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Temporal variation of total mercury concentrations and burdens in the liver of eelpout *Zoarces viviparus* from the Forth Estuary, Scotland: implications for mercury biomonitoring

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ABSTRACT: The utility of fishes as biomonitors for mercury in aquatic environments is widely recognised, with skeletal muscle as the tissue normally chosen for most monitoring programmes. The liver may also be a suitable candidate tissue for monitoring, as it concentrates many pollutants to higher levels than muscle, and is closely involved with processes of metal dynamics, storage, and detoxification. Little consideration has been given previously to the validation of the use of liver for mercury biomonitoring, with regard to quantifying natural temporal variation. This study considered such variation in the eelpout *Zoarces viviparus* L., a resident estuarine fish species collected from a subtidal site near a formerly major industrial mercury discharge in the Forth Estuary, eastern Scotland. Liver and skeletal muscle mercury concentrations, liver weight, and body size variables were measured for 196 individual eelpout in seven 3-month periods. Liver Somatic Index (LSI) was significantly higher, and liver mercury concentrations significantly lower, in the summer than in other seasons. Liver mercury burden (the mass of liver mercury per fish), in contrast, showed only limited significant seasonal variation relative to body size, implying that the observed seasonal variation of liver mercury concentration (by around a factor of 3 between summer and winter) results largely from the dilution of similar burdens by a seasonally growing and shrinking liver. Such natural seasonal variation may confound the use of the muscle/liver mercury concentration ratio, which has been proposed as evidence of fish migration between waters with different levels of mercury contamination. These results also suggest that, as variation in liver mercury concentrations is closely related to seasonal variation in LSI, liver tissue may not be a reliable monitor for mercury in temperate species of marine fishes with seasonally fluctuating liver size.

KEY WORDS: Mercury · Biomonitoring · Liver · Fishes · *Zoarces viviparus* · Forth Estuary, Scotland

INTRODUCTION

The utility of fishes as biomonitors for mercury in aquatic environments is widely recognised (e.g. Phillips 1977, Bryan et al. 1985, Jensen & Cheng 1987, Evans et al. 1993). The purpose of such biomonitoring is usually the provision of continuing assurance of marine foodstuffs with respect to human health, or monitoring in relation to waste disposal or discharge (Franklin 1991). As such, therefore, the tissue of choice for most monitoring programmes has been muscle tissue, this constituting the principal route of exposure of humans to mercury through the diet.

Fish liver has also been used as a target tissue in monitoring programmes for metals in the USA and Denmark (Evans et al. 1993, Jensen & Cheng 1987). The ratio of mercury concentrations in the muscle and liver tissues of cod *Gadus morhua* was proposed by Juhlshamn et al. (1982) to provide possible evidence for migration between waters with different levels of mercury contamination. The liver may be a suitable candidate tissue for such monitoring, as it concentrates

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many pollutants to higher levels than muscle (Evans et al. 1993), and is closely involved with processes of metal dynamics, storage, and detoxification (George 1991). Fish liver is already used routinely as a monitoring tissue for lipid-soluble persistent contaminants, such as aromatic organochlorines (e.g. Kammann et al. 1993).

Elliott et al. (1988) summarised 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 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'. Topping et al. (1975) also recommended that studies of seasonal variation of mercury concentrations should be undertaken for all fish species which are selected for monitoring programmes. This recommendation has been largely disregarded since that time, particularly with respect to the use of liver tissue.

Study area

The Forth Estuary, a large industrialised estuary on the Scottish east coast (Fig. 1), has been described as one of the most contaminated coastal areas in Scotland with respect to trace metals (Davies 1987). The estuary, uniquely in Scotland, has received inputs of mercury for several decades (Elliott & Griffiths 1986). The majority of this mercury has been discharged as unrecovered catalyst in a point-source discharge of effluent from an organic chemical manufacturing complex at Grangemouth in the middle estuary (Fig. 1). The annual total load of mercury discharged in the last 20 yr peaked in 1981 with an input of 6 t, although inputs from this source had fallen almost to zero by December 1992 [S. Hull, Forth River Purification Board (FRPB), pers. comm.].

Study species

The eelpout *Zoarces viviparus* L. is a resident estuarine fish species, found commonly in the estuaries around the North Sea and Baltic Sea (Wheeler 1978). This species has a number of characteristics required of any potential indicator species for metals in the aquatic environment (Phillips 1980), including a restricted home range, reasonable longevity (often longer than 4 yr, based on annuli on otoliths: S. Mathieson pers. obs.), adequate size to provide sufficient tissue for analysis (maximum weight in excess of 100 g in Forth Estuary: S. Mathieson pers. obs.), abundance and ease of collection. The home range of individual eelpout in the Forth Estuary is unknown, although studies of differences in gene frequency between areas in the Danish belt sea area indicate that the Zoarces population has to be conceived as an array of sub-populations, with very little genetic evidence of mixing as a result of migration between areas only a few tens of kilometres apart (Christiansen et al. 1974, Hjorth & Simonsen 1975). These same populations were also the subject of an early study by Schmidt (1917), who presented extensive evidence for localised, phenotypically distinct, populations. The eelpout has been proposed by Jacobsson et al. (1986) as a suitable species for use as an indicator of environmental effects of harmful substances, and has been used by Essink (1980, 1988) as a bioaccumulation monitoring species for mercury. The eelpout was also reported historically as an important food fish in the Forth Estuary (Day

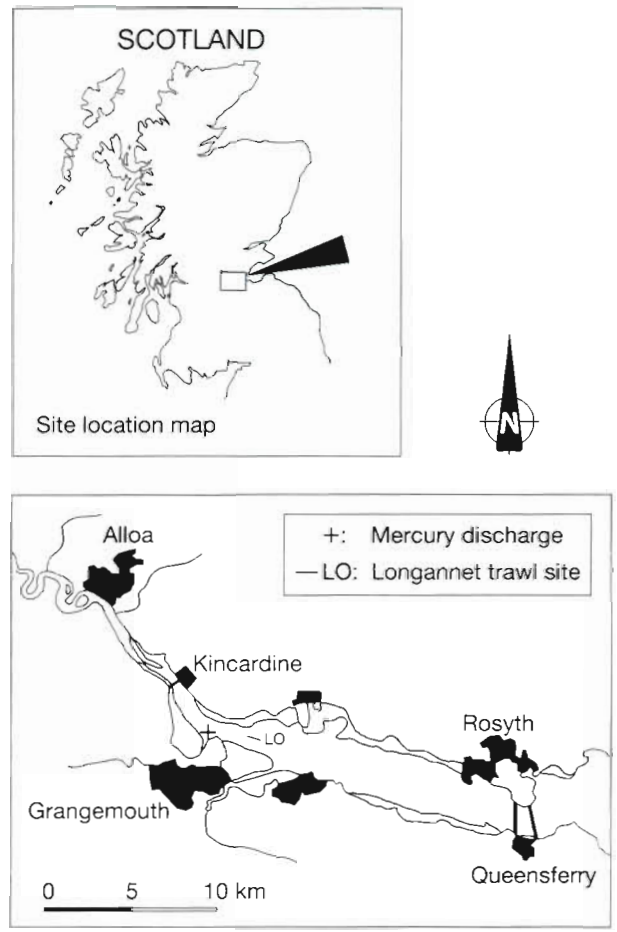

Fig. 1. The Forth Estuary, Scotland, with location of principal mercury discharge and sampling site

1884), and more recently in the Baltic Sea (up to 400 t yr^{-1} in the Gulf of Riga: Soin 1968), and has, therefore, the potential to be a pathway for mercury to man through diet.

As part of a project to assess the utility of the eelpout as a monitoring organism for mercury in estuarine environments (Mathieson 1993), this present study describes the temporal variability of total mercury in liver tissue of this species from a single site in the Forth Estuary, in relation to the seasonal variation of liver size. In addition, total mercury concentrations in skeletal muscle are presented for the same fish, to allow an assessment of the seasonal variation of the muscle/liver mercury concentration ratio. Although much of the mercury stored in fish tissues, and muscle in particular, is in the form of methylmercury (George 1991), total mercury (inorganic+organic forms) is considered here since this is generally the form of mercury for which monitoring is undertaken by the statutory agencies in the UK (S. Hull, FRPB, pers. comm.)

MATERIALS AND METHODS

Sample collection and mercury analysis. Eelpout were collected from the Forth Estuary subtidal area at Longannet (LO: Fig. 1). Samples were collected by trawling a 1000 m track, using an Agassiz frame with a 2 m mouth width and a 15 mm stretched-mesh net, towed by the FRPB research vessel, 'Forth Ranger'. The LO trawl site was located on each occasion using a DECCA navigation system (56° 02.35' N, 03° 39.42' W to 56° 02.35' N, 03° 40.41' W). Sampling was carried out approximately monthly between February 1990 and October 1991. Due to inclement weather and mechanical problems with the survey vessel, no samples were collected between December 1990 and February 1991. The ranges of water temperatures for each season (see Table 2) were obtained from FRPB routine water quality monitoring surveys.

Fish were killed on board the research vessel by a sharp blow to the head. Total body length was recorded to the nearest 1 mm. Each fish was then stored individually in a labelled polythene bag in a refrigerator at 4°C until the vessel returned to port (maximum 3 h). On return to the laboratory, the fresh weight of each fish was then recorded to the nearest 0.01 g. If fish were not dissected immediately, they were re-bagged and placed in a freezer at –20°C until dissection was performed.

The liver was dissected from each fish using stainless steel instruments, weighed (to nearest 0.01 g), and re-frozen (–20°C) in individual labelled polypropylene containers until mercury analysis was carried out. An accurately weighed sub-sample of 0.5 to 1.0 g was taken from larger livers (>1.5 g); other livers were analysed whole. An accurately weighed sub-sample of skeletal muscle (0.1 to 1.0 g) was also sampled from the long post-anal tail of each fish and treated in the same manner as liver samples. Tissue samples were dried to constant weight in Teflon pressure digestion vessels (Valtech Plastics plc, Thirsk, UK). Tissues were digested in the same vessels (sealed), in 5 ml of concentrated nitric acid, at 110°C for 1 h. Cooled solutions were diluted to 10 ml with distilled deionised water (Milli-Q). Mercury was analysed by cold vapour atomic absorption spectrophotometry (CVAAS). A standard reference material (Standard Dogfish Muscle, DORM-1, National Standards Bureau of Canada), subjected to the same preparation and analysis as tissue samples, gave a high recovery of mercury (mean = 92.8%, relative standard deviation = 10.8%, n = 10).

Data treatment and statistical analysis. To facilitate the assessment of temporal variation, samples were grouped in 3-month 'seasons' as follows: December–February (winter), March–May (spring), June–August (summer), September–November (autumn). These are not purely arbitrary divisions, as they reflect the seasonal differences in water temperature in the Forth Estuary (see Table 1), and correspond to relatively distinct periods in the life cycle of the eelpout (e.g. summer: seasonal peaks in liver and testes size; autumn: hatching and rapid growth of viviparous brood in female ovarian cavity; winter: slowed growth of brood and eventual emergence from female).

The Liver Somatic Index (LSI) was calculated for each fish as: 100 × (liver wet weight)/(body fresh weight). For female fish carrying a brood, the weight of the brood was subtracted from the fresh weight prior to calculating the LSI. There were no significant differences between the sexes, when seasonally grouped, in terms of LSI (*t*-tests, all $p > 0.05$), mercury concentrations (*t*-tests, all $p > 0.05$) or liver mercury burdens (with respect to length; analysis of covariance, all $p > 0.05$). Male and female sub-sets were combined, therefore, within seasons. Young-of-the-year fish, recruited into catches in summer only (becoming 1+ fish the following season), were excluded, as their livers provided insufficient material for analysis. Where there was no significant effect of body size (as length or weight) on LSI or liver Hg concentration, comparisons of mean values between seasons were performed using analysis of variance (ANOVA). Where body size had a significant effect (e.g. on liver Hg burden, muscle Hg concentration and, in some seasons, LSI and liver Hg concentration), seasonal samples were compared using an analysis of covariance (ANCOVA). Statistical methods were performed according to the methods in Zar (1984). All differences were taken as significant at the 5% probability level.

RESULTS

Relationships with body size

Data were collected from a total of 196 eelpout in seven 3-month seasons (Table 1). The effect of body size (as both length and weight) on LSI, liver Hg burden, and liver and muscle Hg concentrations was investigated within single seasons using Pearson correlation and linear regression. LSI showed no significant correlation with either length or weight in any season in 1990 (Table 1). In 1991, however, LSI varied significantly with body size in spring and autumn, but not in summer. Liver Hg concentration did not vary significantly with body size in any season, except summer 1991 (Table 1). Liver Hg burdens (the total mass of liver Hg per fish) increased with size of fish, the $\log_{10}$ of Hg burden increasing in a highly significant linear relationship with increasing length in 6 of 7 seasons (Table 2). The $\log_{10}$ of muscle Hg concentration showed an increase with length in all seasons, although linear regressions were significant in only 4 seasons (Table 3). Coefficients of variation (R^2) indicated that length accounted for a maximum of 72% of the variation in muscle Hg concentration in a single season (spring 1991) and, in all other seasons, for less than 40%.

Seasonal variability within years

Seasonal variation was observed in LSI (Fig. 2), with peak mean values in the summers of both 1990 and 1991. Seasonal LSI means were compared within each year, and significant differences were indicated between seasonal means in 1990 (ANOVA, F = 34.674, between-groups df = 3, within-groups df = 130, $p < 0.001$). Multiple comparison testing (Scheffé) indicated that the mean LSI in summer was significantly higher ($p < 0.05$) than those of spring and summer in that year. In 1991, due to a significant correlation between LSI and body size in 2 of the 3 seasons, seasonal differences in the regression of LSI on length were tested using analysis of covariance (ANCOVA) (regression statistics are given in the foot notes in Table 1). The regression elevation (a) for Summer 1991 was significantly higher than those for Spring and Autumn (F = 31.058, df = 2, 56, $p < 0.0005$; Tukey: Su vs Sp, q = 6.708, $p < 0.001$; Su vs Au, q = 8.325, $p < 0.001$).

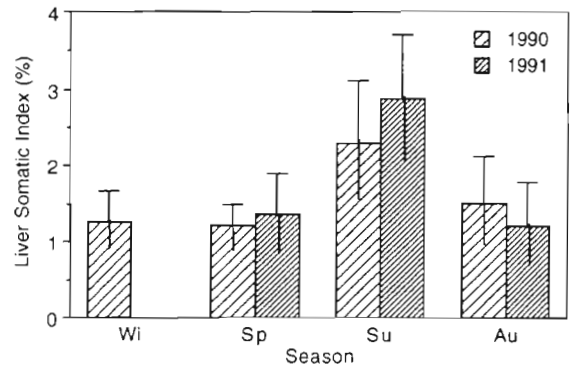

Fig. 2. *Zoarces viviparus*. Seasonal and annual variation of liver somatic index for eelpout from Longannet, Forth Estuary

Liver mercury concentrations also showed seasonal variations (Fig. 3), with a minimum mean value in summer of both years, higher values in spring and autumn, and a maximum mean value in winter of 1990. Differences between seasons were statistically significant in 1990 (ANOVA: F = 13.732, df = 3,130, $p < 0.0001$). A Tukey multiple comparison test indicated that the summer mean was significantly lower than the winter and spring means ($p < 0.05$). Due to a significant effect of body size on liver Hg concentration in summer 1991, differences between the three seasons were investigated using ANCOVA of the regressions of liver Hg concentration on length (regression statistics are given in the footnotes in Table 1). Although a significant difference was indicated between regression slopes (F = 4.359, df = 2, 56, $p < 0.025$), Tukey testing was insufficiently powerful to identify the significant comparisons (all $p > 0.50$). There was no significant correlation between liver and muscle Hg concentrations in any of the 7 seasons (Table 3), and correlation coefficients were extremely low in most cases (0.1 or lower).

Table 1 *Zoarces viviparus*. Seasonal Pearson correlation statistics (r, p) for liver somatic index (LSI) and liver Hg concentration versus measures of body size (length and weight) for eelpout from Longannet, Forth Estuary

Period	Sample size (n)	Pearson correlation coefficient: LSI with: length	LSI with: weight	Liver Hg conc. with: length	Liver Hg conc. with: weight
Winter 1990	31	0.23, ns	0.10, ns	0.012, ns	−0.01, ns
Spring 1990	45	0.02, ns	0.05, ns	0.04, ns	0.10, ns
Summer 1990	52	0.19, ns	0.15, ns	−0.17, ns	−0.23, ns
Autumn 1990	6	0.36, ns	0.46, ns	−0.32, ns	−0.25, ns
Spring 1991[a]	9	0.74, *	0.71, *	0.34, ns	0.24, ns
Summer 1991[a, b]	34	0.03, ns	0.10, ns	−0.48, **	−0.44, **
Autumn 1991[a]	19	0.65, **	0.53, *	0.18, ns	0.16, ns

*$p < 0.05$, **$p < 0.01$; ns: not significant ($p > 0.05$)

[a]Regressions ($y = a + bx$) of LSI on length—Sp: a = −0.04107, b = 0.00879; Su: a = 2.73939, b = 0.00073; Au: a = −0.25872, b = 0.00911

[b]Regressions of liver Hg conc. on length—Sp: a = 0.02712, b = 0.00038; Su: a = 0.27208, b = −0.0010; Au: a = 0.08016, b = 0.00032

Table 2. Body size statistics, linear regressions for $\log_{10}$ liver mercury burden (y) on $\log_{10}$ length (x), and estimated liver mercury burdens of eelpout *Zoarces viviparus*, and water temperatures from Longannet, Forth Estuary. Sample sizes are given in Table 1

Period	Length (mm)		Weight (g)		Regression ($y = a + bx$)			Hg burden (µg)			Water temp.[c]
	Mean	Range	Mean	Range	a	b	R^2 (p)[a]	Estimate[b]	95%	CI	Range (°C)
Winter 1990	174.2	105–282	24.2	4.2–99.9	−8.82	3.32	0.45 (•••)	0.025	0.019	0.033	4.9–6.6
Spring 1990	160.7	100–209	16.4	2.9–35.8	−9.49	3.52	0.41 (•••)	0.014	0.012	0.018	7.2–13.4
Summer 1990	150.9	85–263	27.6	3.0–76.5	−8.37	3.01	0.65 (•••)	0.015	0.012	0.018	13.0–16.8
Autumn 1990	125.4	98–195	10.5	3.6–30.9	−6.41	2.02	0.20 (ns)	0.010	0.002	0.036	12.6[d]
Spring 1991	143.4	102–214	14.3	2.7–35.0	−13.37	5.24	0.83 (•••)	0.011	0.006	0.019	8.6–12.2
Summer 1991	164.9	91–257	34.0	6.6–68.3	−10.81	4.13	0.72 (•••)	0.015	0.012	0.019	13.4–18.8
Autumn 1991	157.3	95–240	18.8	2.7–54.6	−11.98	4.66	0.84 (•••)	0.014	0.011	0.018	5.0–16.0

[a]Two-tailed significance: •••$p < 0.001$, ns: not significant

[b]Estimated Hg burden in liver of 150 mm fish

[c]Surface water temperature from Forth River Purification Board routine water quality sampling, taken at Grangemouth, on south bank opposite Longannet (see Fig. 1)

[d]Identical measurements on 2 separate dates

Table 3. *Zoarces viviparus*. Seasonal linear regression statistics for $\log_{10}$ muscle Hg concentration (y) on length (x), mean muscle: liver Hg concentration ratios, and Pearson correlations (muscle versus liver Hg concentrations) for eelpout described in Table 1

Period	Sample size (n)	Linear regression ($y = a + bx$)			Muscle:liver Hg Ratio		Pearson r	
		a	b	R^2 (p)[a]	Mean	SD	r	p[a, b]
Winter 1990	31	−1.2466	0.0022	0.06 (ns)	1.81	1.57	0.06	ns
Spring 1990	45	−1.7279	0.0049	0.26 (•••)	2.04	2.44	0.26	ns
Summer 1990	52	−1.6183	0.0027	0.24 (•••)	3.64	2.93	−0.03	ns
Autumn 1990	5[c]	−1.4637	0.0011	0.03 (ns)	1.96	1.45	0.10	ns
Spring 1991	9	−1.9173	0.0050	0.72 (••)	1.72	1.34	0.08	ns
Summer 1991	34	−1.0119	−0.0008	0.01 (ns)	2.78	2.18	0.01	ns
Autumn 1991	19	−1.4402	0.0035	0.37 (••)	2.24	1.79	0.07	ns

[a]Significance: •$p < 0.05$, ••$p < 0.01$, •••$p < 0.001$, ns: not significant ($p > 0.05$)

[b]Range of non-significant p values = 0.079 to 0.963

[c]n differs from Table 1 due to a single missing muscle Hg concentration

Total mercury burdens of livers increased in a non-linear fashion with body size, as length. Logarithmic transformation (base 10) of both variables rendered the associations linear. The linear distribution of $\log_{10}$(Hg burden) with $\log_{10}$(total length) is shown for each season in 1990 (Fig. 4a) and 1991 (Fig. 4b), with regression statistics in Table 2. All relationships were statistically significant, except for autumn 1990, which was based on the smallest sample size, and the most restricted range of lengths of any season. Seasonal variation of the regressions was investigated within each year using ANCOVA. In 1990, there was no significant seasonal variation between the slopes of the 4 regressions ($F = 1.616$, df = 3,126, $p > 0.10$). A significant difference among the elevations of these regressions ($F = 5.78$, df = 3,129, $p < 0.0025$) was identified by Tukey testing between winter and spring regressions ($q = 5.03$, df = 4,126, $p < 0.005$). No other comparisons between seasons were significant (all $p > 0.05$). In 1991, a significant difference between the slopes of the regressions from the 3 seasons ($F = 7.61$, df = 2,56, $p < 0.005$) could not be identified by Tukey testing (all

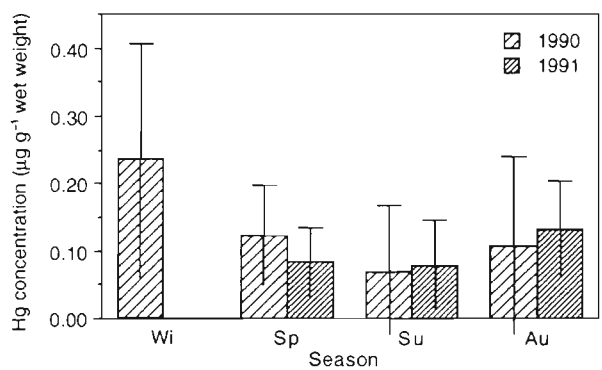

Fig. 3. *Zoarces viviparus*. Seasonal and annual variation of mercury concentrations in the liver of eelpout from Longannet, Forth Estuary

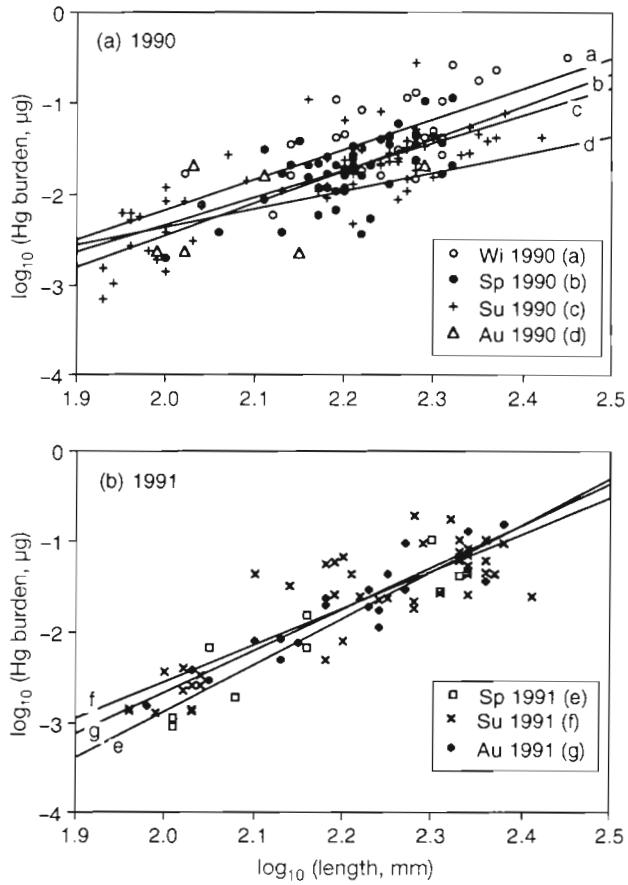

Fig. 4. *Zoarces viviparus*. Variation of liver mercury burden with fish length for eelpout from Longannet, Forth Estuary

$p > 0.05$), indicating that a Type II error had been committed. Estimates of liver mercury burden for a 150 mm fish, calculated from the seasonal regressions, indicate that, allowing for the effects of size, the range of mercury burdens is similar in most seasons.

As the $\log_{10}$ of muscle Hg concentration increased significantly with length in the majority of seasons (Table 3), seasonal differences were investigated by comparing seasonal regressions using ANCOVA. In 1990, seasonal regressions were similar in terms of the regression slopes ($F = 0.896$, df = 3,125, $p > 0.50$), although a significant difference was indicated between regression elevations ($F = 15.521$, df = 3,128, $p < 0.0005$). Tukey testing was not sufficiently powerful, however, to detect a significant difference between the 2 least similar elevations, from winter and spring A significant difference between the 3 seasonal regressions in 1991 (slopes: $F = 4.654$, df = 2,56, $p < 0.025$) lay between the slopes of the spring and summer regressions (Tukey: $q = 3.788$, df = 3,56, $p < 0.05$).

The mean muscle:liver mercury concentration ratio showed a distinct seasonal pattern, with a maximum seasonal value in summer of both years (Table 3). Comparisons between seasons were performed on $\log_{10}$-normalised data, with a significant difference indicated in 1990 (ANOVA: $F = 3.2109$, df = 3,129, $p = 0.025$), between summer and winter means (Scheffé testing, $p < 0.05$). Differences between the means of the 3 seasons in 1991 were, however, not significant (ANOVA: $F = 0.3219$, df = 2,59, $p > 0.50$).

DISCUSSION

Seasonal variation of mercury in the liver

No previous studies of seasonality in fish liver mercury concentrations and burdens can be found in the considerable published literature on mercury in fish. This may reflect a reluctance to explore the use of tissues other than muscle for monitoring mercury, or perhaps that other workers have indeed explored its use, found it unsatisfactory for the purpose, and failed to publish such a negative result. Nonetheless, the important roles played by the liver, as outlined above, in the dynamics and storage of metals in fish make it a tissue with a potential for consideration for use in monitoring programmes for mercury.

An essential part of such monitoring is the exploration of the natural dynamics of mercury in the liver tissue, in relation to biological parameters. The significant seasonal differences observed in mercury concentrations in the liver of eelpout are generally not reflected by such strong seasonal variations in the total liver mercury burdens, although some significant seasonal differences in burdens were noted. If the liver undergoes a large increase in size relative to body weight, such as the increase noted between spring and summer, but the total mercury burden of the liver remains fairly similar (see estimates in Table 2), then a fall in mercury concentration would inevitably result. This suggests that the seasonal variations observed in mercury concentrations are, at least partially, the result of the dilution and concentration of relatively similar seasonal burdens by the significant seasonal growth and shrinkage of the liver.

Seasonal variation of the LSI

The seasonal pattern of variation in the LSI observed for eelpout in this study, with significantly higher values of LSI in summer, has been observed in several studies, of eelpout (Korsgaard & Petersen 1979) and other fish in temperate waters (cod *Gadus morhua*: Jangaard et al. 1967; plaice *Pleuronectes platessa*: Dawson & Grimm 1980, White & Fletcher 1985, George et al. 1990; winter flounder *Pleuronectes americanus*:

Fletcher & King 1978; burbot *Lota lota*: Pulliainen & Korhonen 1990). The seasonal trend in the LSI of eelpout reported in this study follows that of water temperature in the Forth Estuary (Table 2), a relationship also indicated for the LSI of plaice from north-east Scottish coastal waters by George et al. (1990). The seasonal variation of water temperature indicated in that study, from 3–5°C in February to March, to 12–15°C in the period August to September, is similar to that in the Forth Estuary. White & Fletcher (1985) suggested that a 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 a period of intense feeding after spawning. The LSI declined again as the lipid and glycogen stores were mobilised during the winter period of poor feeding and maximum gonad development. Thus, the seasonal feeding cycle of the plaice has an important effect on the seasonal variation in 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 & Tocher 1987).

Seasonal changes in liver size have also been related to the reproductive state of *Zoarces viviparus*. In a study of lipid metabolism in female *Z. viviparus* from Danish waters, Korsgaard & Petersen (1979) observed a large increase in liver lipid content, corresponding to a seasonal summer increase in relative liver size = LSI). The LSI reached its maximum size before the initiation of vitellogenesis, or production of yolk for developing oocytes in the ovary, and liver weight remained high during vitellogenic growth. A decrease in liver lipid content took place simultaneously with a large decrease in the level of vitellogenin and the level of serum lipid. This decline in lipid content also coincided with the decrease of the LSI over the autumn and winter. A summer peak in lipid content of the liver was also reported for both sexes in eelpout from the Gulf of Finland (Pekkarinen 1980). The maximum value of the 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%, similar to the seasonal values recorded for eelpout in this study.

Although the actual uptake mechanisms for both inorganic and organic mercury species remain to be clearly elucidated, it is known that mercury complexes tightly to proteins in the liver (Carty & Malone 1979, George 1991). The limited seasonal variation of the total liver burden of mercury, relative to body size, indicates that seasonal increase of lipid content does not correspond to an equivalent increase in the actual content of mercury in the liver. This suggests that lipids may not play a large role in the storage of mercury in the liver of eelpout.

Implications for use of muscle/liver mercury ratio

The significant seasonal variation of liver mercury concentrations shown for eelpout calls into question the validity of the use of the muscle/liver mercury concentration ratio as an indication of recent mercury exposure in marine fish. The ratio was proposed by Juhlshamn et al. (1982) as a potential indicator of recent mercury contamination of cod. These workers established that, on feeding either inorganic or methylated mercury to cod kept under laboratory conditions, 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. Mean ratios for wild eelpout in this study were always considerably above 1 and, in the summer of 1990, were in excess of 3.6. Although some of the difference in absolute values between the ratios measured in wild eelpout and those reported for wild cod by Juhlshamn et al. (1982) is likely to be due to inter-specific differences in prey, mercury accumulation, and internal dynamics of mercury once taken in, there is also a clear seasonal effect of the summer drop in eelpout liver mercury concentrations (leading to a higher ratio), which means that the difference to the reported ratio for cod is greater in summer than in winter.

The results presented above for eelpout show that the LSI varies considerably between seasons, a natural variation which may be related to seasonal feeding and the reproductive state. Similarly, the mean mercury concentrations in eelpout liver may be more than 3 times higher in winter than in summer, a difference related mainly to the seasonal variation in the LSI, although also, in part, to changes in the liver mercury burden. The LSI of cod has been shown to vary between 2 and 4% of body weight from winter to summer (Jangaard et al. 1967), a similar range to that seen in eelpout. It is possible, therefore, that mercury concentrations in cod liver might show seasonal variations similar to those observed for eelpout, even in an environment relatively uncontaminated by mercury. This highlights the danger of making assumptions about the 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. On the basis of these results, it appears that the application of the muscle/liver ratio to wild populations of eelpout as a measure of recent exposure to mercury is likely to be extremely problematical and cannot be recommended.

Implications for use of liver tissue for biomonitoring

It has been shown here that the use of eelpout liver as a biomonitoring tissue for mercury in estuarine biota is subject to some serious limitations, perhaps leading to the recommendation that liver should be avoided as a routine tissue for monitoring mercury accumulation. It is clear that, in comparison with the use of muscle tissue for monitoring mercury accumulation by fish, liver tissue is likely to provide a greater degree of seasonal variation, partly as a result of the marked and significant changes in liver size with season. This cyclical pattern of annual variation, related to seasonal size changes in the liver, is in addition to any variation of mercury concentrations resulting from variable environmental exposure. The seasonal increase of the LSI of eelpout in summer has been shown by others to be related to an increase in lipid content as a result of vitellogenesis in females (Korsgaard & Petersen 1979) and, possibly, of seasonal increases of feeding activity in both sexes (Pekkarinen 1980).

The seasonal variation of mean concentrations, however, by a factor of around 3 between winter and summer suggests that performing a meaningful comparison of mean concentrations between samples of fish livers may be fraught with difficulties and use of liver should be avoided unless effects of body size and seasonal variability can be accounted for. This study illustrates the necessity of quantifying such natural sources of variation of mercury in different tissues before including them in a programme of monitoring of mercury bioaccumulation.

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