The effect of Prestige oil ingestion on the growth and chemical composition of turbot otoliths

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Abstract

Juvenile turbot (Scophthalmus maximus) were kept in captivity and were fed a prepared food contaminated with five different concentrations of seawater-accommodated fuel oil from 2.4 ± 0.35 to 48.2 ± 2.2 mg g⁻¹ food, with a control group receiving uncontaminated food. The growth and survival of individually tagged fish (N = 202) were measured after a six-week treatment period. The otolith growth rate was measured and otolith composition was determined before and after the treatments using LA-ICPMS. Fish and otolith growth were negatively affected by the fuel oil treatment, and the response decreased with the level of contamination. Otolith growth and element incorporation peaked at mid level exposures and decreased at the highest level. The otolith elemental composition reflected the presence of some elements in the Prestige fuel that may have been incorporated through the diet into the otolith.

Keywords: Prestige; Fuel oil; Otolith growth reduction; Otolith microchemistry; Juvenile turbot; Oil pollution

1. Introduction

In November 2002, the oil tanker Prestige sank 150 miles off the Galician coast (North-western Spain) releasing 39,700 ton of heavy fuel into the marine environment (Albaigés et al., 2006). Research studies have documented that the effects of oil on the morphological development (Hose et al., 1996) and general health of marine fish (Norcross et al., 1996; Marty et al., 1997; Stagg et al., 1998; Brauner et al., 1999; Jewett et al., 2002) may persist for years after oil spills, as in the case of the Exxon Valdez (USA) and the Braer (UK) among others. Somatic growth is reduced in experimental fish and in fish from natural populations exposed to crude oil and/or fed oil-contaminated food or prey (Schwartz, 1985; Norcross et al., 1996; Stagg et al., 1998; Omorogie and Ufodique, 2000; Peterson, 2001; Birtwell and McAllister, 2002). Turbot juveniles fed with food dosed with oil from the Prestige spill also grew more slowly, in both weight and length (Saborido-Rey et al., in press). Pink salmon fed with food contaminated by oil from the Exxon Valdez spill showed reduced otolith and somatic growth (Mortensen and Carls, 1994). However, Gallego et al. (1995) assessed the immediate effect of oil presence in the water on larval herring growth using otolith microstructure analysis, but did not detect any differences between polluted and non-polluted areas in either somatic or otolith growth.

The heavy fuel oil spilled by the tanker Prestige had a low volatile and soluble component; it proved to degrade slowly and persisted in the environment (Albaigés and Bayona, 2003). Oil mass balance models predict that the percent of oil mass at the water surface will decrease with time and eventually, oil dispersion dynamics will result in the accumulation of oil in sea bed sediments as both particulate oil and as dissolved oil in pore water (Gin et al., 2001). Various fish species are potentially affected by an oil spill in coastal waters, especially demersal species. Flatfish populations are of major concern due to their close link with the bottom sand and mud sediments that constitute
their habitat (Stagg et al., 1998; Kirby et al., 1999). Soft sediments are prone to trap oil globules, thus contaminating the natural flatfish habitats. Added to the possible contamination of gills and skin by living in a polluted environment, flatfish are also vulnerable to contamination through the food chain (Gin et al., 2001), from oil-contaminated epibenthic and infaunal prey sources, as shown for other species (Schwartz, 1985; Carls et al., 1996; Wang et al., 1993).

Variations in fish growth may represent a summation of various sub-lethal responses to oil contamination, because growth is an integration of many processes. Short term variations in fish growth rate may be measured by biochemical methods (Belchier et al., 2004), but these only reflect the most recent past. However, variations at the scale of 1–3 days, over the past weeks and months, can be detected in retrospect by analysis of otolith growth and increment widths. Otolith growth reflects both fish growth and metabolism, and therefore is a sensitive indicator of the physiological state of an individual (Morales-Nin, 2000). Otoliths are part of the fish inner ear and grow continuously during a fish’s life. Elements from the water and food are incorporated into the otoliths, giving them the potential to act as life history recorders for the physico-chemical aspects of the environment (de Pontual and Gefen, 2003). Otolith microchemistry has been used for fish population discrimination (Campana, 1999; Swan et al., 2006) and to study the association between fish and water masses (Swan et al., 2003; Gillanders, 2005). A preliminary study showed that the otolitic macula could discriminate against some heavy metals such as Hg (Morales-Nin, 1980), and geographic differences in plaice and sand goby and two species of flatfish and mercury in sand goby and two species of flatfish (Geffen et al., 1998). Dove and Kingsford (1998) used otoliths in relation to body composition was shown for lead and mercury in sand goby and two species of flatfish (Geffen et al., 1998). Dove and Kingsford (1998) used otolith and eye lenses to trace location and pollution due to sewage and industrial waste, being able to detect location using the presence of Ba, Mn and Hg.

In this study, the potential effects of the Prestige oil spill on the otolith growth and elemental composition were evaluated for juvenile turbot (Scophthalmus maximus) (Linnaeus) fed with contaminated food. Turbot are benthic, and as juveniles are found close inshore, primarily on sandy sediments and in shore pools (Wheeler, 1969). Their distribution range and habitat therefore may leave this species vulnerable to oil spills such as the Prestige incident. In addition, because turbot is a valuable aquaculture species, there is considerable knowledge about the physiology and growth patterns, and the protocols for maintaining fish in captivity in good condition are well established. The somatic and otolith growth of juvenile turbot have been studied in the wild (Lagardere et al., 2000; Paulsen and Støttrup, 2004) and detailed studies of otolith biomineralization have also been conducted (Edeyer et al., 2000). Experimental manipulation of chemical composition of turbot otoliths has not yet been studied, and this is the first study linking fuel oil ingestion and otolith composition.

2. Materials and methods

A total of 202 juvenile turbot, approximately 90 mm total length, were collected from a commercial hatchery in June 2004 and transported to the Institute of Marine Research in Vigo, Spain. Fish were randomly distributed among six 250-l cylindrical tanks. Each tank received approximately 21 h$^{-1}$ of filtered seawater at ca. 18 °C, with a 12L:12D photoperiod. Fish were acclimated for 10 days before the experiment was started. The fish were fed a maintenance ration (15 ± 0.01 g) of commercial turbot pellets (2 mm in diameter) three times at day during this period. One week before the start of the experiment, fish were anaesthetised (150 ppm 2-phenoxyethanol), measured (total length, mm), and weighed (to the nearest 0.01 g). Each fish was injected with a solution of SrCl$_2$ at a dosage of 0.01 ml/g, to induce a date mark on the otoliths, and tagged externally with a coded soft VI alfa tag (Northwest Marine Technology, Inc.) before being assigned to a tank. During the experiment, one group (Treatment 1) was fed uncontaminated food, while the other five treatments (Treatments 2–6) were fed food contaminated with different concentrations of Prestige seawater-accommodated fuel oil. Table 1 summarizes the experimental treatment and initial and final fish states. These dosage levels were higher than the levels of Prestige fuel oil detected in the sediment (Franco et al., 2006) to test if its components are able to pass the barriers of the intestine, blood and otolith plasma. Further tests of environmentally relevant dosages would be tested once the permeability of these compartments was evaluated.

The fuel carried by the Prestige corresponds to the M-100 (Russian terminology) or fuel No. 6 (English terminology) characterized by a low volatile and soluble component, high density (0.9753) and high viscosity (611 centiStockes at 50 °C). This fuel has a low degradation rate and is thus very persistent in the environment. It also tends to mix with seawater creating a very viscous emulsion. The emulsion collected from the sea immediately after the Prestige oil spill in November 2002 was used in this experiment and its composition has been characterized by Albáigés and Bayona (2003). Food pellets were made using the collected oil emulsion, and the juvenile turbot were fed three times each day, at 08:30 h, 13:30 h and 18:30 h. The fish in each tank were given a total of 15 g food each day, 4.7 g in the morning and evening feedings and 5.6 g for the midday feeding, and water temperature was recorded three times each day in each tank. Water temperatures varied during the experiment between 15.5 and 19.5 °C, but all tanks experienced the same temperature variations in synchrony.
At the end of the experiment, each fish was killed by anesthetisation in 150 ppm of 2-phenoxyethanol and the lengths and weights were recorded to the nearest mm and mg, respectively. Sagittal otoliths were extracted using Table 1

Summary of fuel oil ingestion experiment, with fuel oil concentration in feed, number of fish (N), length, weight means and standard deviation (in italics) at the start and at the end of the experiment for each tank-treatment

<table>
<thead>
<tr>
<th>Tank</th>
<th>Fuel</th>
<th>Initial values</th>
<th>Final values</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Length (mm)</td>
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<tr>
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<td>0</td>
<td>33</td>
<td>93.58</td>
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<tr>
<td>2</td>
<td>2.4</td>
<td>32</td>
<td>93.66</td>
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<tr>
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<td>0.8</td>
<td>34</td>
<td>94.59</td>
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<tr>
<td>4</td>
<td>1.1</td>
<td>33</td>
<td>93.27</td>
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<tr>
<td>5</td>
<td>1.9</td>
<td>35</td>
<td>90.26</td>
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<tr>
<td>6</td>
<td>2.2</td>
<td>35</td>
<td>93.97</td>
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<tr>
<td>Total</td>
<td></td>
<td>202</td>
<td>93.20</td>
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Fuel oil concentration is given in mg g⁻¹ food. Otolith growth is given as the growth from the Sr marks to the otolith edge and as daily increment growth (µm day⁻¹).

Fig. 1. Scophtalmus maximus sagittal otolith sections from fish in Treatment 4. (a) Scanning electron micrograph showing the Sr mark used to indicate the start of exposure treatments. Scale bar 200 µm. (b) Light micrograph showing the laser ablation spots (inside squares) and the Sr mark. Scale bar 40 µm.
plastic or ceramic forceps, cleaned with ultra pure distilled water and stored dry in plastic vials previously washed with 10% HNO₃.

For laser ablation inductively coupled plasma mass spectrometry analysis the otoliths were mounted with thermoplastic resin on glass slides, several otoliths per slide. Each slide was initially polished with diamond suspension on lapping cloth to reveal a transect through the core of each otolith, although differences in otolith sizes meant that not all otoliths were oriented exactly the same. The slides were cleaned with Milli-Q water in an ultrasonic bath and dried in a clean desiccator. Before further analysis the presence of the Sr mark was determined in several otoliths with an ambient scanning electron microscope (Universitat Illes Balears) using an EDS and the distance from the mark to the otolith edge were measured (Fig. 1a). The post marking otolith growth was measured in all otoliths on light micrographs, where the Sr mark appeared as a clear discontinuity (Fig. 1b).

A 213 nm laser, coupled to a Finnigan Element 2 ICPMS was used to sample three discreet points representing the core area of each otolith (pre-exposure), and three discreet points representing the otolith edge or surface (experimental exposure period). The spot size of the laser samples was 60 μm in diameter and the results of the three ablations in each otolith area were averaged for further analysis. Reference standards were ablated between every 10 sample spots and the results used to correct for instrument drift. Four analytical standards were used: NIST glass 610 and 612, BCR, and NIES fish otolith (Table 2). Seventeen isotopes were measured (²³Na, ²⁵Mg, ²⁵Mg, ⁴³Ca, ⁵²Cr, ⁶⁰Ni, ⁶²Ni, ⁶³Cu, ⁶⁵Zn, ⁶⁶Zn, ⁶⁸Zn, ⁸⁶Sr, ⁸⁸Sr, ¹¹⁴Cd, ²⁰⁸Pb), and from the initial list of isotopes evaluated, 10 isotopes were finally determined to provide reliable information for comparison between fish from different treatments. These isotopes were: ²³Na, ²⁵Mg, ⁵²Cr, ⁶²Ni, ⁶³Cu, ⁶⁶Zn, ⁸⁸Sr, ¹¹⁴Cd and ²⁰⁸Pb. The analytical results were calculated as element concentrations (ng g⁻¹), using Glitter software (ARC National Key Centre for Geochemical Evolution and Metallogeny of Continents, Macquarie University). For each element, all data points below the limits of detection (Table 2) were set to zero. The element concentrations were log transformed (ln(x + 1)) for statistical analyses. Regression analysis was used to test for the effects of fish growth on otolith growth, and fish and otolith growth on the incorporations of different chemical elements. Differences in element concentrations in the core and outer rim of the otoliths were tested between exposure treatments by ANOVA with location on the otolith and tank as fixed factors. Differences between treatments in the final otolith element concentrations were tested by ANCOVA, with individual fish growth increment (in weight and in length) as a covariate to control for the high variation in fish growth within and between the treatments.

Because of the high variability in individual fish responses to the fuel feeding treatments, a multi-variate
approach was also used to compare the pattern of the combined otolith element signal and the tank treatment conditions. These patterns were visualised by multi-dimensional scaling, using the community structure analysis software PRIMER (v.5, Clarke and Warwick, 1994). This approach considers the extent to which similarities in the chemical composition (Bray–Curtis similarity index) can be distinguished between the different treatments. The significance level was settled at $\alpha > 0.05$. MDS is a useful technique for exploring complex relationships especially across a continuum, such as the food dosage treatments experienced by the turbot in this experiment.

3. Results

3.1. Fish and otolith growth

Fish mean weight and length at the beginning of the experiment were not significantly different among treatments (Table 1; ANOVA, $F = 0.75$, $p = 0.586$ for weight; ANOVA, $F = 0.82$, $p = 0.537$ for length). At the end of the experiment, however, mean weight and length varied significantly among treatments (Table 1; ANOVA, $F = 7.59$, $p < 0.001$ for weight; ANOVA, $F = 11.42$, $p < 0.001$ for length). ANCOVA was performed using log-transformation of variables showing no significant differences in the length–weight relationships among Treatments 1–4 or between Treatments 5 and 6. The length–weight relationship of fish in the highest oil concentration Treatments (5 and 6) was significantly different to the length–weight relationships of fish in Treatments 1–4. Accordingly, growth rate, both in length and weight, varied significantly among treatments ($F = 17.97$, $p < 0.01$ for length and $F = 22.28$, $p < 0.01$ for weight). Fish in the control treatment tank (Tank 1) showed significantly higher growth rates compared with the fish in the other treatments ($p < 0.01$). The growth rate of fish in Treatments 2–6 showed no significant differences between adjacent treatments but significant differences occurred in all other comparisons. Fish in Treatments 1–4 grew significantly in both weight and length by the end of the experiment. However, in Treatments 5 and 6, the fish showed no significant growth, and even decreased in mean weight in Treatment 6.

Individual variability in growth was very high, with fish showing different growth patterns, both in weight and length, within each treatment, depending on the initial weight and length (Table 1). Thus, small fish showed small, zero or even negative increments irrespective of the treatment while initially heavier or larger fish grew better. Overall growth was best in Treatment 1 (control, 0 oil contamination), decreasing in the rest as oil concentration increased. Thus, in Treatment 6 fish showed no growth, or very small, both in weight (ANCOVA, $F = 23.52$, $p < 0.01$) and length ($F = 18.30$, $p < 0.01$). The daily fish growth rates were negative in some fish ($n = 15$) and were dependent on initial fish size and on treatment (Fig. 2).

Otolith growth was positive in all fish during the course of the experiment, ranging from 0.5 to several mm per day. Otolith growth also varied between individuals in the different treatments (Table 1) and in relation to fish growth (Fig. 2). The otolith daily growth during the experimental period was not correlated to fish length ($r^2 = 0.008$, $p = 0.44$, $n = 77$) or weight ($r^2 = 0.016$, $p = 0.26$, $n = 77$).

3.2. Otolith microchemistry

The mean values by element and treatment and their standard deviation are included in Table 2. The amount of material deposited in the otolith controls the concentration of the elements incorporated into it. Thus, to determine the effect of the treatment and to take into account the individual variability observed in otolith growth, differences in the concentrations of each element were compared by ANCOVA to control for otolith daily growth rate.

Otolith concentrations of Na, Mg, Mg, Cr, Sr, showed significant effects of fuel exposure, whilst concentrations
of Ni, Zn, Cd and Pb were not significantly affected. Na concentrations increased significantly in relation to otolith growth ($F = 12.03, p = 0.001$) and varied significantly between treatments ($F = 9.41, p = 0.000$). Otolith concentrations of Cr were also significantly dependent on otolith growth ($F = 4.16, p = 0.048$) and treatment ($F = 4.536, p = 0.002$, treatment $2 < 5 < 1,3 < 6 < 4$). The Tukey post hoc tests showed complex dose-related responses in otolith concentrations. Na concentrations followed the pattern Treatment 4 = 6 < 1 < 2 < 3 < 5 ($p$ between 0.041 and 0.0007), with Treatment 2 significantly different from Treatments 1, 3, 4 and 6.

Mg, Cu and Sr concentrations were not influenced by individual otolith growth rate, but there were significant treatment differences (Mg: $F = 2.803, p = 0.028$; Cu: $F = 3.87, p = 0.005$; Sr: $F = 4.024, p = 0.004$). Mg values fluctuated (Treatment 5 > 1, 3, 4 > 2 > 6) so that Treatment 2 was significantly different to 1, 3, 4 and 5, whilst 5 and 6 were significantly different ($p$ between 0.005 and 0.043). Cu concentrations were similar except in Treatment 3 ($3 < 1 < 2 < 4, 5 < 6$), which was significantly different to all other treatments ($p$ between 0.0004 and 0.03). Sr showed an increasing trend with exposure dose (Treatment $1 < 3 < 6 < 2 < 4 < 5$), with treatment 1 significantly different to others except 3, and 3 and 5 also different ($p$ between 0.0003 and 0.019).

Otolith growth rate and fish growth rate may become uncoupled in certain circumstances (Campana, 1990; Mosegaard et al., 1988), thus the treatment comparisons of otolith composition were repeated after controlling for fish weight increment. Na, Mg, Cr and Ni concentrations showed significant treatment differences ($F = 10.4, p = 0.000$; $F = 7.124, p = 0.000$; $F = 3.56, p = 0.006$; $F = 2.94, p = 0.019$; respectively). Na concentrations tended to increase with dosage except for Treatments 4 and 6 ($4 < 6 < 1 < 2 < 3 < 5$). The differences were significant between Treatments 4 and 2, 3, and 5, and Treatments 1–5 and 6–5. Mg concentrations fluctuated, with significantly higher concentrations in the otoliths of fish in Treatment 5 ($5 > 2 < 3, 6, 4, 1$) compared to the other treatments ($p$ between 0.0001 and 0.024). Cr showed similar values except in Treatments 2 and 5 ($2 < 5 < 3, 4, 6, 1$), which were significantly different to all other treatments ($p$ between 0.010 and 0.014). Ni concentrations were low in Treatments 3 and 4 ($3 < 4 < 1, 2, 5, 6$), with Treatment 3 significantly different to 5 and 6 ($p$ between 0.020 and 0.045) (Fig. 3).

The MDS plots show minimal overlap in the patterns of otolith element concentrations between the fish receiving high fuel treatments and low fuel treatments (Fig. 4). The low stress value of 0.04 indicated that the multidimensional model is a good representation of the relationship between the individual fish within each treatment. The distance between points reflects the rank order of the similarities between the individual fish based on the chemical composition of their otoliths. Thus, the closer the symbols representing each treatment, the more similar is the pattern of elemental composition in their otoliths. The otolith elements were most similar between fish in Tanks 4 and 5, whereas the patterns among the fish in Tanks 1–3 were more variable.
Among those fish with positive increase in weight there were significantly higher concentrations of five metals and trace elements depending on the treatment (Pb $F_{5.40} = 2.77$; Na $F_{5.40} = 10.6$; Mg $F_{5.40} = 6.7$; Ni $F_{5.40} = 2.8$; Sr $F_{5.40} = 4.1$; $p < 0.3$). This emphasizes the important interaction of fish growth, otolith growth and food consumption for the resulting otolith elemental concentrations. A PCA analysis was performed with these fish to ascertain the trends in elemental composition (Fig. 5). The data showed a clear grouping along Factor 1, which represents treatment exposure doses and explained 32.69% of the variability in the concentration data. Factor 2 explained at further 20.87% of the variability, and is related to otolith growth, as indicated by the Sr concentration pattern.

4. Discussion

Turbot juveniles exposed to different levels of oil produced water have previously shown sub-lethal physiological stress and complex activity and growth rates dependent on dose (Stephens et al., 2000). In a detailed study of these same experimental turbot, Saborido-Rey et al. (in press) reported a high growth variability and decreasing condition factors and growth rates as the experimental period progressed. This response was related to a reduction in feeding activity, combined with a major reduction in food energy conversion, i.e. food and/or energy assimilation. This may be explained if reduced energy assimilation is the result of alterations in the fish metabolism and tissue damage after assimilation of some of the oil components. Similar effects were found in three species of flatfishes (Moles and Norcross, 1997) after exposure to oil-contaminated sediment.

Otolith growth patterns can be used successfully to investigate both short and longterm effects of oil contamination in situ. Reynolds et al. (2003) used otolith increment counts to calculate the spawning dates of juvenile sea bass following the Sea Empress oil spill, (1996) in comparison to other years, and detected some reduction in abundance of fish spawned during that period. Neither fish growth nor otolith growth were evaluated in relation to oil exposure. Gallego et al. (1995) calculated the otolith growth rates of herring larvae sampled after the Braer oil spill in 1993, but found no difference between contaminated and non-contaminated areas. The growth of the juvenile turbot, and their otoliths, was expected to show stronger responses because the exposure levels were higher, and because the experimental setting ensured that the sample population was exposed and the effects analysed; a difficult assumption for field studies.

Otolith growth also showed a marked variability and was uncoupled from the somatic fish growth in individual turbot. There was a tendency for otolith growth to decrease with exposure to higher doses of fuel oil emulsion in the food. In an experiment with pink salmon given food contaminated with Exxon Valdez fuel, at doses lower than in the present study, otolith growth reduction was evident from week 1, and did not recover after natural feeding was established (Mortensen and Carls, 1994). There is considerably more information about juvenile turbot otolith growth from aquaculture studies as compared to natural environments, but the available field data show high individual variability between otolith weight and fish weight, which increases with fish size (Støttrup et al., 2002; Paulsen and Støttrup, 2004). This high variability was taken into account in the analysis, because element concentration is affected by otolith growth. The measured concentrations in the otolith are affected by otolith growth rate in two ways: the rate of biomineralization directly affects incorporation, and also through the amount of otolith material deposited in relation to the amount sampled with the 60 μm laser spot size.

Some elements were preferentially incorporated depending on the fuel dosage in the food. Prestige fuel was rich in Na (10$^8$ ppm), Al, Ca, Fe, K, Mg, Ti (10$^7$ ppm), Br, Ni, V (10$^6$ ppm), B, Ba, Mn, Mb, Sr, Zn (10 ppm), Ar, Co, Cr, Cu, Li, Se (1 ppm) (CSIC No. 2). The proportion of fish in each treatment with detectable concentrations of elements in their otoliths (above the analytical limits of...
detection (LOD)) was 100% for Na, Mg and Sr. Concentrations of Cr, Ni and Cu were detected in over 80% of the otoliths (except in Tank 2), and the presence of other heavy metals was more irregular (Fig. 6). The behaviour of the different elements is complex and the mechanisms for incorporation differ substantially between elements. Incorporation rate may be linear for some elements but not others, and likely related to otolith growth rate as well as external concentrations and conditions (Geffen et al., 1998).

The mechanisms of element accumulation in fish otoliths are still mostly speculative. Two mechanisms have been described; the incorporation through secretion via the otolithic membrane of small crystallising nuclei to which the elements may stick and be incorporated to the otolith surface; and for blood soluble metals via the epithelial saccular cells (Morales-Nin, 2000 and citations therein). If different metals are accumulated into the otolith via different pathways, the interactions between them would be complex as is suggested by the grouping observed of Cu, Cr and Pb which were opposite to Na (Fig. 5). There is evidence that some of the elements present in the Prestige fuel may have been incorporated through the diet into the otoliths (Table 2). Concentrations of Pb and Cd were not detected in otoliths of fish in Treatment 1, were very high in Treatments 5 and 6 and orders of magnitude higher than in certified otolith reference values (CRM) (Pb = 0.023 mg/kg; Cd = 0.0028 mg/kg; NIES/WAMRL 2000). Other elements were present in higher concentration at the highest dosages, like Zn that increased from concentrations lower than found in the otolith CRMs (Zn = 0.47 mg/kg) to reach higher concentrations in higher dosage treatments. Na on the contrary, did not show a treatment effect and remained at values similar to those in the reference material (Na = 223 mg/kg), whilst it was high in Prestige oil. Na is probably closely regulated due to its function in cell physiology.

Our results are influenced by several related effects. For instance, fish growth individual variability and individual behavioural differences (with big fish feeding more and competing for food with smaller fish) resulted in different growth rates and dosages within the same treatment. Similarly the complex otolith growth and incorporation of elements were interrelated. The results suggest that the very high dosage in Treatment 6, even though the dose was sub-lethal, inhibited growth of the fish (Saborido-Rey et al., in press) as well as the otolith, and thus precluded the incorporation of elements into the otoliths. The concentrations of heavy metals and ultratrace elements in otoliths are very low (Campana, 1999; de Pontual and Geffen, 2003) even after contamination events. Continued improvement in analytical techniques will allow better measurement of contaminants and the fine scale temporal changes that are recorded in the growth of fish otoliths. Thus, we can conclude that the otoliths hold the potential to register past contamination events, although more work is necessary elucidating the mechanisms controlling otolith growth and elemental composition.

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