Vol. 91: 105–115, 1992 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published December 31

Changes in branchial Na^+,K^+-ATPase, metallothionein and P450 1A1 in dab *Limanda limanda* in the German Bight: indicators of sediment contamination?

Ron Stagg[1], Anders Goksøyr[2], Gillian Rodger[1]

[1]Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB9 8DB, Scotland, UK
[2]Laboratory of Marine Molecular Biology, University of Bergen HIB, N-50520 Bergen, Norway

ABSTRACT: Na^+,K^+-ATPase activity, ouabain binding, metallothionein and P450 1A1 were measured in gill tissue from the dab *Limanda limanda* caught from a contaminant gradient in the German Bight during the Bremerhaven Workshop. Metallothionein levels were highest at the most contaminated sites closest to the mouth of the Elbe and lowest at the outermost station on the Dogger Bank. Tissue levels of zinc and cadmium were also elevated at these sites but copper levels did not change along the gradient. P450 1A1 levels, measured with an antibody probe, showed a complex pattern of induction which suggests an inhibitory effect at the most contaminated stations. Na^+,K^+-ATPase was similar in fish from all of the stations but measurement of ouabain binding show that levels of the enzyme are elevated at the innermost and outer stations. Calculation of activity per binding site indicates that the turnover rates of the enzyme were lower at the most contaminated stations. The results show that the role of adaptive responses in the face of inhibition by contaminants is an important consideration in identifying biological effects. The data were analysed with respect to the contaminant burdens in the total and fine particulate fraction (< 63 µm) of the sediment and in the liver of dab caught at each station. The utility of biological effects measurement in the gill as an indicator of environmental contamination is discussed.

INTRODUCTION

The fish gill is central to the functions of gas exchange, osmotic and ionic regulation and acid base balance. It comprises a thin extensive secondary lamellar epithelium which exposes a large surface area to the environment and is continuously irrigated with external water. Counter current blood flow to different parts of the gill is under hormonal and neural control and enables the functional surface area of the gill to be regulated to meet the exchange needs of the fish. It is clear that the gill plays a key role at the interface of the animal with its environment; it is a site of uptake and depuration of contaminants, and a tissue where detoxification and metabolism of contaminants is likely to occur. Two primary detoxification systems which have been studied extensively in fish are the induction of metallothionein and CYP 1A1 (P450 1A1) in response to heavy metal (zinc, cadmium, copper) and organic (polyaromatic hydrocarbons and planar chlorbiphenyls) contaminants respectively. P450 and associated enzyme activity has been detected in fish gills (James & Little 1979, Stegeman et al. 1979, Lindström-Seppa et al. 1981). Indeed recent work (Millar et al. 1989) has shown the induction of P450E (CYP 1A1) in the pillar cells of the gill by immunoblotting techniques following intraperitoneal injection of the model inducer β-napthoflavone (BNF). Several studies have shown the induction of metal binding proteins in the gill in response to waterborne zinc (Kito et al. 1982, Bradley et al. 1985), cadmium (Noel-Lambot et al. 1978, Reichert et al. 1979, Kito et al. 1982, Thomas et al. 1983,

1985, Benson & Birdge 1985, Klaverkamp & Duncan 1987, Olsson & Hogstrand 1987, Olsson et al. 1989) and mercury (Bouquegneau et al. 1975). Induction of metallothionein genes has been shown in gills in response to intraperitoneal injection of cadmium, copper, and zinc (Zafarullah et al. 1989) although in response to lower Cd doses (Norey et al. 1990a, b) induced gene expression, but no increase in metallothionein, could be detected.

Branchial Na^+,K^+-ATPase, a key enzyme in ionic regulation, is sensitive to a variety of contaminants following *in vitro* exposure (Davis & Wedemeyer 1971, Janicki & Kinter 1971, Bouquegneau 1977, Watson & Beamish 1981, Stagg & Shuttleworth 1982, Pinkney et al. 1989, Stagg et al. 1992). However, responses to *in vivo* exposure have been more variable, showing both inhibitory and stimulatory effects, which to some extent depend upon the exposure regime (Leadem et al. 1974, Kuhnert & Kuhnert 1976, Bouquegneau 1977, Verma et al. 1979, Watson & Beamish 1980, Boese et al. 1982, Stagg & Shuttleworth 1982, Watson & Benson 1987, Pinkney et al. 1989). Several studies have shown that increases in the amount of microsomal protein compensate for *in vivo* inhibition of ATPase and lead to enhanced enzyme activity or the maintenance of activity at pre-exposure levels (Sheppard & Simkiss 1978, Watson & Beamish 1980, Lauren & McDonald 1987). Using radiolabelled ligand binding studies in isolated gills, Stagg & Shuttleworth (1982) showed that the number of ouabain binding sites, and therefore total amount of enzyme present, was significantly elevated in response to high concentrations of copper in seawater-adapted flounder *Platichthys flesus*. It was argued that, in the light of copper inhibition of Na^+,K^+-ATPase, overall activity in the gills was maintained by a compensatory increase in the amount of enzyme present. Therefore, it is proposed to use this response to indicate the effects on Na^+,K^+-ATPase (and therefore branchial ion exchange) by simultaneously measuring enzyme activity and the number of ouabain binding sites. In this way the biological effects of the contaminants will be indicated by the activity per enzyme unit (turnover) and the compensatory responses in the gills by changes in amount of Na^+,K^+-ATPase as determined by ouabain binding.

The objectives of this study were to measure Na^+,K^+-ATPase activity, ouabain binding, metallothionein and CYP 1A1 (P450 1A1) in gills of dab (*Limanda limanda*) from the German Bight transect. By measuring these responses in the same individuals it should be possible to compare the induction of detoxification systems with a potentially deleterious biological effect. It was also intended to study the inter-relationships of these responses with chemical contamination in the sediments and in livers of pooled fish from the same sites.

MATERIALS AND METHODS

Samples. Male dab *Limanda limanda* 20 to 25 cm in length were caught at Stns 1, 3, 5, 7 & 9 in the German Bight by fishing from RV 'Victor Hensen' in March 1990. The fish were kept in tanks, supplied with flowing seawater and processed within 2 or 3 h after capture. The fish were killed by a blow to the head and the length and sex recorded. The entire branchial basket was excised and placed in ice-cold heparinised saline to remove clotted blood. Individual gill arches were cut free, placed in a small plastic bag and snap frozen in liquid nitrogen. The gills were subsequently stored at −70 °C until processed.

ATPase. Filaments (100 to 150 mg wet weight) were removed from the arch whilst still frozen, weighed quickly, and then homogenised in 1.5 ml ice cold 100 mM imidazole (pH = 7.8), 0.25 mM sucrose and 5 mM EDTA. The homogenate was centrifuged at 5000 × *g* for 1 min at 4 °C, and the supernatant decanted and kept on ice until assayed.

Na^+,K^+-ATPase activity was measured at 20 °C as the difference between the amount of inorganic phosphate released from the hydrolysis of 2 mM ATP in the presence of 4 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl and that when 0.1 mM ouabain was also present in the incubation buffer (100 mM imidazole-HCl, pH 7.6). Basal or Mg^{++}-ATPase was the activity measured when 4 mM $MgCl_2$ only was present in the incubation buffer. Duplicate assays were run for 30 min using 50 µl of homogenate containing 1.5 to 3.5 mg protein ml^{-1} in a final assay volume of 760 µl. The reaction was stopped by the addition of 740 µl of ice-cold distilled water followed by 3.0 ml of a 1:1 mixture of 1 % lubrol and 1 % ammonium molybdate in 1 N H_2SO_4 to measure inorganic phosphate (Atkinson et al. 1973). The reaction was allowed to develop for 15 min at room temperature and the absorbance read at 290 nm using K_2HPO_4 standards in the range 0 to 1.2 µM. Appropriate blanks were included with each assay to correct for non-enzymatic hydrolysis of ATP. The protein content of the homogenates was determined by the Biorad dye binding method (Bradford 1976) using gamma globulin as standard in 96-well plates read on a Biorad plate reader. ATPase activity was expressed as µmol phosphate mg $protein^{-1}$ h^{-1}.

Ouabain binding was measured by assaying the specific binding of 21,22-^{3}H ouabain (666 to 1180 GBq $mmol^{-1}$; Amersham International) in the 5000 × *g* supernatants used for the Na^+,K^+-ATPase determinations. 80 µl supernatant was incubated in 750 µl of buffer containing 100 mM Imidazole (pH = 7.6), 4 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl and 2 mM ATP and a range of duplicated cold ouabain concentrations. 15.6 pmol^{3}H ouabain (600 000 dpm) was added to each

tube and incubated for 3 h at 20 °C. The binding was terminated by harvesting the membranes on GFC filter discs using a Skatron semi-automatic cell harvester. 250 µl of tissue solubilizer (Optisolve, LKB) was added to each disc, incubated overnight at room temperature, 4.5 ml of scintillant (Quickscint 501; Zinsser Analytic) added and the counts determined in a Tricarb scintillation counter. Counts were recorded for 10 min or to an error of 5 % whichever was the shorter and external standard channels ratio used to correct for quench. The number of binding sites was determined from Scatchard plots of the specific binding at ouabain concentrations from 10^{-8} to 10^{-5} M. Non-specific binding of the radiolabel was determined by measuring the counts bound in the presence of 10^{-4} M ouabain (a concentration sufficient to completely inhibit Na^+,K^+-ATPase activity in gill preparations; Stagg & Shuttleworth 1982) and subtracting these prior to the analysis. Binding was normalised to the protein content of the homogenates and expressed as pmol ouabain bound (mg protein)$^{-1}$.

The turnover of the enzyme per binding site was determined for each fish as the ratio of Na^+,K^+-ATPase activity to ouabain binding. This was expressed as µmol phosphate h^{-1} (pmol ouabain bound)$^{-1}$.

P450 1A1. Aliquots of the 5000 × *g* supernatants prepared for the ATPase determinations were used in an indirect P450 1A1-ELISA as described by Goksøyr (1991) using a polyclonal rabbit anti-cod P450 1A1 IgG. The method gives a semi-quantitative measure of the gill P450 1A1 protein levels. Constant amounts of total protein were used in each determination which were assayed in triplicate and developed for 30 min before stopping the reaction. Results were expressed as absorbance units measured on a Titretek Multiscan MkII plate reader (Flow Laboratories).

Metallothionein. All glassware and Eppendorf tubes used were washed with 10 % nitric acid (Analar, BDH), rinsed with ultra-pure water and dried in a laminar flow cabinet. The gill filaments were cut off the arches while still frozen and weighed rapidly. All subsequent manipulations were carried out at 4 °C with the samples kept on ice. 40 to 180 mg wet weight of filaments from each fish were homogenised in 1 ml of 50 mM TRIS HCl pH 8.0. The homogenate was centrifuged at 10 000 × *g* for 5 min and the resulting supernatant (cytosol) divided into 2 aliquots, one for trace metal analysis and the other for metallothionein extraction. The protein content of the supernatant was measured as described above.

The aliquot for metallothionein determination was heated at 95 °C for 4 min, re-centrifuged at 10 000 × *g* and the supernatant stored at −20 °C. Metallothionein was determined in the heat-stable extract by differential pulse polarography using a modification of the Brdicka (1933) method as described by Olafson & Sim (1979) and Thompson & Cosson (1984). A static mercury drop electrode (EG and G PARC, model 303A) was linked to a polarographic analyser (Princeton Applied Research, model 174A) and analysis was carried out in 10 ml of supporting electrolyte (1.0 M NH_4OH, 1.0 M NH_4Cl and 1.2 mM $[Co(NH_3)_6]Cl_3$), with addition of 100 µl of Triton X-100 (0.0125 % v/v) to the sample cup. Oxygen-free nitrogen was passed through the sample cup for 2 min prior to each measurement and the analysis calibrated using 0.05 to 0.35 µg rabbit MTI standard (Sigma). 10 to 15 µl of sample was analysed in duplicate and the results expressed as µg rabbit MT equivalents normalised to the protein content of the 10 000 × *g* supernatant. The cytosol samples for trace metal analysis were acid digested in concentrated nitric acid (Aristar, BDH) and then analysed using graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 3030, Zeeman background correction) for cadmium, copper and zinc. A reference material, Tort-1 lobster hepatopancreas (National Research Council of Canada), was also analysed simultaneously.

Data analyses. Data are presented as back-transformed means ± SE of log-transformed variables. A 1-way ANOVA on log-transformed data was used to compare different sites and individual means compared using a least significant difference test. Correlation analysis of Zn, Cd and Cu concentrations in the gills with metallothionein data was carried out on log-transformed data. The only data describing chemical contamination of the samples are for pooled female dab livers and sediment from each of the sites studied (Cofino et al. 1992) except for the metal analysis carried out in conjunction with the metallothionein measurements. Consequently, correlation analysis was inappropriate (only 4 degrees of freedom since only 5 stations were studied) and principal component analyses of the correlation matrices of the log transformed data were used to make semi-quantitative descriptions of the relationship between contamination and observed effects.

RESULTS

Eight fish were analysed at Stns 3, 5, 7 & 9 but only 4 fish of the appropriate sex and size were obtained from Stn 1.

The residual Mg^{++}-ATPase activity in gill homogenates varied between 3.12 and 4.63 µmol P_i (mg protein)$^{-1}$ h^{-1} and showed significant differences ($p = 0.001$) between stations being higher at Stns 3 & 9 compared to 1 & 7 (Fig. 1). Branchial Na^+,K^+-ATPase activity was between 4.5 and 6.2 µmol P_i (mg protein)$^{-1}$

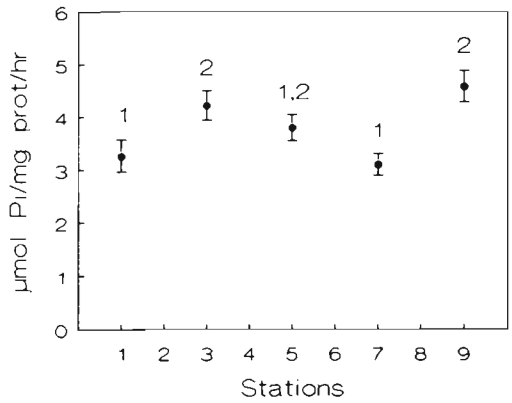


Fig. 1. *Limanda limanda*. Mg^{++}-ATPase activity in gills from dab caught in the German Bight. Values are the back-transformed means ± SE of log-transformed data. Means which share the same number are not significantly different

h^{-1} (Fig. 2a) but although there was a tendency for values to be lower in the middle of the transect the differences were not significant. In contrast, there were differences in the specific binding of ouabain to the gill membranes at different stations (Fig. 2b). The number of binding sites was significantly elevated ($p = 0.002$) at the innermost stations (1 & 3) and the outer station (9) compared to Stn 7. These data indicate that the amount of enzyme present in the gill is greater at the innermost and outer stations on the transect. Calculation of the turnover of Na^+,K^+-ATPase from the ratio of the total tissue activity and the number of ouabain binding sites (Fig. 2c) would suggest that Na^+,K^+-ATPase activity per enzyme unit is greater at Stns 5 & 7 compared to the others and is lowest at Stn 9.

The expression of P450 1A1 in the gills was significantly different ($p = 0.001$) at stations along the transect. Relative levels of the protein measured as absorbance units in the ELISA were lower at Stns 1 & 7 compared to the other stations sampled on the transect (Fig. 3). This would suggest induction of P450 1A1 at Stns 3, 5 & 9.

Normalisation of metal and metallothionein levels to wet tissue weights was difficult because of variable amounts of water associated with the gills and consequent errors in the weight determinations. Expression of the data as a function of the protein content gave a similar pattern of mean values but the error terms were lower. The protein content of the 10 000 × *g* supernatant did not vary significantly between sites.

The concentrations of zinc (Fig. 4a; $p < 0.001$) and cadmium (Fig. 4b; $p = 0.05$) in the gill showed significant differences between stations with levels higher at Stns 1 & 3 compared to the outermost stations. Copper concentrations in the gill did not show any differences between stations (Fig. 4c; $p = 0.579$). Analysis of the

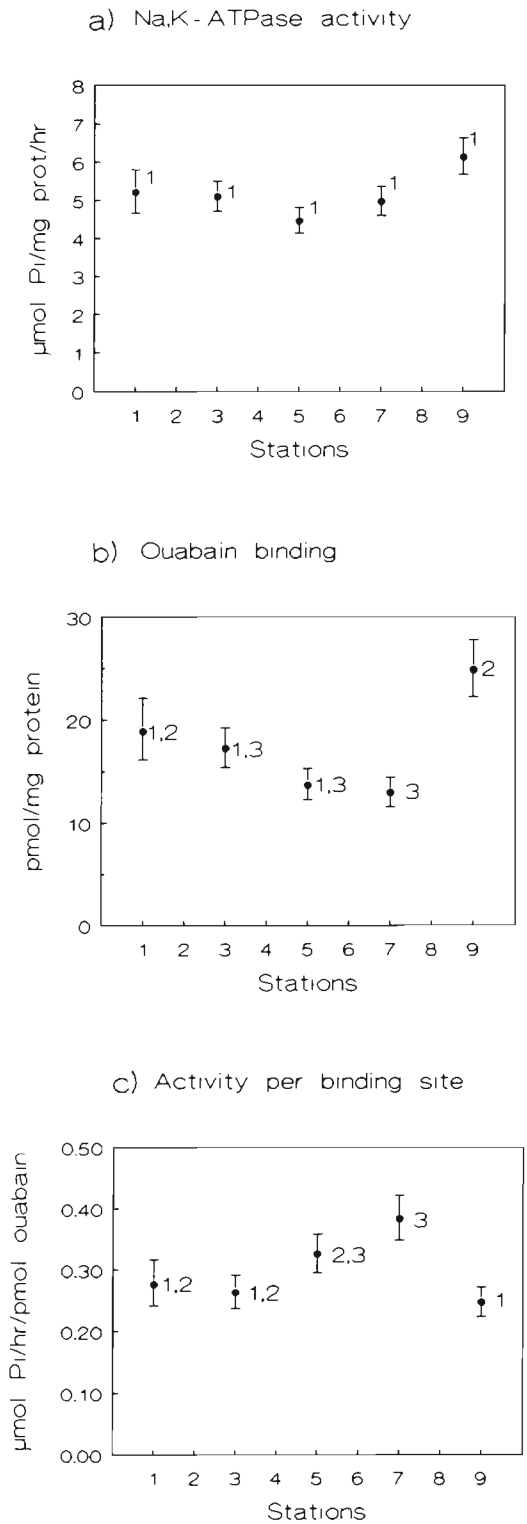


Fig. 2. *Limanda limanda*. Na^+,K^+-ATPase activity in gills from the dab caught in the German Bight. (a) Total tissue activity [µmol P_i (mg protein)$^{-1}$ h^{-1}]; (b) ouabain binding [pmol (mg protein)$^{-1}$]; (c) turnover [µmol P_i h^{-1} (pmol ouabain bound)$^{-1}$]. Values are back-transformed means ± SE of log-transformed data. Means which share the same number are not significantly different

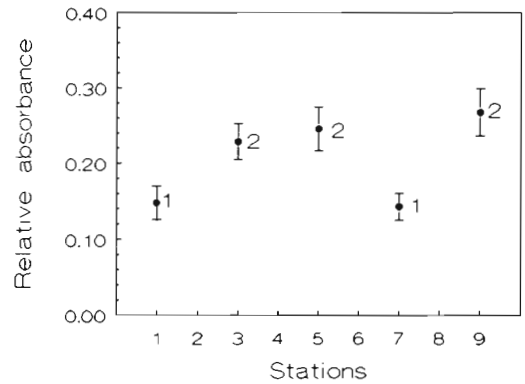
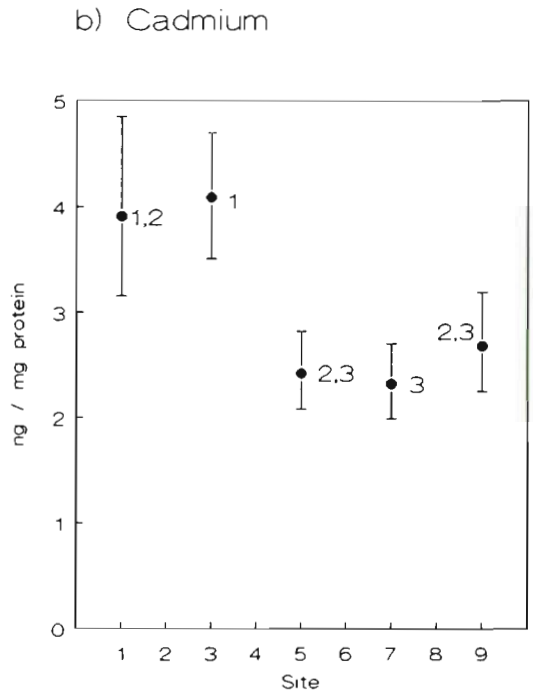
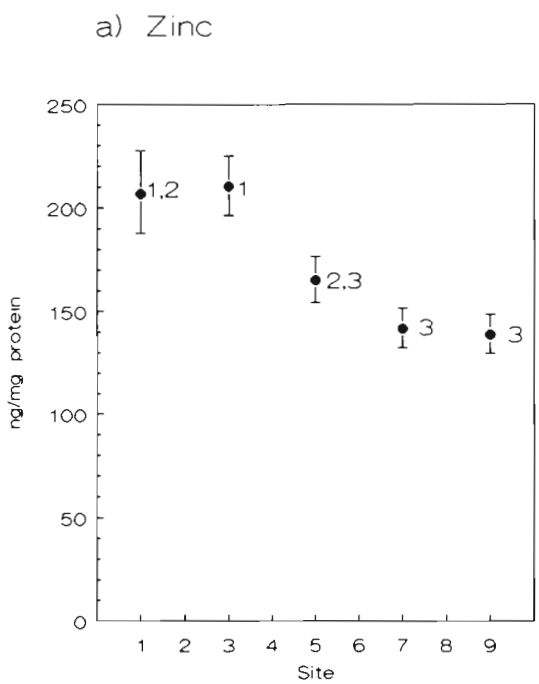


Fig. 3. *Limanda limanda*. P450 1A1 (CYP 1A1) protein levels (relative absorbance units) in gills from dab caught in the German Bight. Values are back-transformed means ± SE of log-transformed data. Means which share the same number are not significantly different

relationship of metal concentrations in the gill to that in either the total or the < 63 µm fraction of the sediment is shown in Table 1. Although none of these correlations were statistically significant the coefficients were highest for zinc and cadmium in the total sediment when compared to the < 63 µm fraction.

There were very clear differences ($p < 0.001$) in gill metallothionein levels (Fig. 5a) which were highest at Stn 1 and lowest at Stn 9. There was also a significant correlation between the levels of metallothionein and the tissue burden of metals when this was expressed as the sum of the molar concentrations of zinc, cadmium and copper on an individual fish basis (Fig. 5b). It should be noted that since zinc was present in the gills at a much higher concentration than cadmium or copper it is the predominant metal 'driving' this relationship.





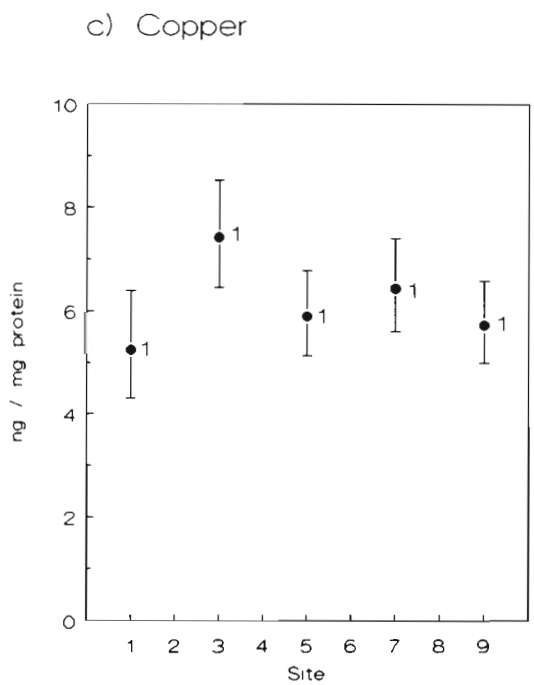


Fig. 4. *Limanda limanda*. Metal concentrations [ng (mg protein)$^{-1}$] in gills from dab caught in the German Bight. (a) Zinc, (b) cadmium, (c) copper. Values are the back-transformed means ± SE of log-transformed data. No significant differences were found between means

Table 1 Correlation matrix for zinc, cadmium and copper concentrations in gills from dab *Limanda limanda* with metal concentrations in the sediments at 5 stations in the German Bight

	Total sediment		< 63 µm fraction	
	r	p	r	p
Zn	0.869	0.1–0.05	0.464	> 0.1
Cd	0.746	> 0.1	0.513	> 0.1
Cu	0.114	> 0.1	0.085	> 0.1

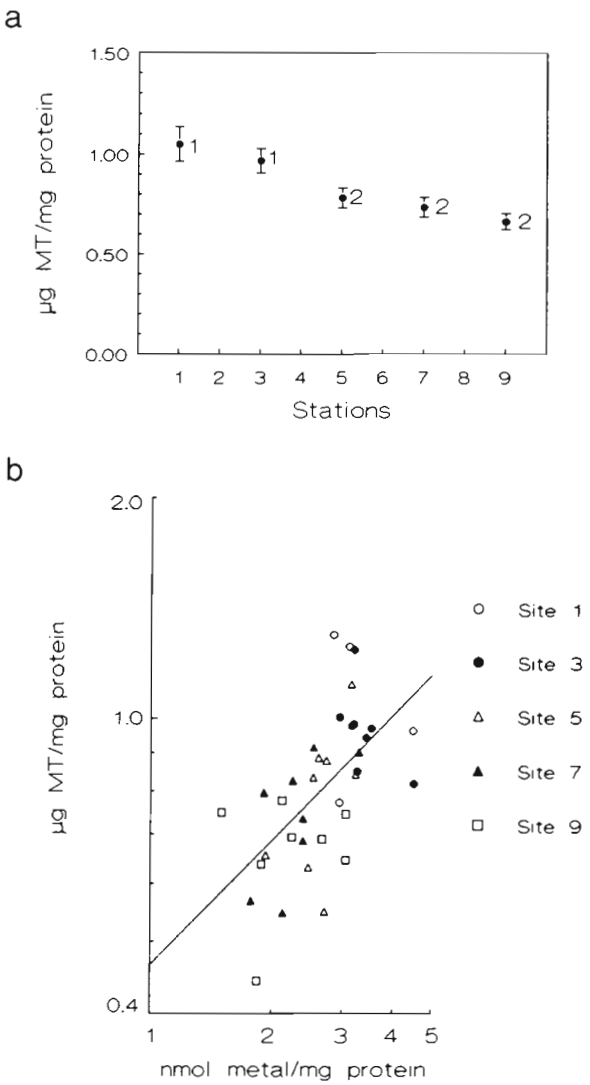


Fig. 5. *Limanda limanda*. (a) Metallothionein [µg (mg protein)$^{-1}$] in gills from dab caught in the German Bight. Values are the back-transformed means ± SE of log-transformed data. Means which share the same number are not significantly different. (b) Regression of the sum of the molar concentrations of zinc, cadmium and copper and the level of metallothionein in the gills of dab caught in the German Bight ($p < 0.001$)

Relationship to sediment and fish chemistry

Principal components analyses of the correlation matrix between P450 1A1, Na^+,K^+-ATPase turnover (activity per ouabain binding site) and the concentrations of chlorinated biphenyls (CB's) measured in the sediments (both total and < 63 µm fraction) and pooled livers from female fish are shown in Fig. 6. In each case the analysis shows a strong inverse relationship between the induction of P450 1A1 and the inhibition of Na^+,K^+-ATPase turnover. The relationship between the CB's and the 2 biological variables was dependant on the matrix in which the contaminants were measured. In livers and total sediments (Fig. 6a, b) the CB's were grouped in a cluster along the first principal component and the correlations were weak, particularly for Na^+,K^+-ATPase turnover. However for CB's in the < 63 µm fraction of the sediment there was much greater resolution of the different CB congeners on the second principal component (Fig. 6c). For example CB105 had the highest correlation with both Na^+,K^+-ATPase turnover (negative) and P450 1A1 (positive). Some of the CB's had negative correlations with P450 1A1 (e.g. CB 28). The site ordination (Fig. 6c) showed that Stns 1, 3, 5 & 9 were separated from Stn 7 along the first principal component and that Stns 1, 3, 5 & 9 were separated from each other along the second principal component. Stn 7 was associated with high Na^+,K^+-ATPase turnover, low P450 1A1, and low CB concentrations whereas Stn 9 had a low Na^+,K^+-ATPase turnover, high P450 1A1 and high levels of the congeners CB 105, 52, 180 and 118 in the sediment fraction.

Analysis of the principal components for metallothionein, the turnover of Na^+,K^+-ATPase and metals for liver, total sediment and the < 63 µm fraction of the sediment are shown in Fig. 7. The results show little correlation between Na^+,K^+-ATPase turnover and metallothionein. When contaminants were measured in liver (Fig. 7a) both zinc and copper were only weakly correlated with branchial metallothionein whereas cadmium showed a strong negative correlation. Na^+,K^+-ATPase turnover had strong positive correlations with zinc and copper but very weak correlations with mercury and cadmium. Principal component analyses of sediment metal concentrations showed that metallothionein had high correlations with all metals in the total sediments (Fig. 7b), particularly copper, cadmium and lead, whereas Na^+,K^+-ATPase turnover had very weak correlations only. In contrast metal concentrations found in the < 63 µm fraction (Fig. 7c) showed negative correlations of metallothionein with copper and lead and only weak positive correlations with zinc, cadmium and mercury. Again this contrasted with Na^+,K^+-ATPase turnover

which showed a strong negative correlation with Cd, Zn, Hg and Pb but not with Cu in the < 63 µm fraction.

DISCUSSION

ATPase

Na^+,K^+-ATP ase activity was not significantly different at the 5 stations studied although there were small changes which followed the expected contamination gradient (Fig. 2a). However, measurement of the binding of ^{3}H-ouabain shows that the level of enzyme present was significantly higher at Stns 1 & 9 compared to Stn 7 (Fig. 2b). Regulation of Na^+,K^+-ATPase can be both short term, principally through changes in intracellular sodium and enzyme turnover, or longer term through the biogenesis of new enzyme (Gick et al. 1988). In the context of field measurements over the geographical scales sampled in this study it is likely that longer term modulation of enzyme activity will be important. In the mammalian kidney, corticosteroid and thyroid hormones modulate sodium pump density through the activation of the Na^+,K^+-ATPase gene and the induction of specific mRNA (Gick et al. 1988). Corticosteroids are believed to regulate Na^+,K^+-ATPase in the teleost gill (Butler & Carmichael 1972, Forrest et al. 1973, Madsen 1990) both through increases in the numbers of chloride cells and the level of enzyme in each cell. It is likely therefore that changes in the levels of ouabain binding reflect modulation of branchial Na^+,K^+-ATPase along the contaminant gradient studied. The turnover rate of Na^+,K^+-ATPase changed significantly at different sites with stations in the middle of the gradient (Stns 5 & 7) having significantly higher rates when compared to the inner and outer stations (Fig. 2c). In general if one considers both the total sediment and the < 63 µm fraction Stns 7 & 8 had the lowest levels of contamination (Cofino et al. 1992) and the highest rate of Na^+,K^+-ATPase activity per ouabain binding site. More specific analysis of the relationship of Na^+,K^+-ATPase turnover to contamination at each site shows only weak correlations between the levels of organic conta-

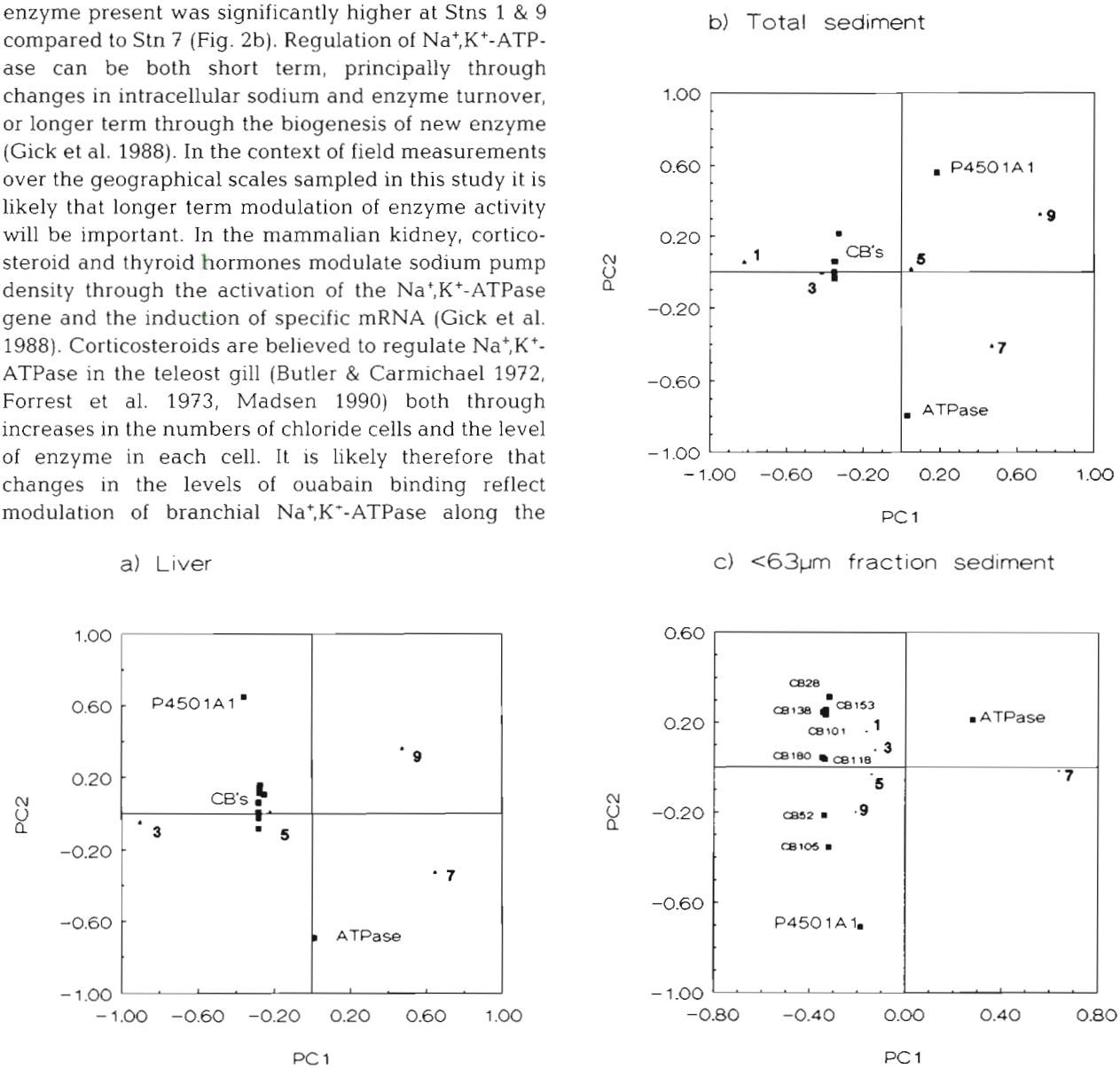
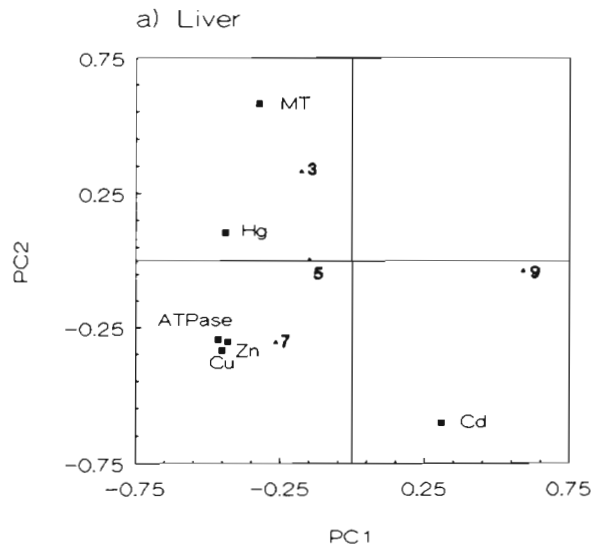


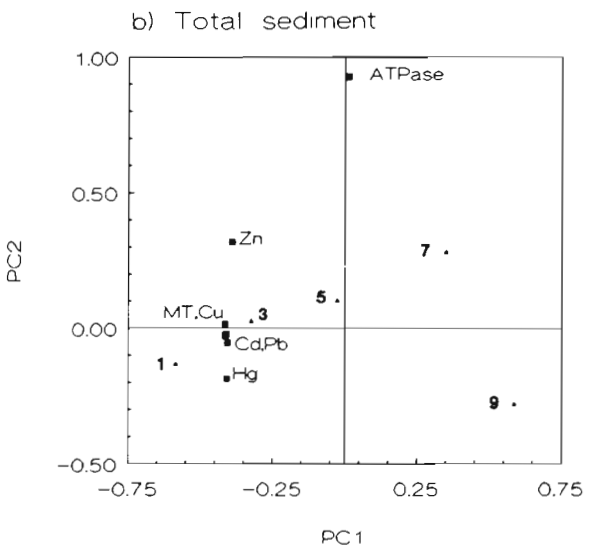
Fig. 6. Principal component analysis of Na^+,K^+-ATPase turnover, P450 1A1, and chlorbiphenyls in (a) liver, (b) total sediment, and (c) < 63 µm fraction of the sediment for each station in the German Bight. Also shown is the site ordination for each of the stations sampled

minants (Fig. 6a, b) and the metals cadmium, zinc, mercury and lead (Fig. 7a, b) in liver and total sediments but strong negative correlations when the contaminant concentrations in the < 63 µm fraction of the sediment are considered (Figs. 6c & 7c). Therefore, it is tempting to conclude that the accumulation of contaminants in the < 63 µm fraction of the sediment was associated with an inhibitory effect on Na^+,K^+-ATPase turnover.

This type of response is consistent with other studies which have shown that following *in vivo* exposure compensatory changes occur in Na^+,K^+-ATPase (Watson & Beamish 1980, Stagg & Shuttleworth 1982, Lauren & McDonald 1987). In response to the inhibitory actions of contaminants the gills have responded by increasing the amount of enzyme so that the overall levels of Na^+,K^+-ATPase are maintained. The work described here is, therefore, a clear demonstration of the way in which homeostatic mechanisms can maintain cellular capacity in the face of contaminant-induced perturbation. Clearly more sensitive indices of contaminant effect will be obtained by probing the underlying mechanism of enzyme regulation than merely by measuring enzyme activity per se.

Residual Mg^{++}-ATPase activity did not show a consistent response to the contaminant gradient but since this is likely to be a mixture of non-specific ATPase activities interpretation of these data is difficult. It is worth noting, however, that a very high correlation ($p < 0.01$) was observed between P450 1A1 and Mg^{++}-ATPase at the stations studied.





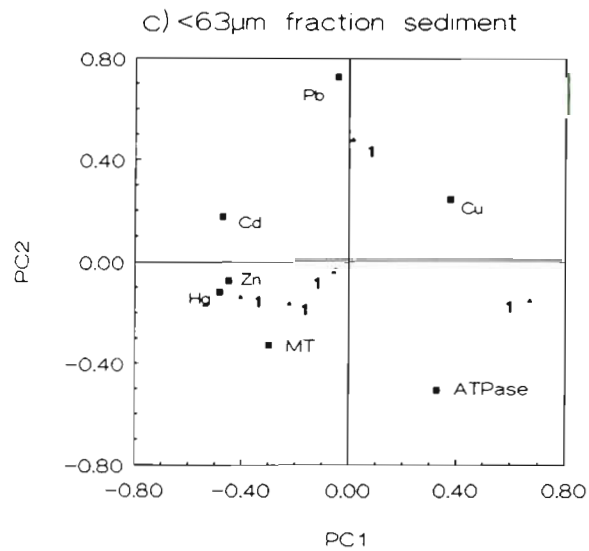


Fig. 7. Principal component analysis of Na^+, K^+-ATPase turnover, metallothionein and heavy metals in (a) liver, (b) total sediment, and (c) < 63 µm fraction of the sediment for each station in the German Bight. Also shown is the site ordination for each of the stations sampled

P4501A

Levels of this protein were significantly higher at Stns 3, 5 & 9 compared to Stns 1 & 7. Induction of this isoform of P450 in liver is associated with contamination from planar micro-organic contaminants and PAH's (Goksøyr & Forlin 1992). In gills induction of P450 1A1 has been observed following both injection with model inducers (Millar et al. 1989) and environmental exposure (James & Little 1979, Stegeman et al. 1979, Lindstrom-Seppa et al. 1981). The relative levels of branchial P450 1A1 at the sites studied was surprising given the expected contaminant gradient.

Since induction of P450 1A1 has been associated with elevated levels of polyaromatic hydrocarbons and planar organochlorine compounds it was envisaged that higher levels would be expected at the innermost stations and perhaps Stn 9 as a reflection of the defined chemical gradients (Cofino et al. 1992). However, the German Bight gradient is very complex and a variety of toxicants have been shown to inhibit expression and/or activity of P450 systems even in the presence of inducers. For example George (1989) showed that injection of cadmium inhibited both ethoxyresorufin o-deethylase (EROD) activity and P450 1A1 expression in plaice *Pleuronectes platessa* liver. The highest concentrations of contaminants were found in the sediments at Stns 1 & 3 and this, combined with the intimate relationship between the gill epithelium and the environment, may explain the lower P4501 A1 expression in the gills at these stations.

Correlations between P450 1A1 levels and the different CB congeners in liver, total sediment and the < 63 µm fraction of sediments show that in relation to CB's in total sediment and liver there are only weak correlations with P450 1A1. However in relation to CB's in the < 63 µm fraction there was much greater resolution of individual congeners. It is interesting to compare the ranking of these congeners with the accepted toxicity ranking of CB's based on their capacity to induce EROD activity in model systems (Voogt et al. 1991). Of the CB's measured CB 105 has the highest toxicity ranking and also the highest correlation with P450 1A1 in the gill.

Metallothionein

The use of polarography for the measurement of metallothionein is well established (Olafson & Sim 1979, Thompson & Cosson 1984). However, the method used in this study is non-specific in that it will measure all sulphydryl-rich proteins in the heat stable, cytoplasmic fraction. That this is a good estimate of the metallothionein levels is confirmed by the studies of Hogstrand & Haux (1990) who showed a close linear relationship between polarographic and radioimmunoassay methods of measurement.

It is clear from this study that stations in the German Bight closest to the Elbe and showing the greatest degree of contamination of the total sediment with metals (Cofino et al. 1992) also had elevated levels of zinc and cadmium in the gills (Fig. 4). The concentration of copper in the gills was not significantly different between stations despite the elevated levels of copper at the coastal stations. Branchial metallothionein levels were also significantly elevated at the innermost stations on the transect (Fig. 5a) and there was a clear relationship between the sum of the molar concentrations of cadmium, copper and zinc and metallothionein in the gills (Fig. 5b). Since zinc was present at much higher concentrations of copper or cadmium it was predominant in driving this relationship. It is interesting to compare these branchial responses with those found in the liver of the same fish (Hylland et al. 1992). Here it has been suggested that other factors in addition to environmental metal contamination, such as vitellogenesis, rates of accumulation in food organisms and migration, have played a modulatory response in determining the overall environmental response. Therefore, effects in the gill provide a better indicator of the effects of environmental contaminants, possibly as a consequence of the intimate relationship between the gill and the environment and/or the rapid turnover of tissue within the gill.

Relationship to sediment contamination

Both organic micropollutants (Ernst et al. 1988) and heavy metals (Salomons & Forstner 1984) have a high affinity for sorption to fine particulates in sediment. It has therefore been argued that measurement of the contaminant burden in the fine particulate fraction will give an accurate measurement of the input of the contaminants into the sediment (Ernst et al. 1988, Cofino et al. 1992). For this reason contaminant concentrations are often measured in both the total and the < 63 µm fraction of the sediment (Cofino et al. 1992). The relative biological significance of the measurements in either of these matrices is unclear. However, in the context of the present results principal components and correlation analyses of the biological effects response in gills have revealed that P450 1A1 and Na^+,K^+-ATPase have highest correlations (positive and negative respectively) with contaminants in the < 63 µm fraction but that metallothionein and metal concentrations in the gill were more closely correlated with total sediment metal burdens. This may imply that factors governing availability and subsequent effect are different for metal and organic contaminants respectively and is obviously an area which merits further study.

In conclusion, it is clear that this study has demonstrated the utility of the gill as a tissue which responds directly to environmental contamination as indicated by the sedimentary burden at each of the sites studied, and the complementary nature of the responses measured in detoxication systems and by effects on Na^+,K^+-ATPase turnover. Finally, the results also demonstrate that studies of adaptive responses by enzyme systems may be more sensitive indicators to use in pollution monitoring than measurement of activity alone.

Acknowledgements. We are grateful to Rob Fryer for help with the principal components analysis.

LITERATURE CITED

Atkinson, A., Gatemby, A. O., Lowe, A. G. (1973). The determination of inorganic orthophosphate in biological systems. Biochim. Biophys. Acta 320: 195–204

Benson, W. H., Birdge, W. J. (1985). Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. Environ. Toxicol. Chem. 4: 209–217

Boese, B. L., Johnson, V. G., Chapman, D. E., Ridlington, J. W., Randall, R. (1982). Effects of petroleum refinery wastewater exposure on gill ATPase and selected blood parameters in the Pacific staghorn sculpin (*Leptocottus armatus*). Comp. Biochem. Physiol. 71C (1): 63–67

Bouquegneau, J. M. (1977). ATPase activity in mercury intoxicated eels. Experentia 33: 941–943

Bouquegneau, J. M., Gerday, C., Disteche, A. (1975). Fish mercury-binding thionein related to adaptation mechanisms. FEBS Lett. 55: 173–177

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–251

Bradley, R. W., Duquesnay, C., Sprague, J. B. (1985). Acclimation of rainbow trout, *Salmo gairdneri* Richardson, to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish Biol. 27: 367–379

Brdicka, R. (1933). Polarographic studies with the dropping mercury cathode. Part XXXI. A new test for proteins in the presence of cobalt salts in ammoniacal solutions of ammonium chloride. Collect. Czech. Chem. Commun. 5: 112–128

Butler, D. G., Carmichael, F. J (1972). (Na^+-K^+)-ATPase activity in eel (*Anguilla rostrata*) gills in relation to changes in environmental salinity: role of adrenocortical steroids. Gen. comp. Endocrinol. 19: 421–427

Cofino, W. P., Smedes, F., de Jong, S. A., Abarnou, A., Boon, J. P., Oostingh, I., Davies, I. M., Klungsøyr, J., Wilhelmsen, S., Law, R. J., Whinnett, J. A., Schmidt, D., Wilson, S. (1992). The chemistry programme. Mar. Ecol. Prog. Ser. 91: 47–56

Davis, P. W., Wedemeyer, G. A. (1971). Na^+,K^+ activated-ATP ase inhibition in rainbow trout: a site for organochlorine pesticide toxicity? Comp. Biochem. Physiol. 40B: 823–827

Ernst, W., Boon, J. P., Weber, K. (1988). Occurrence and fate of micropollutants in the North Sea. In: Salomons, W., Bayne, B. L., Duursma, E. K., Forstner, U. (eds.) Pollution of the North Sea: an assessment. Springer-Verlag, Berlin, p. 284–299

Forrest, J. N., Cohen, A. D., Schon, D. A., Epstein, F. H. (1973). Na transport and Na-K-ATPase in gills during adaptation to seawater: effects of cortisol. Am. J Physiol. 224(3): 709–713

George, S. G. (1989). Cadmium effects on plaice liver xenobiotic and metal detoxication systems: dose response. Aquat. Toxicol. 15: 303–310

Gick, G. G., Ismail-Beigi, F., Edelman, I. S. (1988). Hormonal regulation of Na,K-ATPase. Prog. clin. biol. Res. 268B: 277–295

Goksøyr, A. (1991). A semi-quantitative cytochrome P450IA1 ELISA: a simple method for studing the monooxygenase response in environmental monitoring and ecotoxicological testing of fish. Sci. Tot. Environ. 101: 255–262

Goksøyr, A., Forlin, L. (1992). Biotransformation enzymes in fish aquatic toxicology and environmental monitoring. Aquat. Toxicol. 22: 287–312

Hogstrand, C., Haux, C. (1990). Comparison of polarography and radioimmunoassay for the quantification of metallothionein in perch (Perca fluviatilis). Mar. environ. Res. 28: 187–190

Hylland, K., Haux, C., Hogstrand, C. (1992). Hepatic metallothionein and heavy metals in dab *Limanda limanda* from the German Bight. Mar. Ecol. Prog. Ser. 91: 89–96

James, M. O., Little, P. J. (1979). Polyhalogenated hydrocarbons and phenobarbitol: evaluation as inducers of drug metabolising enzymes in the sheepshead *Archosargus probatocephalus*. Chem. Biol. Interact. 36: 229–248

Janicki, R. H., Kinter, W.B. (1971). DDT inhibits Na^+,K^+, Mg^{2+}-ATPase in the intestinal mucosae and gills of marine teleosts. Nature, New Biol. 233: 148–149

Klaverkamp, J. F., Duncan, D. A. (1987). Acclimation to cadmium toxicity by white suckers: cadmium binding capacity and metal distribution in gill and liver cytosol. Environ. Toxicol. Chem. 6: 275–289

Kito, H., Tazawa, T., Ose, Y., Sato, T., Ishikawa, T. (1982). Formation of metallothionein in fish. Comp. Biochem. Physiol. 73C: 129–134

Kuhnert, P. M., Kuhnert, B. R. (1976). The effects of in vivo chromium exposure on Na/K- and Mg-ATPase activity in several tissues of the rainbow trout (*Salmo gairdneri*). Bull. environ. Contam. Toxicol. 15, 383–390

Lauren, D. J., McDonald, D. G. (1987). Acclimation to copper by rainbow trout, *Salmo gairdneri*: biochemistry. Can. J. Fish. Aquat. Sci. 44: 105–111

Leadem, T P., Campbell, R. D., Johnson, D. W. (1974). Osmoregulatory response to DDT and varying salinities in *Salmo gairdneri*. I. Gill ATPases. Comp. Biochem. Physiol. 49A: 197–205

Lindström-Seppa, P., Koivusaari, U., Hanninen, O. (1981). Extrahepatic xenobiotic metabolism in North-European freshwater fish. Comp. Biochem. Physiol 69C: 259–263

Madsen, S. S. (1990). Effect of repetitive cortisol and thyroxine injections on chloride cell number and Na^+/K^+-ATPase in gills of freshwater acclimated rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 95A: 171–175

Millar, M. R., Hinton, D. E., Stegeman, J. J., (1989). Cytochrome P-450E induction and localisation in gill pillar (endothelial) cells of scup and rainbow trout. Aquat. Toxicol. 14: 307–322

Noel-Lambot, F., Gerday, C. H., Disteche, A. (1978). Distribution of Cd, Zn and Cu in liver and gills of the eel *Anguilla anguilla* with special reference to metallothioneins. Comp. Biochem. Physiol. 61C: 177–187

Norey, C. G., Cryer, A., Kay, J. (1990a). Induction of metallothionein gene expression by cadmium and the retention of the toxic metal in the tissues of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 97C: 215–220

Norey, C. G., Cryer, A., Kay, J. (1990b) A comparison of cadmium-induced metallothionein gene expression and Me^{2+} distribution in the tissues of cadmium sensitive (rainbow trout; *Salmo gairdneri*) and tolerant (stone loach; *Noemacheilus barbatulus*) species of freshwater fish. Comp. Biochem. Physiol. 97C: 221–225

Olsson, P.-E., Larsson, A., Maage, A., Haux, C., Bonham, K. (1989). Induction of metallothionein synthesis in rainbow trout (*Salmo gairdneri*) during long-term exposure to waterborne cadmium. Fish Physiol. Biochem. 6: 221–229

Olsson, P.-E., Hogstrand, C. (1987). Subcellular distribution and binding of cadmium to metallothionein in tissues of rainbow trout after exposure to ^{109}Cd in water. Environ. Toxicol. Chem. 6: 867–874

Olafson, R. W., Sim, R. G. (1979). An electrochemical approch to quantitation and characterization of metallothioneins. Anal. Biochem 100: 343–351

Pinkney, A. E., Wright, D. A., Jepson, M. A., Towle, D. W. (1989). Effects of tributyl tin compounds on ionic regulation and gill ATPase activity in estuarine fish. Comp. Biochem. Physiol. 92C(1): 125–129

Reichert, W. L., Federighi, D. A., Malins, D. C. (1979). Uptake and metabolism of lead and cadmium in coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 63C: 229–234

Salomons, U., Forstner, U. (1984). Metals in the hydrocycle. Springer-Verlag, Berlin

Sheppard, K., Simkiss, K. (1978). The effects of heavy metal ions on Ca^{2+} ATPase extracted from fish gills. Comp. Biochem. Physiol 61B: 69–72

Stagg, R. M., Rusin, J., Brown, F. (1992). Na^{+},K^{+}-ATPase activity in the gills of the flounder (*Platichthys flesus L.*) in relation to mercury contamination in the Firth of Forth. Environ. Res. 33: 255–266

Stagg, R. M., Shuttleworth, T. J. (1982). The effects of copper on ionic regulation by the gills of the seawater-adapted flounder (*Platichthys flesus L.*). J. comp. Physiol. 149(1): 83–90

Stegeman, J. J, Binder, R. L., Orren, A. (1979). Hepatic and extrahepatic microsomal electron transport components and mixed function oxidases in the marine fish *Stenotomus versicolor*. Biochem. Pharmac. 28: 3431–3439

Thompson, J., Cosson, R. (1984). An improved electrochemical method for the quantification of metallothionein in marine organisms. Mar. environ. Res. 11: 137–152

Thomas, D. G., Brown, M. W., Shurben, A., Solbe, De L. G., Cryer, A., Kay, J. (1985). A comparison of the sequestration of cadmium and zinc in the tissues of rainbow trout (*Salmo gairdneri*) following exposure to the metals singly or in combination. Comp. Biochem. Physiol. 82C: 55–62

Thomas, D. G., Cryer, A., Solbe, De L. G., Kay, J (1983). A comparison of the accumulation and protein binding of environmental cadmium in the gills, kidney and liver of rainbow trout (*Salmo gairdneri* Richardson). Comp. Biochem. Physiol. 76C: 241–246

Verma, S. R., Mohan, D., Tyagi, A. K., Dalela, R. C. (1979). In vivo response of ATPases in few tissues of the fish *Mystus vittatus* (Ham.) to the synthetic detergent Swacofix E45 (ABS). Bull. environ. Contam. Toxicol. 22: 327–331

Voogt, De P., Wells, D. E., Reutergardh, L., Brinkman, UATh (1991). Biological activity, determination and occurrence of planar, mon- and di-ortho PCB's. Int. J. anal. Chem. 40: 1–46

Watson, C. F., Benson, W. H. (1987). Comparative activity of gill ATPase in three freshwater teleosts exposed to cadmium. Ecotox. Environ. Saf. 14: 252–259

Watson, T. A., Beamish, F. W. H. (1980). Effects of zinc on branchial ATPase activity in vivo in rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 66C: 77–82

Watson, T. A., Beamish, F. W. H. (1981). The effects of zinc on branchial adenosine triphosphatase enzymes in vitro from rainbow trout, *Salmo gairdneri*. Comp. Biochem. Physiol. 68C: 167–173

Zafarullah, M., Olsson, P.-E., Gedamu, L. (1989). Endogenous and heavy-metal-ion-induced metallothionein gene expression on salmonid tissues and cell lines. Gene 83: 85–93