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Concentrations of Mercury and Other Toxic Elements in Orange Roughy, *Hoplostethus atlanticus*, from the Mid-Atlantic Ridge

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Abstract Concentrations of the elements mercury, arsenic, cadmium and lead were measured in the muscle tissue of Orange roughy (*Hoplostethus atlanticus*) obtained from the Mid-Atlantic Ridge during the Mar-Eco expedition in the North Atlantic Ocean in 2004. The age of the fish varied from 1 to 139 years. To the best of our knowledge, the concentration of the heavy metals presented here is for one of the oldest fish in the literature, in addition to the fact that very little information on arsenic in Orange roughy has been previously published. The concentration of mercury in the fillet of the fish varied between 0.06 and 1.1 μg g^{-1} w.w. Mercury was the only element that was positively correlated to the age. The concentrations of mercury were found to be below the maximum limits for Orange roughy set by EU at 1.0 μg g^{-1} w.w, except for a 134 year fish sample with a concentration of 1.1 μg g^{-1} w.w.

Keywords Contamination · Heavy metals · Orange roughy

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The Orange roughy is a deepsea fish with habitats found over oceanic ridges (Lack et al. 2003) and steep continental slopes (Lack et al. 2003; Clark et al. 2000). The species has a relatively slow growth rate (Clark 1999; Lorance et al. 2002) and a low natural mortality (Bulman and Koslow 1995). Through studies of otolith zones and analysis of radio-isotope ratios, it has been shown that Orange roughy might have life span well over 100 years (Lack et al. 2003; Clark et al. 2000; Bull et al. 2001) and reaches maturity between 23 and 40 years of age (Lack et al. 2003). As an opportunistic high level carnivore (Van den Broek and Tracey 1981), it feeds mainly on benthopelagic or mesopelagic species (Bulman and Koslow 1995; Rosecchi et al. 1988).

Orange roughy has become a popular consumer product in recent years due to the fillet's firm texture (van den Broek and Tracey 1981; Lack et al. 2003), and has been a target for deepsea fisheries since 1978 (McClatchie and Coombs 2005). Reported annual nominal catches range from ~5,000 tons in 1979, to a peak of ~95,000 tons in 1990 with a decrease to ~35,000 tons in 2002 (Marine Resources Service, Fishery Resources Division, and FAO Fisheries Department 2005). The United States of America (USA) is the largest market for Orange roughy, although the species is also a widely used consumer product in Japan, Germany, Spain and France (Lack et al. 2003).

Seafood contains essential nutrients associated with various beneficial health effects, and is regarded as an important part of a healthy diet. Seafood is a good source of proteins, omega-3 fatty acids, vitamin D, vitamin B_{12}, selenium and iodine (Alexander et al. 2007). Nevertheless, seafood also contributes substantially to undesirable substances such as toxic elements. The contaminants which bioaccumulate are difficult to excrete and may therefore increase the risk of unwanted health effects for the human population. Since the Orange roughy is a high level carnivore and has an extremely long life span, it is a food item with a high risk of being exposed to pollutants over a long time-span of more than 100 years. As a result, it may therefore be capable of accumulating large quantities of toxic elements. Because of this, and considering the food safety of the fish species, the present study evaluated the effect of age on the concentration of the toxic elements (As, Cd, Hg and Pb) in the muscle tissue of Orange roughy sampled in the Mid-Atlantic Ridge.

Materials and Methods

Twenty-five of Orange roughy were obtained during the Mar-Eco expedition along the Mid-Atlantic Ridge in 2004 and stored at -40°C until dissection. The individuals were obtained from the Faraday Seamount, superstation 53, trawling station 376 (49°51′72N, 29°37′74W) by the G.O. *Sars* on 15 July 2004 (Bergstad et al. 2008). Muscle tissue samples were obtained from 12 Orange roughy made available for this investigation, eight of which were juveniles of 4–8 g, while the other four were adults of 380–5,650 g. Muscle tissue from the mature individuals was cut from the left side, directly in front of the dorsal fin, while muscle tissue from the immature individuals was cut as a fillet. Samples were stored at −20°C until a workup could be performed. Otoliths from the immature and adult female fish were removed and used for age determination. The tissue samples from the mature individuals were homogenized and freeze-dried. The tissue samples from the immature individuals were not freeze-dried due to the small sample size.

For the determination of the elements, subsamples of approximately 0.2 g dry weight were accurately weighed and subjected to microwave-assisted wet digestion using 2.0 mL of concentrated nitric acid and 0.50 mL 30% (w/w) of hydrogen peroxide in an Ethos Pro microwave with the following temperature program (1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 400 W, and 5 min at 650 W). The digests were diluted to a final volume of 25 mL with distilled water, and the sample solutions were analysed by Inductively coupled plasma mass spectrometer (ICPMS) and used as an element specific detector. Rhodium served as an internal standard in order to correct for instrumental drift. For the determination of Hg, gold was added, in order to stabilize the element (Julshamn et al. 2007).

The trueness of the element determinations were verified by the analysis of CRM Dorm-2 (dogfish muscle) from the National Research Council of Canada. The obtained values (mean ± 95% uncertainty) for Dorm-2 were 17.9 ± 0.4 μg As g^{-1}, 0.043 ± 0.001 μg Cd g^{-1}, 4.5 ± 0.2 mg μg Hg g^{-1}, 0.057 ± 0.005 μg Pb g^{-1}. The results agreed well with the certified values for Dorm-2 (18.0 ± 1.1 μg As g^{-1}, 0.043 ± 0.008 μg Cd g^{-1}, 4.64 ± 0.26 μg Hg g^{-1}, and 0.065 ± 0.008 μg Pb g^{-1}). The limit of quantification (LOQ) for the elements was estimated at 0.01 μg As g^{-1}, 0.002 μg Cd g^{-1}, 0.002 μg Hg g^{-1} and 0.005 μg Pb g^{-1} dry weight (6 SD) from at least 20 repeated analyses of reagent blanks. The trueness of the method was also established by participating in proficiency tests. At least once a year, the laboratory participates in a national proficiency test. A sample of fillet of swordfish was tested in 2010 with good results. The elements showed a *z*-score between −1 and +1. The repeatability as a relative standard deviation (RSD) (%) was found to be 10% and better. One Dorm-2 subsample accompanied each series of the samples throughout the digestion and analysis processes. The results of the CRM analysis were verified against control-charts made prior to the investigation of the samples. One CRM solution was reanalysed at the end of the ICPMS sequence in order to determine if the concentrations of these solutions changed throughout the analysis. A five-point external calibration curve was used. The calibration curves obtained during the analyses had a correlation coefficient (r^2) value of better than 0.999.The analytical method used in the present study are accredited in accordance with EN-ISO 17025 by the Norwegian accreditation authorities.

Otoliths were sent to the National Institute of Water and Atmospheric Research Ltd., Wellington, New Zealand for age determination (Tracey 2007). Thin sections from the longitudinal sectioning of the otoliths and images were prepared, and zone counts were performed.

Chem Station (Agilent Technologies, Inc.) was used for the calculations of calibration curves and concentrations. Excel was utilized for calculations of multiplication factors and statistics, such as Q tests and t tests.

Results and Discussion

In addition to the total weights, the ages of the specimens are also tabulated in Table 1. All the immature fish were found to be between 1 and 2 years of age. The Orange roughy is known to be a long living deep-sea species, as shown by the two oldest individuals in the present study (134 and 139 years). As far as we know, there has never before been an analysis conducted for metals in fish this old. The weight of the fish recorded in Table 1 clearly demonstrates that the Orange roughy has a slow growth rate.

The immature fish have arsenic concentrations ranging from 0.59 to 0.83 μg g^{-1} wet weight (w.w.), whereas adult fish have arsenic concentrations between 0.96 and 1.8 μg g^{-1} (Table 1). To the best of the authors'

Table 1 Weight, age and heavy metal concentration (μg g^{-1} w.w.) in Orange roughy sampled from the Mid-Atlantic Ridge

Fish no.	N[a]	Weight (g)	Age (year)	As (μg g^{-1})	Cd (μg g^{-1})	Hg (μg g^{-1})	Pb (μg g^{-1})
1	1	6	1	0.73	0.078	0.10	<0.01
2	1	4	1+	0.83	0.098	0.078	<0.01
3	1	4	1+	0.60	0.17	0.11	0.16
4	1	5	2	0.64	0.046	0.098	<0.01
5	1	6	2	0.59	0.093	0.094	<0.01
6	1	8	2	0.74	0.032	0.067	0.046
7	1	8	2+	0.68	0.046	0.058	0.050
8	3	377	n.d.	0.50 ± 0.05	0.011 ± 0.003	0.24 ± 0.02	0.098 ± 0.025
9	6	4,060	87	1.5 ± 0.3	0.007 ± 0.001	0.55 ± 0.04	<0.01
10	3	4,270	78	0.96 ± 0.10	0.006 ± 0.001	0.76 ± 0.010	<0.01
11	3	5,520	134	1.1 ± 0.1	0.039 ± 0.006	1.1 ± 0.15	<0.01
12	3	5,650	139	1.8 ± 0.2	0.019 ± 0.004	0.85 ± 0.07	<0.01

n.d. not determined

[a] Number of replicates

knowledge, no comparable data is available on the total arsenic in Orange roughy. The total arsenic concentrations found in the present study are rather low compared to other deep-water fish species investigated in the Norwegian surveillance program for undesirable substances, including fish species such as Greenland halibut (*Reinhardtius hippoglossoides*) (3.2–18 mg/kg w.w.), tusk (*Brosme brosme*) (0.2–5.1 mg/kg w.w.) and ling (*Molva molva*) (1–5.1 mg/kg w.w.) (Julshamn et al. 2004). Those results are in agreement with data reported from other European countries (FSA 2005; EFSA 2009; Sirot et al. 2009).

With concentrations ranging from 0.032 to 0.17 μg Cd g^{-1} w.w., the muscle of the juvenile individuals are generally found to have higher concentrations of Cd than the adults (0.006–0.039 μg g^{-1} w.w.). The concentrations found in adult fish in the present study appear to be somewhat higher than those obtained for adult Orange roughy from the Rockwall Trough and Hebridean Continental Slope in the North Atlantic Ocean, which ranged from not detected to 0.01 μg g^{-1} w.w. (Cronin et al. 1998). However, the age range in the present study is much wider than that of the study performed by Cronin et al. (1998) (11–70+ years) and would therefore be likely to encompass a wider range of concentrations. The cadmium concentrations obtained in this study seem to be higher than those found in marine fish muscle sampled off the coast of Norway, ranging between 0.001 and 0.01 μg g^{-1} w.w. (Julshamn et al. 2004). The cadmium concentrations in the muscle of Orange roughy were well below the upper limit of cadmium in fish muscle as set by the EU at 0.05 μg g^{-1} w.w. (EC 2008). The Tolerable Weekly Intake (TWI), is the amount of a given substance which can be consumed every week over the course of a lifetime without causing any appreciable health effects for consumers. EFSA has established a TWI of 2.5 μg per kg b.w. Consumption of a 200 g fillet of Orange roughy with a Cd concentration of 0.04 μg g^{-1} w.w. will give an intake of approximately 8 μg Cd, which is equal to 5% of the TWI for a 60 kg person.

The immature individuals contained Hg concentrations in the range of 0.058–0.11 μg g^{-1} w.w., while the adult fish contained concentrations ranging from 0.51 to 1.1 μg g^{-1} w.w. (Table 1). Unlike arsenic and cadmium, the juvenile individuals had concentrations of mercury below those found for all the mature individuals. A strong positive correlation ($r^2 = 0.95$) between Hg concentration and weight was found (Fig. 1).

The Hg concentrations in the present study were in the same range as those reported by Van den Broek and Tracey (1981), who determined the mean mercury concentrations in the muscle of Orange roughy around New Zealand ranging from 0.05 to 0.62 μg g^{-1} w.w. with the fish weight ranging from 0.28 to 1.99 kg. They also concluded that

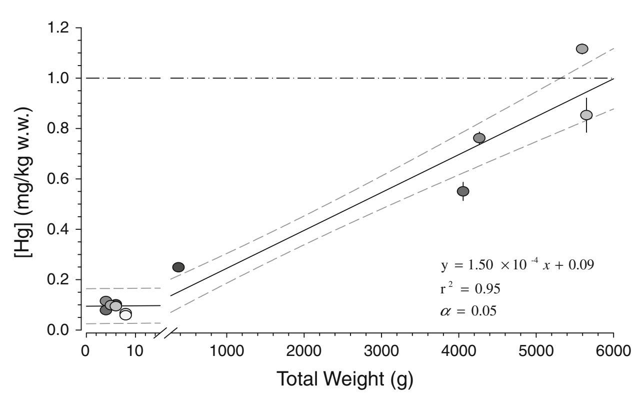

Fig. 1 Orange roughy samples plotted as total mercury concentration versus weight of the fish

significant differences exist between groups of Orange roughy at different geologic locations, which would account for any differences between different populations. Cronin et al. (1998) found mercury concentrations in the muscle of Orange roughy from the North Atlantic Ocean ranging from 0.11 to 0.86 μg g^{-1} w.w., where the fish were between the ages 11 and 70 years. They also investigated five other deep-sea fish species that showed lower mercury concentrations than those found in Orange roughy (Cronin et al. 1998). The mercury concentrations in the muscle of Orange roughy were on the same level as the upper limit of mercury in some of the carnivore fish muscle set by the EU at 1.0 μg g^{-1} w.w. (EC 2008). EFSA has established a TWI of 1.6 μg per kg b.w. Consumption of a 200 g fillet of Orange roughy with an Hg concentration of 1.0 μg g^{-1} w.w. will give an intake of approximately 200 μg of Hg, which is equal to 200% of the TWI for a 60 kg person, provided that the Hg is present as $MeHg^{+}$.

Lead was only found to be present in the muscle tissue of four individuals. Three of these were juveniles, while the fourth was a fish weighing 377 g (Table 1). These concentrations are lower than those found by Cronin et al. (1998), who reported concentrations up to 0.66 μg g^{-1} w.w. in the muscle tissue of Orange roughy, with a median value of 0.010 μg g^{-1} w.w. This median value was found to be an order of magnitude lower than concentrations found in *C. mediterraneus*, *C. labiatus* and *C. armatus*, and at the same level as *C. rupestris* and *M. berglax*. Negative correlations were found between lead concentration, length and weight (Cronin et al. 1998). Low concentrations of lead are generally found in the muscle of fish (i.e. less than 0.01 mg/kg w. w.) (Stange et al. 1996; Julshamn et al. 2004). The lead concentrations in the muscle of Orange roughy were well below the upper limit of lead in fish muscle as set by the EU at 0.4 μg g^{-1} w.w. (EC 2008). EFSA has established a TWI of 25 μg per kg b.w. Consumption of a 200 g fillet of Orange roughy with a Pb concentration of 0.1 μg g^{-1} w.w. will give an intake of approximately 20 μg Pb, which is equal to 1.3% of the TWI for a 60 kg person.

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