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Iron status of adult dogfish (*Scyliorhinus canicula*) tissues and sources of iron during embryonic development

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Concentrations of iron in embryos and yolk sacs of dogfish *Scyliorhinus canicula* (Elasmobranchii: Squaliformes) reared in natural and artificial (iron-free and iron-augmented) seawater were measured and compared with the iron content of adult dogfish tissues in order to investigate the iron budget of dogfish embryos. No difference in iron concentration between sexes was observed in adult dogfish. Liver iron concentration ($89.6 \pm 5.8\ \mu g\ g^{-1}$) showed the highest value among the three tissues investigated and was significantly higher than that measured in muscle ($22.6 \pm 2.1\ \mu g\ g^{-1}$), or developing eggs from females ($40.5 \pm 2.5\ \mu g\ g^{-1}$). The iron concentration in the entire contents of early eggcases ($27.1 \pm 4.8\ \mu g\ g^{-1}$) was lower, but not significantly different from that of eggs taken from the females. Dogfish yolk sacs from eggcases maintained in (a) natural seawater, (b) artificial seawater, and (c) artificial seawater with supplementary iron, for periods of up to six months, did not show any differences in iron content. Developing embryos from the artificial seawater with supplementary iron treatment demonstrated elevated iron concentrations ($62.4 \pm 12.0\ \mu g\ g^{-1}$) when compared with those from the remaining two treatments (35.8 ± 5.8 and $35.1 \pm 2.7\ \mu g\ g^{-1}$ respectively). The results identify maternal investment as the primary contributor to the iron burden of juvenile dogfish, and demonstrate the ability of the embryos to supplement this supply from their environment.

INTRODUCTION

The lesser-spotted dogfish (*Scyliorhinus canicula*, L.) is a common demersal small shark found on muddy and sandy substrata in Atlantic coastal European waters (including the Mediterranean), at depths up to 400 m. Compared with other marine fish species it has a low vitellogenic rate and the nutritive demands of egg yolk production (vitellogenesis) are distributed throughout the year (Craik, 1978). Mature females lay large eggs surrounded by jelly contained within a collagen eggcase and the embryo utilizes the yolk within the eggcase prior to hatching, deriving its nutrition from material provided by the mother (Lechenault et al., 1993). The eggcase is closed during the first few months of embryonic development but is open to the surrounding seawater in the later stages (Diez & Davenport, 1987).

Despite the extent of information available on dogfish reproductive biology, little attention has been focused on maternal-embryonic nutrient transfer, although some investigations on yolk utilization have been carried out (Diez & Davenport, 1990; Lechenault et al., 1993). The present study set out to test the hypothesis that dogfish embryos satisfy a proportion of their iron requirement from environmental sources. Iron is an essential trace element and a constituent of haemoglobin and myoglobin and a key component in many enzymes, being toxic to fish only at very high concentrations (Alam & Maughan, 1992). Fish normally accumulate metals through the gills in respiration, across permeable external surfaces (skin and gills), through ingested food or by maternal transfer (Baker et al., 1997). Trophic accumulation is the major pathway in the uptake of metals found in low concentrations in seawater for most adult fish species, but in juveniles prior to exogenous feeding, the gills, skin and maternal transfer provide the primary sources of metals including iron. Trace metal concentrations in aquatic animals have been well documented, due to their various positions in the aquatic food chain (Alam et al., 2000) and also because of the risk they may pose to humans when consumed as food (Kargin, 1996) and this work is confined, for the most part, to adult fish. There has also been some interest to date in the effects of elevated metal concentrations on elasmobranch fish (Crespo et al., 1981; Vas, 1987), again with little work on the early life stages. There is evidence, however, that juvenile fish take up metals, including iron, from the surrounding water (Alam & Maughan, 1992; Tsioros et al., 1996).

To investigate the iron budget of dogfish embryos we measured the iron content of yolk and embryos reared in natural seawater and artificial seawaters, either iron-free or containing iron, and compared these with the iron content of adult dogfish tissue (muscle, liver and individual developing eggs).

MATERIALS AND METHODS

Adult dogfish (11 females and 4 males) were collected from the south-west coast of Ireland during the summer months. The fish were dissected and samples of liver, muscle and whole eggs (from females) removed for analysis of iron concentrations. Tissue samples were stored frozen in clean, labelled polyethylene containers prior to analysis.

Dogfish eggcases were supplied by the Marine Biological Station Millport, Scotland. Prior to establishment of the

eggcases in the different treatment groups the contents of four of the eggcases were stored for iron analysis. Eggcases were maintained in groups of 20 for six months in 20-l aerated aquaria, suspended by the case tendrils in one of three seawater treatments. The first was natural seawater (NSW) collected from the south coast of Ireland, with the other two treatments being artificial seawater, one containing additional iron (ASW Fe) and the other being iron-free (ASW). The artificial seawater was made up in the laboratory using deionized water, and each litre contained the following salts (in order of addition): $CaCl_2$ (1.2 g), $MgCl_2$ (4.6 g), $MgSO_4$ (5.8 g), KCl (0.57 g), $NaHCO_3$ (0.21 g), NaBr (0.06 g) and NaCl (23.6 g). For the treatment containing iron (ASW Fe) the iron was added to the artificial seawater at the time of manufacture at a concentration of 0.1 mg/l. Dissolved iron concentration was subsequently monitored weekly using a HACH DR/2010 portable datalogging spectrophotometer. Where dissolved iron concentration was observed to have reduced over time additional iron was added to maintain a large excess of iron thus maximizing iron availability. The lowest recorded iron concentration in seawater over the course of the experiment was 0.04 mg l^{-1}. Oxygen, nitrite and pH were also monitored on a weekly basis in all tanks. KOH pellets were used when necessary to stabilize the pH of the artificial seawater.

Four months from the beginning of the experiment a sub-sample of eggcases was removed from each treatment for analysis of iron concentrations in embryos and yolks. The developmental stage of these eggcases was assigned according to Ballard et al. (1993) and all were seen to be at stage 30 or higher, i.e. in the stages leading to pre-hatching or later. After six months all remaining animals were removed for analysis of iron concentrations. All embryos and yolk sacs were frozen for subsequent analysis of iron concentrations. A small number of animals were collected as they hatched during the course of the trial and were preserved for analysis of iron concentrations. During preliminary analysis no significant differences were found between iron concentrations in embryos collected at the four-month or six-month stages of the trial. For this reason data for all yolks and embryos analysed for iron have been pooled for each treatment.

Iron measurement

In the laboratory, the samples were prepared for wet digestion by thawing at room temperature, rinsing with deionized water and drying on absorbent paper before weighing. Each sample was dried to a constant weight at 70°C and weighed into a glass digestor tube and digested with 10 ml HNO_3 in a Tecator digestor for three hours at 100°C. Once cooled, the sample was diluted to 50 ml with deionized water in a volumetric flask. The digested sample solution was analysed specifically for Fe by acetylene-air-flame atomic absorption spectrophotometry using a SpectraAA-300 spectrophotometer. Working standard solutions were prepared by successive dilution of the stock standard solution, to the desired concentration, with 5 ml HNO_3 and deionized water. Procedural blanks were also prepared in the laboratory and were treated in the same manner as the samples and run concurrently with the samples. Quality assurance was performed with standard reference materials from the National Research Council of Canada (DORM-2, dogfish muscle reference material for trace metals). The standards were digested and analysed in the same manner as the samples with each analytical run.

Data Analysis

Results are expressed on a dry weight basis and data are reported as means ±standard error (SE). Differences in iron concentration between groups were investigated using a T-test. Where the number of groups to be compared was greater than two, a one-way ANOVA was used, and where significant differences were observed this was followed by a Tukey test to identify which means differed significantly.

RESULTS

Adult dogfish

The concentration of iron and the moisture content of adult muscle and liver tissue and of eggs from mature females are shown in Table 1. Significant variation in iron concentration was found amongst all five tissues analysed ($F_{4,34}=41.74$, $P<0.001$), but no significant difference was seen in iron concentrations between male and female muscle tissue, or between male and female liver tissue (Table 1). Significant differences were observed between iron concentration in muscle and liver tissue, irrespective of sex (Tukey $P<0.05$). Iron was found in the lowest concentration in muscle tissue (both male and female), and liver tissue was seen to have significantly higher concentrations of iron compared to muscle tissue or the eggs from adult females.

Juvenile dogfish (embryos and yolks)

The mean weight of all dogfish embryos collected in this study was 2.1 ±0.3 g, and the mean length was 7.9 ±0.4 cm (N=33). No significant difference in weight or length between embryos from the three treatment groups was observed (weight $F_{2,30}=1.59$, $P>0.05$; length $F_{2,30}=1.73$, $P>0.05$). The relationship between length and weight of dogfish embryos in this study was described as follows:

$$W = 0.003\ L^{3.002} \quad (1)$$

where W is the weight (g) and L is the length (cm) of embryos (Figure 1). This relationship indicates isometric growth of embryonic dogfish up to the hatching stage.

Table 1. *Iron concentration and moisture content of adult dogfish tissue (mean ±standard error; N=number).*

Tissue	N	Iron content (μg g^{-1} dry weight)	Moisture content (%)
Eggs	10	40.54 ±2.50	52.52 ±6.07
Female muscle	11	24.56 ±10.7	79.96 ±1.01
Male muscle	3	15.57 ±1.23	77.61 ±0.64
Female liver	11	84.61 ±6.55	62.46 ±2.42
Male liver	4	103.34 ±2.36	54.12 ±3.76

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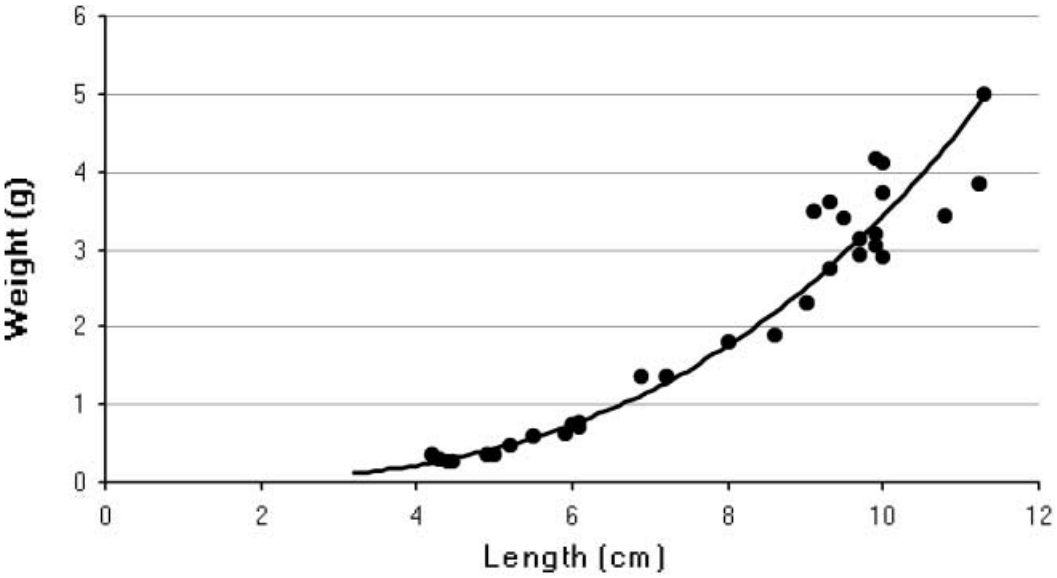

Figure 1. The relationship between length (cm) and weight (g) of dogfish (*Scyliorhinus canicula*) embryos.

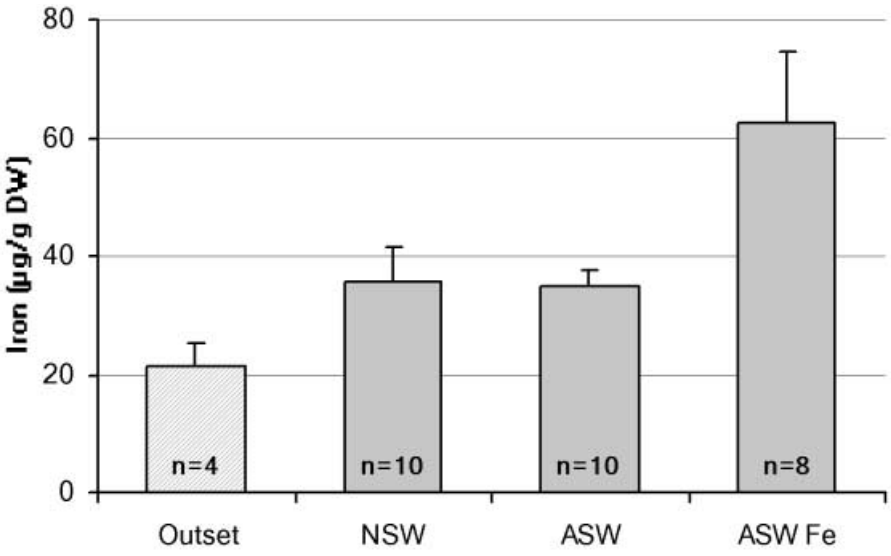

Figure 2. Concentrations of iron ($\mu g\ g^{-1}$ dry weight, mean ±SE) in dogfish embryos following maintenance for six months in three different seawater treatments compared with the contents of a sample of eggcases taken at the outset of the experiment. NSW, natural seawater; ASW, artificial seawater; ASW Fe, artificial seawater with added iron.

The mean moisture content of the dogfish embryos collected in this study was 77.16 (±1.18)% (N=40), and for the yolks was 42.75 (±0.58)% (N=16). The iron concentration in dogfish embryos reared in artificial seawater enriched with iron ($62.4 \pm 12.0\ \mu g\ g^{-1}$ dry weight) was significantly higher than in embryos from the other two treatment groups (35.1 ± 2.7, $35.8 \pm 5.8\ \mu g\ g^{-1}$ dry weight) ($F_{2,25}=4.41$, $P<0.05$, Figure 2). No significant effect of maintenance treatment on iron concentration in the yolks was seen ($F_{2,12}=0.98$, $P>0.05$, Figure 3). The iron concentration of eggcase contents at the outset of the experiment did not differ significantly from that of individual eggs taken from mature females ($t=2.47$, df=4, $P>0.05$). It was not possible to compare total iron values as the weights of individual eggs were not available because the eggs were pooled to achieve a sample weight in which iron could be measured. The concentrations of iron in all embryos and yolks were within the range measured in the various adult dogfish tissues. The concentrations were not significantly different from the iron concentration of the eggcase contents at the outset of the experiment, with the exception of embryos from the treatment group maintained in artificial seawater enriched with iron. The iron concentrations of the embryos from this treatment group were significantly higher than the iron concentration of the eggcase contents ($T=2.0$, df=10, $P<0.05$). Iron concentrations in embryos from the remaining two treatment groups were higher, and iron concentratons in yolks from all treatment groups were lower than the concentrations measured in the eggcase contents at the outset of the study, although differences were not statistically significant.

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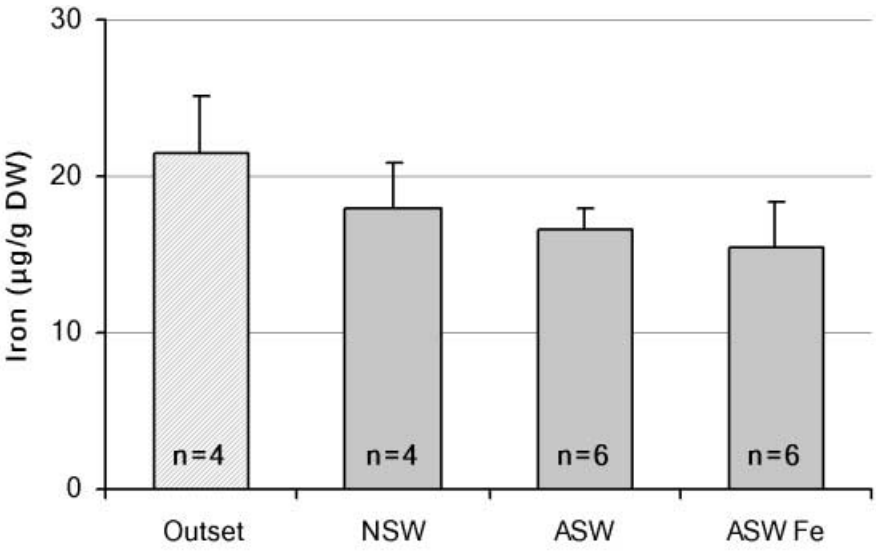

Figure 3. Concentrations of iron (μg g^{-1} dry weight, mean ±SE) in yolk sacs of dogfish embryos following maintenance for six months in three different seawater treatments and in the contents of a sample of eggcases taken at the outset of the experiment. NSW, natural seawater; ASW, artificial seawater; ASW Fe, artificial seawater with added iron.

DISCUSSION

Adult dogfish

The observed inter-individual variation in iron concentration in adult dogfish tissue was high, as is common in studies of this kind (Catsiki & Strogyloudi, 1999). Due to the lack of studies on metal concentrations in dogfish it is not possible to compare the iron concentrations observed in this study with those of dogfish from different areas. In the context of iron concentrations in other fish species, the iron concentration in dogfish muscle observed in this study is similar to concentrations previously reported for a number of species (Catsiki & Strogyloudi, 1999; Alam et al., 2000). High spatial and temporal variation in iron concentration in fish tissue is reported and concentrations considerably higher than those in this study have also been reported. The iron concentration in dogfish livers in this study was higher than values reported in the literature for other fish species (Manera et al., 2000), but again large inter-individual and inter-specific differences are characteristic of iron concentrations in fish liver tissue (Chen & Chen, 2001).

Sex differences in iron concentration of dogfish liver or muscle tissue, were not observed in this study, and are not common in shark species (Vas, 1987). Livers of females of some fish species have, however, been shown to have significantly higher iron concentrations than those of males (Tsioros et al., 1996). For both male and female dogfish, iron concentration variation between different tissues showed that the concentration in the liver was significantly higher than that in the muscle and that in the eggs collected from mature females. Different concentrations of metals in different fish tissues are commonly reported (Catsiki & Strogyloudi, 1999; Wepener et al., 2001), and depend on the particular metal being analysed, the fish species under investigation and also upon environmental factors (Avenant-Oldewage & Marx, 2000). Metal concentrations in fish species are most commonly measured either in muscle (flesh) due to its importance as human food, and its position as a final target tissue for metal storage, or in liver tissue which is widely accepted as an indicator organ of metal pollution because of its storage and detoxification functions. Lower concentrations of trace metals, including iron, in muscle tissue compared to liver tissue are well documented (Kargin, 1996; Avenant-Oldewage & Marx, 2000; Chen & Chen, 2001) and are primarily a consequence of the important role played by the liver in metal metabolism.

There are few reports on the provision of iron to eggs by female fish during vitellogenesis. The results of this study demonstrate a clear transfer of iron from female dogfish to the eggs that they produce. The concentration of iron in eggs collected from gravid females was almost twice that measured in adult female muscle tissue, yet lower than that measured in adult female liver tissue.

Juvenile dogfish (embryos and yolks)

The iron concentration of early eggcase contents in this study was not significantly different from that of the yolk sacs of later eggcases, or from developing embryos from the two treatments containing no supplementary iron which supports the theory that during vitellogenesis the mother provides the iron required for embryonic development. Iron concentrations in whole embryonic dogfish were within the range observed for adult dogfish tissue. In this study yolk sacs from dogfish eggcases maintained in all three treatments had similar iron concentrations (Figure 3) with no significant effect of the iron concentration in the surrounding water. By contrast, the developing dogfish embryos from different treatment groups did show significant variation. The average iron concentration of dogfish embryos was almost twice that observed in the yolk. The iron concentration of embryos in artificial seawater with supplementary iron was almost double that measured in embryos maintained in the other two treatments (Figure 2). Taken together with the yolk data, these findings indicate that embryos can take up iron directly from their environment.

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The results indicate that dogfish embryos can in fact accumulate iron from environmental sources to supplement what maternal transfer provides, a phenomenon also observed in embryonic sea lamprey (*Petromyzon marinus*) (Tsioros et al., 1996). As this study was carried out in the stages prior to exogenous feeding, the only environmental source of iron available to the dogfish embryos was in the seawater used in the study. It has been demonstrated that external gill filaments of developing elasmobranch embryos are capable of molecular uptake from external fluids (Hamlett et al., 1985). External gill filaments develop in *Scyliorhinus canicula* embryos in the stages immediately preceding what is known as pre-hatching. Hamlett et al. (1985) demonstrated that external gill filaments act as a nutrient absorptive site in addition to providing a respiratory surface, the former being significant only in non-oviparous species.

Although supplementary iron was added directly to the seawater in this study, it is reasonable to assume that this enhanced level became available within the eggcases themselves even during the early stages of embryonic development. It has been demonstrated that fluids within the eggcase are similar in composition to the surrounding seawater (Mellinger et al., 1986), which is due to the permeable nature of the eggcase wall. Although a very strong structure the eggcase wall is extremely complex and is highly permeable to small molecules while remaining impermeable to large molecules and microorganisms (Knight et al., 1996). The results of this study suggest that female dogfish supply an adequate amount of iron to eggs during vitellogenesis, but that dogfish embryos may incorporate additional iron into their tissues as they develop, if iron is available to them.

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REFERENCES

Alam, M.K. & Maughan, O.E., 1992. The effect of malathion, diazinon, and various concentrations of zinc, copper, nickel, lead, iron, and mercury on fish. *Biological Trace Element Research*, **34**, 225–236.

Alam, S.K., Brim, M.S., Carmody, G.A. & Parauka, F.M., 2000. Concentrations of heavy and trace metals in muscle and blood of juvenile Gulf sturgeon (*Acipenser oxyrinchus desotoi*) from the Suwannee River, Florida. *Journal of Environmental Science and Health, Part A*, **35**, 645–660.

Avenant-Oldewage, A. & Marx, H., 2000. Bioaccumulation of chromium, copper and iron in the organs and tissues of *Clarias garienpinus* in the Olifants River, Kruger National Park. *Water SA*, **26**, 569–582.

Baker, R.T.M., Martin, P. & Davies, S.J., 1997. Ingestion of sublethal levels of iron sulphate by African catfish affects growth and tissue lipid peroxidation. *Aquatic Toxicology*, **40**, 51–61.

Ballard, W.W., Mellinger, J. & Lechenault, H., 1993. A series of normal stages for development of *Scyliorhinus canicula* (*Chondrichthyes: Scyliorhinidae*). *Journal of Experimental Biology*, **267**, 318–336.

Catsiki, V.-A. & Strogyloudi, E., 1999. Survey of metal levels in common fish species from Greek waters. *The Science of the Total Environment*, **237**, 387–400.

Chen, Y.C. & Chen, M.H., 2001. Heavy metal concentrations in nine species of fishes caught in coastal waters off Ann-Ping, SW Taiwan. *Journal of Food and Drug Analysis*, **9**, 107–114.

Craik, J.C.A., 1978. An annual cycle of vitellogenesis in the elasmobranch *Scyliorhinus canicula*. *Journal of the Marine Biological Association of the United Kingdom*, **58**, 719–726.

Crespo, S., Soriano, E., Sampera, C. & Balash, J., 1981. Zinc and copper distribution in excretory organs of the dogfish *Scyliorhinus canicula* and chloride cell response following treatment with zinc sulphate. *Marine Biology*, **65**, 117–123.

Diez, J.M. & Davenport, J., 1987. Embryonic respiration in the dogfish *Scyliorhinus canicula* L. *Journal of the Marine Biological Association of the United Kingdom*, **67**, 249–261.

Diez, J.M. & Davenport, J., 1990. Energy exchange between the yolk and embryo of Dogfish (*Scyliorhinus canicula* L.). Eggs held under normoxic, hypoxic and transiently anoxic conditions. *Comparative Biochemistry and Physiology*, **96B**, 825–830.

Hamlett, W.C., Allen, D.J., Stribling, M.D., Schwartz, F.J. & DiDio, L.J.A., 1985. Permeability of embryonic shark external gill filaments. Electron microscope observations using horseradish peroxidase as a macromolecular tracer. *Journal of Submicroscopic Cytology*, **17**, 31–40.

Kargin, F., 1996. Seasonal changes in levels of heavy metals in tissues of *Mullus barbatus* and *Sparus aurata* collected from Iskenderun Gulf (Turkey). *Water Air and Soil Pollution*, **90**, 557–562.

Knight, D.P., Feng, D. & Stewart, M., 1996. Structure and function of the salachian egg case. *Biological Reviews*, **71**, 81–111.

Lechenault, H., Wrisez, F. & Mellinger, J., 1993. Yolk utilization in *Scyliorhinus canicula*, an oviparous dogfish. *Environmental Biology of Fishes*, **38**, 241–252.

Manera, M., Serra, R., Isani, G. & Carpené, E., 2000. Macrophage aggregates in gilthead sea bream fed copper, iron and zinc enriched diets. *Journal of Fish Biology*, **57**, 457–465.

Mellinger, J., Wrisez, F. & Alluchon-Gerard, M.-J., 1986. Developmental biology of an oviparous shark, *Scyliorhinus canicula*. In *Indo-Pacific fish biology* (ed. T. Uyeno et al.), pp. 310–332. Tokyo: Ichthyology Society of Japan.

Tsioros, K.K., Holmes, J.A. & Youson, J.H., 1996. Origin of iron in sea lamprey, *Petromyzon marinus*, tissues during embryogenesis and early larval life. *Canadian Journal of Zoology*, **74**, 2206–2210.

Vas, P., 1987. Observations on trace metal concentrations in a carcharhinid shark, *Galeorhinus galeus*, from Liverpool Bay. *Marine Pollution Bulletin*, **18**, 193–194.

Wepener, V., Vuren, J.H.J. van & Preez, H.H. du, 2001. Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of a freshwater teleost, *Tilapia sparrmanii*. *Water SA*, **27**, 99–108.

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