Short Communication

The relation between concentrations of ovarian trace elements and the body size of Atlantic cod *Gadus morhua*

A. Bang, P. Grønkjær, and B. Lorenzen

Bang, A., Grønkjær, P., and Lorenzen, B. 2008. The relation between concentrations of ovarian trace elements and the body size of Atlantic cod *Gadus morhua*. – ICES Journal of Marine Science, 65: 1191–1197.

Trace metals in the ovaries of fish are transferred from the female via the yolk to the offspring, which makes the early life stages susceptible to deleterious effects of potentially toxic elements contained in the ovaries. Here, the concentrations of 13 elements from the ovaries of 133 ripe female North Sea cod *Gadus morhua* weighing 0.2–18 kg were correlated with female size, accounting for differences in maturity and condition. Most elements were negatively correlated with the size variables weight, length and, especially, ovarian dry weight. Further, they were negatively correlated with maturity and condition. Many of the trace elements showed true size-dependence, but the correlations were generally weak. A linear discriminant analysis separated "small" and "large" fish at a length of 85 cm based on concentrations of Co, Mn, Se, and Zn, and correctly assigned 78 of 102 small fish and 23 of 31 large fish to their respective size category. This corresponds to an overall classification success of 75.9%. The results suggest that embryos and early larvae from small females are exposed to higher levels of potentially harmful metals. If the differences in trace element concentration influence survival success, this will add to the negative effects of size distribution truncation and declines in size-at-maturity experienced by many populations of cod.

Keywords: bioaccumulation, elemental fingerprint, gonads, maternal transfer, North Sea, size-dependence.

Received 31 October 2007; accepted 11 May 2008; advance access publication 3 June 2008.

A. Bang and P. Grønkjær: Marine Ecology, Department of Biological Sciences, University of Aarhus, Finlandsgade 14, 8200 Aarhus N, Denmark. B. Lorenzen: Plant Biology, Department of Biological Sciences, University of Aarhus, Ole Worms Allé 1135, 8000 Aarhus C, Denmark. Correspondence to A. Bang: tel: +45 89424379; fax: +45 89424387; e-mail: anders.bang@biology.au.dk.

Introduction

Biological factors are important in determining metal accumulation and, hence, toxicity, in marine fish (Ansari *et al.*, 2004). Potentially important biological factors are the kinetics of metal turnover, metal regulation and storage, feeding strategies, growth (including the effects of size and age), sex, and maturation (Langston and Spence, 1995). Of these, the most extensively studied biological factor is body size. However, for fish, there is no consistent relationship between the concentration of different metals and body or organ size (Powell *et al.*, 1981; Coetzee *et al.*, 2002; Canli and Atli, 2003; Anan *et al.*, 2005). It is clear that different metals have their own characteristics and that there are large variations in the metal uptake of different tissues (Brim *et al.*, 2001). Therefore, analyses of whole fish yield little insight into the fate of metals and their ultimate effects.

The main focus of metal toxicological study has been on the heavy metals Cd, Cu, Hg, Pb, and Zn, and their accumulation in white muscle tissue as well as various internal organs such as the liver (e.g. Greig and Wenzloff, 1977; Hellou *et al.*, 1992; Auounsson, 1999; Mormede and Davies, 2001; Anan *et al.*, 2005). Few studies have examined size-related trace metal content in ovaries, and these only examined metal accumulation as a function of ovary or fish size, often with few fish and a limited size spectrum (Hellou *et al.*, 1992, 1996; Márquez *et al.*, 1998). However, a large part of the variation in metal concentrations can be ascribed to maturity stage (Julshamn and Brækkan, 1977; Miramand *et al.*, 1991), and likely also condition through its effects on resource allocation to the ovaries (Kjesbu *et al.*, 1991).

Ovarian element levels are relevant because the early life stages are more susceptible to the deleterious effects of contaminants than the adult stages, e.g. both non-essential and essential metals may alter embryonic development of fish causing retardation of normal development, disability of organs, or mortality (Weis and Weis, 1989). Consequently, any size-dependence in ovarian trace element concentrations may have implications for offspring survival, so a model for predicting the heavy metal exposure for offspring of females of different size is warranted, considering the large changes in size distribution, size-at-age, and size-at-maturity of commercial fish associated with fishing (Cardinale and Modin, 1999). Moreover, any size-related trend in ovarian trace metal concentration is likely to be imprinted in the trace element concentration of the eggs and the newly hatched larvae. In that case, the trace element concentration of the early life stages can potentially be used to reveal maternal size and become an important tool in field-based investigations

of relationship between female size, reproductive output, and survival of the offspring.

Here, the concentrations of 20 elements in the ovaries of 133 ripe female North Sea cod *Gadus morhua* weighing from 0.2 to 18 kg were analysed and correlated with female size, accounting for differences in maturation stage and condition. This was done to assess the impact of female size on the trace element concentration of the offspring, and to evaluate the possibility of using trace element concentration of individual field-caught eggs or larvae as an indicator of maternal size.

Material and methods

Sample collection

In all, 133 ripe female cod were caught in the North Sea, the Skagerrak, and the Kattegat (Figure 1) during the main spawning season from January to February of 2006. The fish were collected as part of the annual ICES monitoring programme 'International Bottom Trawl Surveys', which samples each ICES statistical rectangle at least twice annually (ICES, 2004). The samples were supplemented with commercial catches to increase the number of very large cod available for the analysis. Cod from the three areas were analysed together because the dynamics of the populations in the three areas are remarkably similar (Chen *et al.*, 2005), and there is no evidence to suspect the presence of separate stocks (K. Brander, ICES, pers. comm.). Moreover, within the sampling area, the size range and distribution of cod were quite similar (i.e. large cod were not just taken from one subarea).

On the day of capture, the total length (from the tip of the snout to the tip of the caudal fin) and wet weight (total body weight and ovary weight) of each fish was measured. A four-stage maturity key was used to assess the state of the ovaries (ICES, 2004). All mature prespawning females without running eggs (stage two) were included in the analyses. No further subsampling was performed. As oocyte-size distribution is homogenous in cod ovaries (Kjesbu *et al.*, 1990), a small sample of one of the ovary lobes was taken as representative and frozen on board the ship at -20°C in individual polyethylene bags until their subsequent laboratory analysis ashore.

Element analyses

In the laboratory, the ovary samples were rinsed in ultra-filtered water (18.2 MΩ cm) to remove traces of blood and particulates. Wet weight was recorded and the samples subsequently freeze-dried to constant weight in polyethylene bags (96 h). Dry weight was then recorded and the water content of the ovaries determined by the individual differences in wet and dry mass. After drying, samples were gently crushed inside their respective bags by hand, allowing the selective removal of the oocytes without including the surrounding connective tissue layers. The ovary samples (now only oocytes) were digested in a microwave sample preparation system (Anton Paar Multiwave 3000) as follows. Replicates of each sample with a known weight ($\sim$1 g dry weight) were transferred to Teflon fluoropolymer (PFA) vessels, and 4.0 ml HNO_3 (65%), 2.3 ml H_2O_2 (30%), and 0.5 ml HCL (36–38%) were added. The high-pressure vessels were closed and the microwave oven programmed to increase intensity to 1400 W during a 20 min. ramping period, followed by a 25 min temperature controlled (max. 210°C) intensity of maximum 1400 W. Replicates of each ovary sample were pooled following digestion (always from the same run) to provide enough material for analyses with ICP-OES. The digests were transferred to acid-washed polyethylene flasks and ultra-filtered water was added to a final volume of 50 ml. The flasks were stored cool until element analysis. In each digestion run (16 vessels), two

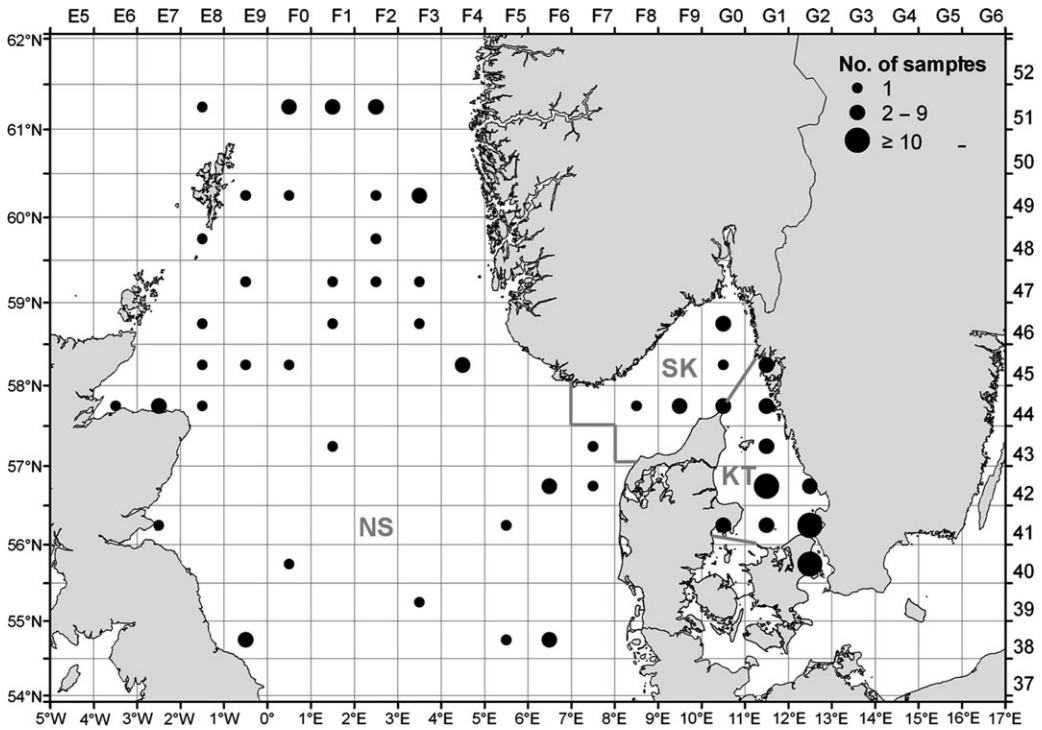

Figure 1. Map showing the distribution of the cod sampled. The cod were caught by bottom trawl in the main spawning season of 2006, January/February. NS, North Sea; SK, Skagerrak; KT, Kattegat.

procedural blanks were analysed and a duplicate analysis of a random ovary sample performed to determine the precision of analysis.

Elements were quantified with ICP-OES (Perkin Elmer Optima 2000DV) equipped with a Meinhard concentric nebulizer and a cyclonic spray chamber. Limits of detection were defined as 3σ of the procedural blank values. All samples were measured three times and only elements with relative standard deviation (RSD) $<10\%$ were accepted. Drift of the instrument was controlled by remeasuring standards after every tenth sample. Further, ovary samples were analysed randomly with respect to the size of the fish. In all, 20 elements, As, B, Ba, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sr, Zn, and V, were analysed. Concentrations are expressed as μg element g^{-1} dry weight ovary.

Statistical analyses

Size measures were length, weight, and ovary dry weight. Maturity was represented by the gonadosomatic index (GSI), which was calculated as $GSI = 100 \times OW/(W - OW)$, where OW is ovary wet weight (g) and W whole body wet weight (g). A measure of fish condition was represented by a somatic Fulton's condition factor, K, which was calculated as $K = 100 \times (W - OW)/L^3$, where L is the total length of the fish.

All data were initially checked for normality (Kolmogorov–Smirnov test) and log-transformed where appropriate, except GSI, which was arcsine-transformed. To test for a relation between size and ovarian trace element concentrations, simple bivariate correlations (Pearson product-moment correlations) were calculated. Further, to control for confounding effects of GSI and condition, partial correlations were subsequently calculated, holding these traits constant.

Linear discriminant analysis, DFA, was used to develop elemental fingerprints and hence to classify fish from different size classes (based on total length). Only those elements that showed a significant relationship with fish size were included. To simulate classification by discriminant function rules to an unknown test set, a jackknifed classification matrix was computed. The goal was to separate "small" and "large" fish, but no specific *a priori* boundary between the two groups exist. Instead, the two size groups which showed the most significant statistical separation were found by an iterative process. Minimum group size was set to 20 fish to ensure a robust test.

Results

Somatic and ovary trace element concentration data are listed in Table 1. The cod sampled had a wide size range and included a notable number of very large animals (Figure 2). Of the 20 elements analysed, two failed to meet the procedural standards; Ba showed poor recovery in the duplicate samples, and Ni consistently had RSD values $>10\%$. Further, five elements were consistently below the detection limits of the apparatus (B, Cs, Mo, Pb, and V). This left 13 elements for further analyses (Table 1).

Simple bivariate correlations between biological variables and trace element concentrations are presented below the diagonal in Table 2. From the extensive correlation matrix, several general relations are evident. First, most trace elements correlated with the size variables and the strongest relationship was with ovary dry weight. Second, the correlations were negative, i.e. the larger the fish, the lower the relative quantity of trace element in the ovaries. Third, several trace elements correlated significantly with the maturity stage of the fish (GSI), but weakly with the condition of the fish. Finally, the trace elements were positively correlated with each other, which is attributed to their common dependence on size, maturation, and condition.

Table 1. Biological data and element concentrations (μg g^{-1} dry weight ovary) in 133 North Sea cod.

Variable	Mean	s.e.	Range
Length (cm)	67	1.8	26–117
Weight (kg)	4.677	0.380	0.190–17.900
Ovary dry weight (g)	488	53.0	6–3 825
GSI (%)	12.2	0.53	0.8–31.6
Ovary water content (%)	75.0	0.46	68.4–88.9
Fulton's *K*	0.99	0.011	0.77–1.35
As	2.68	0.101	0.34–8.54
B	<0.022	–	–
Cd	0.0394	0.00180	0.0048–0.142
Co	0.0604	0.00281	0.014–0.173
Cr	0.478	0.00513	0.355–0.733
Cs	<0.00061	–	–
Cu	4.08	0.0870	2.36–8.76
Fe	34.8	0.953	18.9–77.2
Li	0.114	0.0045	0.051–0.339
Mg	460.3	13.4	267.6–1 262
Mn	4.03	0.168	1.01–14.81
Mo	<0.067	–	–
Pb	<0.060	–	–
Rb	6.55	0.213	3.77–22.30
Se	3.79	0.0805	2.06–6.61
Sr	2.40	0.145	0.85–16.9
Zn	164.1	3.08	113.7–377.6
V	<0.0066	–	–

Partial correlations are presented above the diagonal in Table 2. When the effects of maturity stage and condition are accounted for Co, Mn, and Se still correlated negatively with all size measures, whereas Cr, Fe, and Rb correlated negatively with

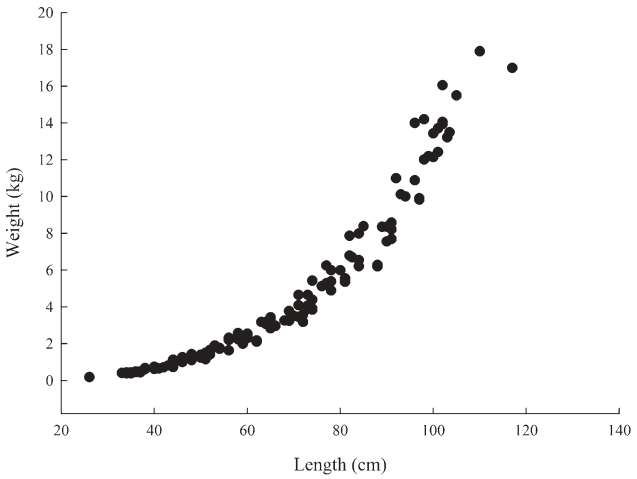

Figure 2. Plot of fish length against fish weight to show size distribution of sampled cod ($n = 133$). Data fit a power curve, $y = 6.19 \times 10^{-6} x^{3.14}$, $r^2 = 0.99$.

Table 2. Pearson product-moment correlations, *r*, between biological variables and trace elements.

Variable	SL	WW	GDW	GSI	K	As	Cd	Co	Cr	Cu	Fe	Li	Mg	Mn	Rb	Se	Sr	Zn
Length (SL)		0.98**	0.96**	–	–	−0.01	0.02	−0.23**	−0.12	0.01	−0.11	−0.01	0.05	−0.17*	−0.14	−0.26**	0.09	0.20*
Weight (WW)	0.98**		0.98**	–	–	−0.02	0.01	−0.20*	−0.10	−0.01	−0.11	−0.01	0.04	−0.18*	−0.13	−0.25**	0.06	0.20*
Ovary DW (GDW)	0.88**	0.89**		–	–	−0.05	−0.04	−0.24**	−0.18*	−0.06	−0.18*	−0.04	−0.04	−0.23**	−0.21*	−0.25**	0.04	0.13
GSI	0.27**	0.28**	0.66**		–	–	–	–	–	–	–	–	–	–	–	–	–	–
Condition (K)	0.27**	0.33**	0.13	−0.24**		–	–	–	–	–	–	–	–	–	–	–	–	–
As	−0.07	−0.08	−0.25**	−0.38**	0.19*		0.81**	0.09	0.08	−0.06	−0.05	0.06	0.00	0.09	−0.08	0.09	0.25**	0.16
Cd	−0.05	−0.06	−0.24**	−0.38**	0.20*	0.84**		0.02	0.02	−0.03	−0.07	0.19*	−0.09	−0.03	−0.13	0.18*	0.36**	0.24**
Co	−0.31**	−0.29**	−0.30**	−0.11	−0.19*	0.08	0.03		0.28**	0.29**	0.31**	0.01	0.17	0.33**	0.21*	0.36**	0.13	0.17
Cr	−0.28**	−0.28**	−0.49**	−0.53**	0.01	0.26**	0.22*	0.34**		0.54**	0.46**	−0.25**	0.60*	0.42**	0.67*	0.08	−0.16	0.36**
Cu	−0.17*	−0.19*	−0.42**	−0.55**	0.06	0.17	0.19*	0.31**	0.69**		0.47**	−0.16	0.64*	0.33**	0.62*	−0.07	−0.06	0.30**
Fe	−0.24**	−0.26**	−0.40**	−0.35**	−0.08	0.08	0.07	0.50**	0.58**	0.56**		−0.13	0.58*	0.40**	0.60*	−0.09	−0.05	0.10
Li	0.02	0.01	0.14	0.34**	−0.27**	−0.15	−0.07	0.33**	−0.19*	−0.21*	0.16		−0.27*	−0.19*	−0.22*	0.12	0.53**	0.15
Mg	−0.17*	−0.20*	−0.45**	−0.57**	−0.02	0.20*	0.14	0.39**	0.73**	0.73**	0.74**	−0.01		0.44**	0.83*	−0.09	−0.15	0.42**
Mn	−0.32**	−0.34**	−0.43**	−0.32**	−0.18*	0.19*	0.09	0.34**	0.52**	0.46**	0.45**	−0.17	0.52**		0.48*	0.19*	−0.15	0.15
Rb	−0.30**	−0.32**	−0.42**	−0.28**	−0.25**	0.02	−0.01	0.51**	0.64**	0.56**	0.75**	0.31**	0.84**	0.45**		−0.03	−0.19*	0.33**
Se	−0.36**	−0.36**	−0.48**	−0.45**	0.03	0.25**	0.32**	0.37**	0.32**	0.22*	0.15	−0.03	0.25**	0.31**	0.17*		0.13	0.17
Sr	0.11	0.09	0.10	0.09	0.01	0.17	0.27**	0.24**	−0.13	−0.07	0.07	0.54**	−0.03	−0.15	0.05	0.10		0.10
Zn	−0.02	−0.03	−0.35**	−0.65**	0.21*	0.37**	0.42**	0.23**	0.60**	0.57**	0.35**	−0.09	0.64**	0.33**	0.42**	0.42**	0.04	

Correlations below the diagonal are bivariate, and correlations above the diagonal are partial. Statistical significance is marked as *$p < 0.05$ and **$p < 0.01$.

ovary weight only. Finally, Zn correlated positively with length and weight.

The linear discriminant analysis separated "small" and "large" fish at 85 cm TL using data on Co, Mn, Se, and Zn concentrations. A MANOVA confirmed a difference in the elemental fingerprint between the two groups ($p < 0.001$). The analysis correctly assigned 78 of 102 fish $\leq$85 cm TL and 23 of 31 fish $>$85 cm TL to the correct size category. This corresponds to 75.9% of the individuals being correctly assigned.

Discussion

We have presented information on the amount of 18 trace metals in the ovaries of ripe North Sea cod (Table 1). The highest concentrations are those of biologically essential elements such as Mg, Zn, and Fe, which are important components of certain enzymes or involved in oxygen transport. Generally, element levels are of the same order of magnitude and therefore comparable with concentrations found in cod from other areas (Julshamn and Brækkan, 1977; Hellou *et al*., 1992).

A significant negative relationship between the levels of 11 elements in the ovary and organ size (Table 2, below diagonal) was demonstrated. Furthermore, a significant negative relationship between the levels of eight elements and fish size was found. Elements with and without known metabolic functions exhibited a relation with size, and there were no trends concerning atomic number or element group. Negative associations between tissue size and specific element concentrations are supported by the literature and have been reported for many fish species (Hellou *et al*., 1992; Coetzee *et al*., 2002; Canli and Atli, 2003; Anan *et al*., 2005). The most probable explanation is the decreased weight-specific metabolic rate with increased fish size and the dilution of tissue metal concentrations attributable to growth (Langston and Spence, 1995). A lowered metabolic activity in older fish means a smaller relative uptake of elements. Consequently, such fish are able to regulate their metal content through excretion and are proposed to be in a steady state with ambient levels (except when ambient levels are higher than the capacity of these factors). On the contrary, smaller fish are not able to compensate for their relatively high intake of elements through regulatory processes (Langston and Spence, 1995).

There was a high degree of positive inter-element correlation. This is probably a consequence of the common dependence on fish and organ size, but also indicates similar transport, utilization, and/or elimination rates of these elements. No clear relation between the condition of the fish and element levels was evident (Table 2). When in poor condition, cod give priority to egg size rather than number, i.e. egg size is maintained at the expense of the number of eggs spawned (Kjesbu *et al*., 1996). Hence, condition should not affect mass-specific element levels. Conversely, the GSI correlated negatively with trace element levels, as seen before for cod (Julshamn and Brækkan, 1977) and red mullet (*Mullus surmuletus*; Miramand *et al*., 1991), but was itself correlated with fish size. This and the effect of condition was removed by calculating partial correlations and revealed that some trace elements were still associated with size beyond what could be accounted for through their relationship with GSI% and condition (Table 2, above diagonal). In particular, Co, Cr, Fe, Mn, Rb, and Se all correlated negatively with one or more of the size measures. Interestingly, Zn displayed a positive relationship with size. Zinc levels in the ovary are higher than in any other organ in cod (Julshamn and Brækkan, 1977), and this seems to be a general phenomenon across species (Hamza-Chaffai *et al*., 1996). Zinc plays an important role in reproduction and sexual maturation, so is actively transported to and stored in the developing ovary (Ansari *et al*., 2004).

Many of the trace elements examined showed true size-dependence, but the individual correlations were generally weak and size alone could explain no more than 10% of the variation in trace element levels (Table 2). Although size and metabolic capacity are clearly important factors in determining the trace element concentration of the individual organism, various physical factors may interact, e.g. ambient level and bioavailability. The cod we used were sampled from a relatively large area (Figure 1), and although ecologically similar, the existence of subpopulations and genetic differences between individual organisms in, for instance, metabolic capacity cannot be ruled out (Knutsen *et al*., 2003; Nielsen *et al*., 2005). More importantly, the individual correlation analyses assume that the relationship between size and element level is linear. This is highly conceivable when considering metabolic intensity within a species past the juvenile stage (Glazier, 2005). However, there are several factors which change non-linearly with fish size and age, and so may adversely influence the analyses. These factors include habitat (Heessen and Daan, 1994), diet, and trophic level (Greenstreet, 1996; Jennings *et al*., 2002), which are likely to influence the accumulation of trace elements in fish.

Our present analysis shows that the gonads of small spawning female cod have higher trace element concentrations than larger females, and hence that the eggs and early larvae from small females are likely to be more exposed to potential detrimental effects of, for example, toxic heavy metals. The fresh-water literature particularly abounds with studies showing significant sublethal effects of metals on development, hatchability, behaviour, and secondary mortality (e.g. Williams and Holdway, 2000; Vosyliene *et al*., 2003; Kusch *et al*., 2007), which ultimately may influence the population dynamics of the fish. Because many marine fish populations, including cod, have experienced a significant reduction in size-at-maturity and truncation of the size distribution towards younger ages (Cardinale and Modin, 1999; Yoneda and Wright, 2004; Barot *et al*., 2004), the potential effect of trace element concentration on recruitment and population dynamics is likely to be augmented. This further adds to the negative effects of fisheries-induced changes in size structure of marine fish populations.

Some studies have indicated that offspring from older or larger females survive better than the offspring from small females, and hence that large fish are crucial to the reproductive success of the populations (e.g. Trippel, 1998; Berkeley *et al*., 2004). However, a field-based test of the survival of offspring from large vs. small females is extremely difficult to construct, because it would entail tracing whether an individual egg or larva is the offspring of a large or a small female. Our study gives an indication that trace elements may be used to this end. Based on four trace elements, it was possible to classify gonads to large ($>$85 cm) or small ($\leq$85 cm) females. Hence, it may be possible technically to classify individual eggs or early larvae to two or more size groups of females. Especially interesting in this respect is the development in otolith microchemistry, which is already being used to trace individual larval and adult fish back to their natal regions based on the trace element composition of the core of the otolith (Warner *et al*., 2005). It is conceivable that the trace

element composition of the core also carries a maternal fingerprint that may be used to reveal maternal origin.

In conclusion, we have presented element levels from a large number and wide size range of cod in a study focusing solely on ovary composition. We have shown a significant negative relation between fish size and the ovarian content of the trace elements Co, Cr, Fe, Mn, Rb, and Se, after accounting for the interacting factors maturation stage and condition. As the only exception, Zn levels exhibit a positive relationship with size. The negative relationship between fish size and trace element concentration suggests that embryos and early larvae from small females are exposed to higher levels of potentially harmful metals, but it is unknown to what extent this may negatively impact their development, behaviour, and ultimately survival.

Acknowledgements

We thank the technicians and scientists who took part in the IBTS surveys and helped supply ovary samples. B. Andersen for help with preparation and analyses of samples, and Janeck Scott-Fordsmand for help with data analysis. The project was financed by a research grant from the Carlsberg Foundation, and grant support from the Elisabeth & Knud Petersens Foundation and the Hartmann Brothers Foundation.

References

Anan, Y., Kunito, T., Tanabe, S., Mitrofanov, I., and Aubrey, D. G. 2005. Trace element accumulation in fishes collected from coastal waters of the Caspian Sea. Marine Pollution Bulletin, 51: 882–888.

Ansari, T. M., Marr, I. L., and Tariq, N. 2004. Heavy metals in marine pollution perspective—a mini review. Journal of Applied Sciences, 4: 1–20.

Auounsson, G. A. 1999. The effect of nutritional status of Icelandic cod (*Gadus morhua*) on macroconstituents and trace elements in the liver. Journal of the Marine Research Institute Reykjavík, 16: 111–129.

Barot, S., Heino, M., O'Brien, L., and Dieckmann, U. 2004. Long-term trend in the maturation reaction norm of two cod stocks. Ecological Applications, 14: 1257–1271.

Berkeley, S. A., Chapman, C., and Sogard, S. M. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology, 85: 1258–1264.

Brim, M. S., Alam, S. K., and Jenkins, L. G. 2001. Organochlorine pesticides and heavy metals in muscle and ovaries of Gulf coast striped bass (*Morone saxatilis*) from the Apalachicola River, Florida, USA. Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Wastes, 36: 15–27.

Canli, M., and Atli, G. 2003. The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, 121: 129–136.

Cardinale, M., and Modin, J. 1999. Changes in size-at-maturity of Baltic cod (*Gadus morhua*) during a period of large variations in stock size and environmental conditions. Fisheries Research, 41: 285–295.

Chen, Q., Chan, K-S., Lekve, K., Torstensen, E., Gjøsæter, J., Ottersen, G., and Stenseth, N. C. 2005. Population dynamics of cod *Gadus morhua* in the North Sea region: biological density-dependent and climatic density-independent effects. Marine Ecology Progress Series, 302: 219–232.

Coetzee, L., du Preez, H. H., and van Vuuren, J. H. J. 2002. Metal concentrations in *Clarias gariepinus* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. Water SA, 28: 433–448.

Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. Biological Reviews, 80: 611–662.

Greig, R. A., and Wenzloff, D. R. 1977. Trace metals in finfish from the New York Bight and Long Island Sound. Marine Pollution Bulletin, 8: 198–200.

Greenstreet, S. P. R. 1996. Estimation of the daily consumption of food by fish in the North Sea in each quarter of the year. Scottish Fisheries Research Report, 55. Fisheries Research Services, Aberdeen. 16 pp.

Hamza-Chaffai, A., Roméo, M., and El Abed, A. 1996. Heavy metals in different fishes from the middle eastern coast of Tunisia. Bulletin of Environmental Contamination and Toxicology, 56: 766–773.

Heessen, H., and Daan, N. 1994. Cod distribution and temperature in the North Sea. ICES Marine Science Symposia, 198: 244–253.

Hellou, J., Warren, W. G., Payne, J. F., Belkhode, S., and Lobel, P. 1992. Heavy metals and other elements in three tissues of cod, *Gadus morhua* from the Northwest Atlantic. Marine Pollution Bulletin, 24: 452–458.

Hellou, J., Zitko, V., Friel, J., and Alkanani, T. 1996. Distribution of elements in tissues of yellowtail flounder *Pleuronectes ferruginea*. The Science of the Total Environment, 181: 137–146.

ICES. 2004. Manual for the International Bottom Trawl Survey, Revision VII. ICES Document CM 2004/D: 05.

Jennings, S., Greenstreet, S. P. R., Hill, L., Piet, G. J., Pinnegar, J. K., and Warr, K. J. 2002. Long-term trends in the trophic structure of the North Sea fish community: evidence from stable-isotope analysis, size-spectra and community metrics. Marine Biology, 141: 1085–1097.

Julshamn, K., and Brækkan, O. R. 1977. The relation between the concentration of some main elements and the stages of maturation of ovaries in cod (*Gadus morhua*). Fiskeridirektoratets Skrifter Serie: Errnæring, 1: 1–15.

Kjesbu, O. S., Klungsøyr, J., Kryvi, H., Witthames, P. R., and Greer Walker, M. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Canadian Journal of Fisheries and Aquatic Sciences, 48: 2333–2343.

Kjesbu, O. S., Solemdal, P., Bratland, P., and Fonn, M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 53: 610–620.

Kjesbu, O. S., Witthames, P. R., Solemdal, P., and Greer Walker, M. 1990. Ovulatory rhythm and a method to determine the stage of spawning in Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 47: 1185–1193.

Knutsen, H., Jorde, P. E., André, C., and Stenseth, N. C. 2003. Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Molecular Ecology, 12: 385–394.

Kusch, R. C., Krone, P. H., and Chivers, D. P. 2007. Chronic exposure to low concentrations of waterborne cadmium during embryonic and larval development results in the long-term hindrance of anti-predator behaviour in zebrafish. Environmental Toxicology and Chemistry, 27: 705–710.

Langston, W. J., and Spence, S. K. 1995. Biological factors in metal concentrations observed in aquatic organisms. *In* Metal Speciation and Bioavailability in Aquatic Systems, pp. 407–478. Ed. by A. Tessier and D. R. Turner. John Wiley, New York. 679 pp.

Márquez, M., Vodopivez, C., Casaux, R., and Curtosi, A. 1998. Metal (Fe, Zn, Mn and Cu) levels in the Antarctic fish *Notothenia coriiceps*. Polar Biology, 20: 404–408.

Miramand, P., Lafaurie, M., Fowler, S. W., Lemaire, P., Guary, J. C., and Bentley, D. 1991. Reproductive cycle and heavy metals in the organs of red mullet, *Mullus barbatus* (L.), from the northwestern Mediterranean. The Science of the Total Environment, 103: 47–56.

Mormede, S., and Davies, I. M. 2001. Heavy metal concentrations in commercial deep-sea fish from the Rockall Trough. Continental Shelf Research, 21: 899–916.

Nielsen, E. E., Grønkjær, P., Meldrup, D., and Paulsen, H. 2005. Retention of juveniles within a hybrid zone between North Sea and Baltic Sea Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 62: 2219–2225.

Powell, J. H., Powell, R. E., and Fielder, D. R. 1981. Trace element concentrations in tropical marine fish at Bougainville Island, Papa New Guinea. Water, Air, and Soil Pollution, 16: 143–158.

Trippel, E. A. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Transactions of the American Fisheries Society, 127: 339–359.

Vosyliene, M. Z., Kazlauskiene, N., and Svecevicius, G. 2003. Effect of a heavy metal model mixture on biological parameters of rainbow trout *Oncorhynchus mykiss*. Environmental Science and Pollution Research, 10: 103–107.

Warner, R. R., Swearer, S. E., Caselle, J. E., Sheehy, M., and Paradis, G. 2005. Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnology and Oceanography, 50: 1529–1542.

Weis, J. S., and Weis, P. 1989. Effects of environmental pollutants on early fish development. Reviews in Aquatic Sciences, 1: 45–73.

Williams, N. D., and Holdway, D. A. 2000. The effects of pulse-exposed cadmium and zinc on embryo hatchability, larval development, and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). Environmental Toxicology, 15: 165–173.

Yoneda, M., and Wright, P. J. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology Progress Series, 276: 237–248.

doi:10.1093/icesjms/fsn094