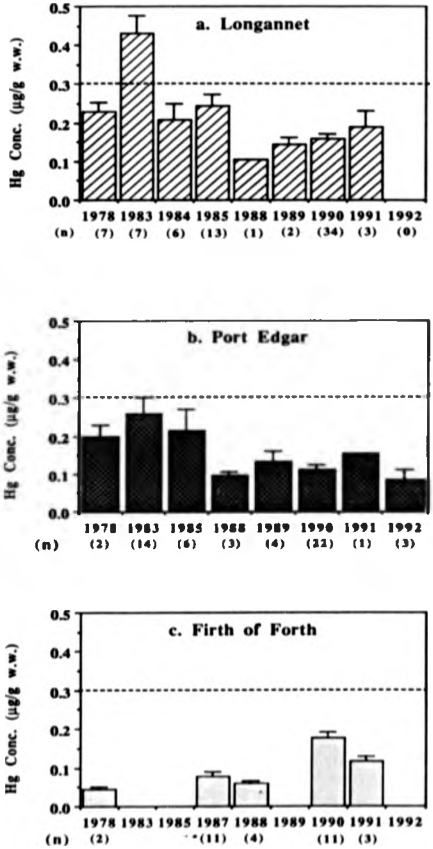


Fig. 5.2 Annual variation of mean mercury concentration(+ sem) in eelpout muscle (restricted length class 155-194 mm.) at sites in the Estuary (a, b), and Firth of Forth (c), for the period 1978-1992. Dotted line shows EC EQS for mercury in fish muscle.

three sites in the footnote of Table 5.2. Statistically-significant differences were observed between-years in all three sites.

The mean mercury concentration at LO in 1983 was statistically-significantly higher (Scheffe tests, $P<0.05$) than in 1978, 1984, 1985 and 1990. The mean mercury concentration in 1985 was also significantly higher than in 1990. The only statistically-significant difference between years at PE lay between 1983 and 1990. Although subject to less variability during the study period than the other two sites, the mean muscle mercury concentration in Firth of Forth eelpout showed significant differences between years. The difference between 1987 and 1990 means was statistically-significant (Scheffe F-test, $P<0.05$), as was the difference between 1990 and 1991.

(b) Spatial Variability Within Years

Mean mercury concentrations were compared between sites within years by analysis of variance (ANOVA: Zar, 1984). Comparison of sites was possible in 1983, 1985, 1990 and 1991.

1983: A significant difference was observed between Longannet and Port Edgar ($F=6.3414$, between groups degrees of freedom=1, within groups df=19, $P=0.0212$).

1985: There was no significant difference between the same sites ($F=1.409$, between groups df =1, within groups df =11, $P=0.2602$).

1990: Differences between mean mercury concentrations of Longannet, Port Edgar and Kingstone Hudds (in the Firth of Forth) were statistically significant ($F=17.397$, between groups degrees of freedom=2, within groups d.f.=52, $P=0.0001$). Scheffe multiple comparison (S) tests revealed that differences of mean mercury concentrations were significantly between all three sites (LO vs PE: Scheffe $S=4.706$, $P<0.05$; LO vs KH: Scheffe $S=17.220$, $P<0.05$; PE vs KH: $S=5.476$, $P<0.05$). The difference between LO and PE was not significant at the 1% level ($P>0.01$).

1991: The difference between LO and KH was statistically-significant ($F=6.833$, between groups degrees of freedom=1, within groups d.f.=7, $P=0.0347$).

Table 5.2 Between-Year comparisons (Scheffe S-test)[1] of mean skeletal muscle mercury concentrations for eelpout from sites in the Forth Estuary and Firth of Forth

Longannet				
Year	1983	1984	1985	1990
1978	4.8814[2]	0.0513	0.263	0.906
(significance)	(*)	(ns)	(ns)	(ns)
1983		5.4386	2.8169	14.2412
(sig.)		(*)	(*)	(*)
1984			0.5172	0.3692
(sig.)			(ns)	(ns)
1985				2.5994
(sig.)				(*)

Port Edgar			
Year	1985	1989	1990
1983	0.2322[2]	1.4356	5.098
(significance)	(ns)	(ns)	(*)
1985		0.4375	1.3584
(sig.)		(ns)	(ns)
1989			0.029
(sig.)			(ns)

Kingstone Hudds, Firth of Forth			
Year	1988	1990	1991
1987	0.7338[2]	5.5057	0.4127
(significance)	(ns)	(*)	(ns)
1988		0.6892	1.5321
(sig.)		(ns)	(ns)
1990			5.5045
(sig.)			(*)

Notes. [1] All within site comparisons of annual means by analysis of variance revealed a significant difference between years: (Longannet: overall ANOVA: F= 15.259, between groups degrees of freedom=4, within group d.f.=56, P= 0.0001); Port Edgar: overall ANOVA: F= 5.599, between groups d.f.=3, within group d.f.=42, P= 0.0025; Firth of Forth: overall ANOVA: F= 8.2317, between groups d.f.=3, within group d.f.=27, P= 0.0002).

[2] Values of Scheffe S-statistic given, with significance of statistic: * = P<0.05, ns = no significant difference

5.3.2 An estimation of the Environmental Capacity of the Forth Estuary for mercury inputs with respect to mercury in eelpout skeletal muscle

(a) Correlation between mercury input to Forth tidal waters and eelpout muscle mercury concentrations at two sites in the Forth estuary

The mercury levels measured in eelpout tissues during the period 1978 to 1992, in fish of restricted length class 155-194 mm., were related to known inputs of mercury to Forth tidal waters during the same period by calculation of the Pearson correlation coefficient, r (Zar, 1984). The correlation between mercury concentrations and mercury input was considered for the mercury input in the year before the year of capture (Y_1), the mean mercury input in the two years (Y_2), and three years (Y_3) before the year of capture. Table 5.3 summarizes the linear correlation results relating eelpout skeletal muscle mercury concentration to mercury inputs into Forth tidal waters. The data for annual total mercury inputs to the Forth were described in Chapter 1.

Positive linear correlations of muscle mercury concentrations with mercury inputs were statistically-significant for both Longannet and Port Edgar, whether inputs were considered as values Y_1, Y_2 or Y_3, as above (see Table 5.3). There were differences in the degree of correlation, however, between the different input values and mercury concentration, especially for fish from Longannet.

The mercury concentrations in muscle of Longannet fish showed the highest correlation with the mean mercury input over the two years previous to the year of capture (Y_2), and correlation was lowest with mercury inputs over the previous three years (Y_3). Statistical similarity of the three correlation coefficients was tested, using the chi-square (χ^2) statistic (Zar, 1984), and the differences were found to be highly non-significant (χ^2 =0.798, P>0.50). For Port Edgar fish, the degree of correlation was very similar, whether mercury input was considered as values Y_1, Y_2 or Y_3. The differences between the correlation coefficients also tested highly non-significant for the Port Edgar samples (χ^2 =0.005, P>0.50). It was decided to calculate the Environmental Capacity of the two sites based on the mean mercury inputs over two years (Y_2), as this value gave the highest correlation with muscle mercury concentrations at Longannet.

Muscle mercury concentration in eelpout skeletal muscle increases in a linear fashion with increasing values of mercury input, taken as the mean of the two years prior to capture. This relationship is shown for Longannet in Figure 5.3a, and Port Edgar in Figure 5.3b. Regression statistics describing the relationship at each site are given in Table 5.4. The slope (b) of the best-fit line for Longannet is steeper than that for Port Edgar, meaning that the rate of increase of muscle mercury concentrations with mercury inputs is higher at the former site.

Table 5.3 Pearson correlation statistics for eelpout muscle mercury concentrations with mercury inputs for two sites in the Forth Estuary

Site	Sample Size	Mercury Input[1]	Pearson Correlation Coefficient, r	Value of F-statistic	Significance (P) of Correlation[2]
Longannet	68	Y_1	0.6121	39.540	0.0001
"	"	Y_2	0.6829	57.663	0.0001
"	"	Y_3	0.5973	36.602	0.0001
Port Edgar	55	Y_1	0.5299	20.693	0.0001
"	"	Y_2	0.5195	19.586	0.0001
"	"	Y_3	0.5242	20.077	0.0001

Notes. [1]. The mercury input values Y_1, Y_2 and Y_3 are fully explained in the text

[2]. P calculated from two-tailed F-test (after Zar, 1984)

Table 5.4 Linear regression statistics[1] relating muscle mercury concentration of Forth Estuary eelpout to mean annual mercury input to Forth tidal waters over the two years prior to capture (Y_3)

Site	Sample Size	a	b	Value of t statistic	Significance (P) of regression[2]
Longannet	68	0.1142	0.0001	7.5936	0.0001
Port Edgar	55	0.1071	0.00004	4.4527	0.0001

Note. [1]. The linear regression equation takes the form :
Muscle mercury concentration ($\mu g.g^{-1}$) = a + b* (mean mercury input, Y_3, tonnes/year)
[2]. P calculated from two-tailed t-test (after Zar, 1984)

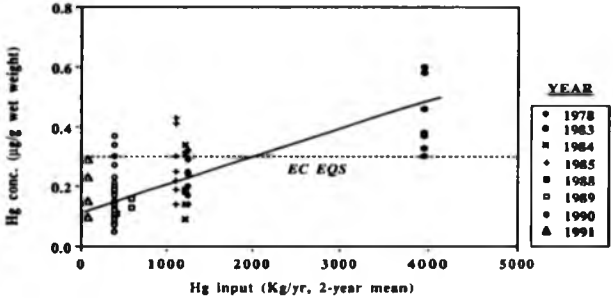


Fig. 5.3a Relationship between mercury input on eelpout skeletal muscle mercury concentrations at Longannet, Forth Estuary.

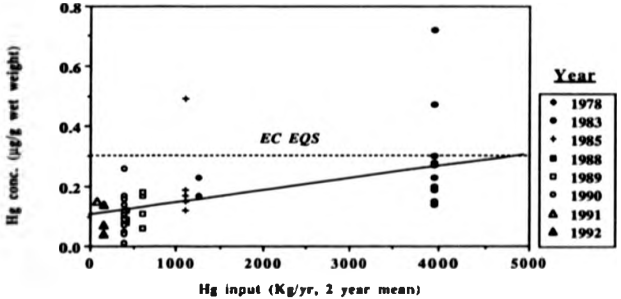


Fig. 5.3b Relationship between Hg input and eelpout skeletal muscle mercury concentrations at Port Edgar, Forth Estuary.

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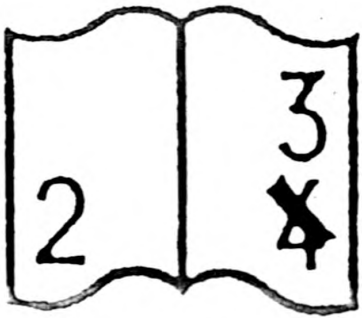

(b) Calculation of the Environmental Capacity of the Forth Estuary for mercury inputs with respect to fish muscle mercury concentrations

The dotted line on Figures 5.3a and 5.3b represents the maximum acceptable mercury concentration in fish muscle, of 0.3 $\mu g.g^{-1}$ wet weight, established as an Environmental Quality Standard by the European Community (see 5.1 Introduction). This maximum acceptable mercury concentration in muscle was related to an annual mercury input using the regression equations ($y = a + bx$) described in Table 5.3. The regression equation for each site was re-arranged such that : $x = (y - a) / b$.

By substituting 0.3 for y, and appropriate values of the regression equation constants a and b from Table 5.3, an inverse prediction was carried out. This predicted the value of two-year mean mercury input to the estuary (x) at which, according to the regression equation relating the two variables, the muscle mercury concentration would equal the EQS value of 0.3 $\mu g.g^{-1}$. At Longannet the value of x was 1858 kg $Hg.year^{-1}$, while at Port Edgar the value was much higher, at 4822.5 kg $Hg.year^{-1}$.

5.4 Discussion

5.4.1 Trend monitoring of mercury levels in eelpout

It was recommended by Jensen (1982) that annual trends in metal concentrations of fish tissues required to be examined over relatively long time periods. Indeed, he suggested that results from up to 10 years might be necessary to allow the results to be interpreted in terms of natural variability between years, which could be considerable. Such a dataset has now been collected for the eelpout in the Forth Estuary, covering the period 1978 to 1992. This is the longest time period covered by a bioaccumulation study of mercury in the Forth, although gaps exist in the time series.

It is evident from the results presented here that there has been a clear trend of decreasing mercury concentrations in eelpout skeletal muscle since the early 1980's. This trend has been particularly marked in fish from the Longannet site (LO), closest to the ICI discharge of mercury. This pattern is similar to those presented in Chapter 2, for flounder (*Platichthys flesus*), mussel (*Mytilus edulis*), and bladder wrack (*Fucus vesiculosus*) from the Forth Estuary over the same time period. Mercury inputs to the Forth Estuary were greatly reduced over this period, as described in Chapter 2. Mean mercury levels in skeletal muscle of eelpout from LO were clearly well above the background concentrations measured in eelpout from the Firth of Forth in the early 1980's. A similar decrease in mercury levels of eelpout was reported from the Ems Estuary in the Netherlands, a trend which was correlated with reduced inputs of mercury to the estuary (Essink, 1980, 1985, 1988).

Although no causal link has been established between mercury inputs and fish tissue levels in the Forth Estuary, the results of the FRPB monitoring programmes for mercury in flounder, mussel and bladder wrack, in addition to the results presented here for eelpout, provide strong circumstancial evidence that reductions in mercury inputs to the Forth Estuary over the 1980's have led to a considerable reduction in the mercury burden of the biota of the estuary. A trend of decreasing environmental mercury levels is not evident from analyses of water and sediment mercury concentrations over the same period. It is possible that a temporal trend is masked by the high natural variability of mercury concentrations in these components.

Unlike the measurement of mercury levels in flounder muscle, the analysis of mercury levels in eelpout muscle is not a statutory requirement for the FRPB. It is recommended, however, that eelpout should continue to be taken for mercury analysis each year, from the two sites, LO and PE, studied here. In addition, eelpout should also be collected from the Firth of Forth as a guide to background concentrations. Up to 10 individuals from each site should be collected in Spring, of the length range 155-194 mm. Such a study would be useful as the discharge of mercury from ICI has practically ceased, and it might be expected that mercury levels in the

biota would continue to fall. There is, however, as a result of decades of mercury discharges, a considerable legacy of mercury contamination in the estuary, mostly in the sediments and suspended material,(Elliott and Griffiths, 1986). It is not possible to predict how quickly this mercury will become unavailable to the biota. It has been shown here that, even in the Firth of Forth, fairly distant from the major input of mercury to the Forth, there are significant differences between years in the mercury concentrations of eelpout skeletal muscle. The existing dataset of mercury in eelpout, therefore, provides a reference for interpreting future fluctuations in the bioavailability of mercury in the Forth Estuary.

5.4.2 Estimation of the Environmental Capacity of the Forth Estuary for inputs of mercury

The estimation of a much lower value of Environmental Capacity for mercury inputs at Longannet compared to Port Edgar is not unexpected. This implies that, for the same input of mercury, higher mercury levels will accumulate in Longannet fish than in Port Edgar. The former site is much closer to the principal point-source input of mercury, and both environmental and biota mercury levels have historically been higher at this site than at Port Edgar (Elliott and Griffiths, 1986).

Although the simple empirical relationships calculated are purely correlative, and causality cannot be assumed, they can be used for predictive purposes. The value of 1858 kg Hg per year, calculated here as the Environmental Capacity at Longannet for mercury inputs, would be equivalent to a daily input of 5.1 kg of mercury. The conditions of the consent to discharge issued by the Forth River Purification Board permit the discharge of about 4 kg Hg per day from Grangemouth (Davies, 1987), although in 1986 the mean daily discharge was reduced to below 1 kg per day. Thus, even if the maximum permitted amount of mercury were discharged each day, it is predicted by this linear model that the mean levels of mercury in eelpout skeletal muscle at Longannet should not exceed the EQS value. As this calculation of Environmental Capacity has been carried out *a posteriori* , after mercury discharges have been greatly reduced from unacceptable levels, it provides a useful confirmation of this prediction. Mean mercury levels in the fish at Longannet did indeed only exceed the EQS value when mercury inputs to the Forth were unacceptably high. It was pointed out by Krom and Cohen (1991) that almost all estimates of Environmental Capacity in the literature have been carried out *a posteriori*, as here. The principal reason for this seems to be the extreme complexity of predictive models required to relate the input flux to the concentration in the target species. Most estimates of Environmental Capacity, therefore, involve an existing pollutant input, and address the question, what will happen if there is a change (generally an increase) in existing inputs to the area. Krom and Cohen, in reviewing several published examples of Evironmental Capacity estimation, point out that the input has often changed by a considerable amount by the time the estimation is made, and environmental consequences have been studied. The assumption is also

generally made that the existing (often simple empirical) relationship between the pollutant input and concentration at the target will remain valid. The possible problem suggested, that of a delayed abrupt response, has been avoided in this study, by relating the concentration to several measures of input over different time periods. It has been suggested that the above constraints place severe limits on the usefulness of the Environmental Capacity concept (Krom and Cohen, 1991).

The simple linear relationships described for the two sites in the Forth Estuary, between mercury inputs and eelpout muscle mercury levels, are highly dependent on a single year with very high mercury inputs. Nevertheless, the relationship, although a crude simplification of the natural situation, is significant. The results highlight the effect of site selection on the estimation of environmental capacity for mercury. Selection of a sampling site further from the disharge point would imply that the estuary could take higher inputs of mercury before unacceptable conditions occured, in thiscase, the accumulation of mercury in muscle of fish to unacceptably high levels. Selection of a restricted length range of shorter fish would also suggest that the discharge of higher mercury inputs were possible. It is necessary, therefore, when estimating Environmental Capacity, to interpret the results in terms of both the sampling site and the selection criteria for the fish used for the exercise.

This study provides confirmation that, with a simple definition of acceptability of impact, such as the meeting of the EQS for mercury in fish muscle, a meaningful estimation of the Environmental Capacity of an estuarine area for a single persistent contaminant is possible. This confirms the findings of Preston and Portmann (1981), who first demonstrated the practicality of the approach, using accumulation of mercury in flounders to estimate the Environmental Capacity of UK coastal sites to receive mercury inputs. The application of the concept to mercury inputs to Haifa Bay, Israel, was not so successful, however (Krom *et al.*, 1991), the authors having difficulty in defining a suitable level of acceptable effect. The calculation of Environmental Capacity was also unable to clarify whether the pollution abatement measures installed had led to a sufficient clean up of the area.

Acknowledgement of the Environmental Capacity concept promotes acceptance of the distinction between contamination and pollution of the marine environment (Portmann and Lloyd, 1986). The prescribing of a certain value of Environmental Capacity, for a substance or activity, to an environment is not intended as a polluter's charter, but rather as a way of setting an absolute maximum value, the exceeding of which will lead to unacceptable environmental or human health impacts (Stebbing, 1992). The conflicting requirements of environmental protection and waste disposal depend on the subjective assessment of acceptability. This is perhaps where future debate on the concept should be focused.

Chapter 6

General Discussion

6.1 General Discussion

It is clear that the presence of mercury in marine food chains leading to humans has led to a considerable number of human deaths, particularly during the two most serious and well-documented incidents at Minimata and Niigata in Japan (Takizawa, 1979). The guideline limits established for mercury concentrations in seafood products following these incidents are based on observations by the World Health Organisation, that certain social and ethnic groups with high rates of seafood consumption, may be at risk from chronic methylmercury poisoning (GESAMP, 1986a). Such risks can be calculated for a proportion of the population, for mercury concentrations in the upper range encountered in marine food products. The desire to avoid repetition of epidemic incidents like Minimata, and chronic methylmercury poisoning from lower level, longer term exposure from dietary sources, has led to the evolution of many national and international monitoring programmes for mercury in the marine environment. Organisations such as the International Council for the Exploration of the Seas (ICES) have co-ordinated the development, quality assurance and reporting of such programmes (ICES, 1984). Results of some of these programmes have been described in Chapters 1 and 2.

In a recent summary statement on the state of the marine environment, GESAMP (1990) concluded that trace metals like mercury, which occur both naturally and as a result of human activities, are now of less concern than in previous years, except where high levels occur near contamination sources. GESAMP recommended, however, that their discharge should be kept under surveillance and monitoring should be continued to ensure compliance with current acceptable limits.

Recommendations have been made by several sources, both Governmental (e.g. FAO: Bernhard, 1976) and academic (Phillips, 1977, 1980; Bryan *et al.*, 1985), in relation to the selection of specific biota as monitors of mercury bioaccumulation. There has been much emphasis, by these and other workers (e.g. Elliott *et al.*, 1988), on the need for quantification of variability in tissue mercury concentrations from sources other than the degree of environmental mercury exposure. Indeed, the monitoring programmes which contribute to the main national and international monitoring programmes for mercury do take account of these recommendations, and samples are collected in the same season each year. Sampling site and organism size are normally also standardised between years (e.g. the monitoring programe for mercury in mussels and flounders, carried out annually by the Forth River Purification Board, and summarised in Chapter 2). Despite this, many workers continue to report mercury concentrations in tissues of fish species, and interpret these results in terms of degree of environmental exposure to mercury, without consideration of the effects of other sources of variability. The failure of many workers to consider differences between the sexes as such a source of variability was highlighted in Chapter 2, as was the paucity of studies in the literature concerning the seasonal variability of mercury concentrations.

While seasonal patterns of variation of skeletal muscle mercury concentrations were not statistically significant in this study, they were negatively correlated to fish condition. It is possible that fish species which undergo greater seasonal variation in condition than eelpout could show significant seasonal variation of skeletal muscle mercury concentrations, without significant seasonal variations in the bioavailability of mercury. Seasonal variation of mercury concentrations in liver were, however, statistically significant. Without some knowledge of seasonal patterns of liver growth and total mercury burdens, this variability could easily be misinterpreted as the result of changes in mercury exposure. Similar variability was also observed for testes tissue. Hence, the necessity is clear for the eliminating or quantifying of variation, due to factors such as sex or seasonal changes in organ or tissue size, before embarking on use of any particular tissues of a species for mercury monitoring.

Such an assessment, of the variability of mercury concentrations related to biological, temporal and spatial factors, was undertaken here for tissues of the eelpout from sites in the Forth Estuary and Firth of Forth (and for a sample of eelpout from the Firth of Clyde). The use of this species for mercury bioaccumulation monitoring in the Forth Estuary was suggested originally by Elliott and Griffiths (1986), following the observation that eelpout from sites of different environmental mercury levels had mercury concentrations in skeletal muscle which correlated aproximately with these levels. A key requirement for an indicator organism for mercury in a particular environment is that there should be a simple correlation between mercury concentrations in the tissue studied, and those in the water or sediment of its environment (Phillips, 1977). Murray and Portmann (1982) addressed the problem of the large variability of mercury concentrations in water and sediment, and the difficulty of correlating these levels with those of indicator species. They highlighted the need to establish the relationships between inputs, levels of exposure and accumulation and trends in contaminant concentrations in fish and shellfish. This has been attempted in Chapter 5, with an analysis of temporal trends, and a description of a linear relationship between mercury inputs to the Forth, and mercury concentrations in the skeletal muscle of eelpout. The elimination of sex and season as a source of variation of mercury levels, as described in Chapter 2, allowed this study to be conducted. Variability due to size was easily controlled by the selection of fish from a restricted length class.

In relation to the need for studies of accumulation highlighted by Murray and Portmann (1982), there is also a need for more laboratory based studies, for example, on the causes of variability in tissue mass in relation to seasonally varying factors such as water temperature. Useful lines of enquiry might address more of the causes of the large natural variability of mercury burden between individual fish from the same environment. The laboratory study of methylmercury dynamics in eelpout presented in Chapter 4, although very limited in scope, did provide some useful insights into aspects of mercury accumulation by this species, which confirmed assumptions in previous chapters, based on correlative relationships. Of particular note was the

very limited study of methylmercury transfer between maternal tissues and those of the intraovarian brood, and the observation that a portion of the methylmercury administered to the intraperitoneal cavity is tranferred to the ovarian fluid. This provided support to the hypothesis that mercury transfer between the mother and her brood occurs, at least in later stages of brood development, via the brood's consumption of cellular material in the fluid.

Although trends of mercury inputs and environmental mercury levels in UK coastal waters, such as the Forth Estuary, are downward, there is clearly no room for complacency on the global scale. Examples were presented in Chapter 1 of many mercury contaminated aquatic areas worldwide. The continuing widespread use of mercury for numerous industrial and agricultural processes means that discharges of mercury to the marine enviroment are likely to continue for the foreseeable future. In addition, geological 'hot spots' of naturally high environmental mercury levels (such as the 'mercury anomaly' of the Mediterranean), and the naturally high mercury levels in large and long-lived fish, even from areas without known mercury contamination, mean that certain key social and ethnic groups, such as fishermen and their families, are at a continuing risk of chronic methylmercury poisoning from marine dietary sources. It is to be hoped that the world never again has to face another epidemic of acute methylmercury poisoning such as those at Minimata and Niigata. The continuation of monitoring programmes for mercury in fish and shellfish, particularly if targeted in key areas and on key food species, provides one means of ensuring this. It is hoped that the work presented in this thesis will contribute to the accuracy and effectiveness of such programmes.

Chapter 7

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