

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.

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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; Omoregie 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. Flat-fish populations are of major concern due to their close link with the bottom sand and mud sediments that constitute

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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 Geffen, 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 otolith macula could discriminate against some heavy metals such as Hg (Morales-Nin, 1980), and geographic differences in plaice and whiting otolith composition reflected sewage dumping patterns in the Irish Sea (Geffen et al., 2003). If contaminants are incorporated into otoliths, then their time keeping properties can also be exploited to interpret past pollution events. The complex nature of metal concentrations in 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. $18\text{ }^{\circ}\text{C}$, with a 12L:12D photoperiod. Fish were acclimated for 10 days before the experiment was started. The fish were fed a maintenance ration ($15 \pm 0.01\text{ g}$) of commercial turbot pellets (2 mm in diameter) three times at day for 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 $50\text{ }^{\circ}\text{C}$). 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 Albaigés 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 $19.5\text{ }^{\circ}\text{C}$, but all tanks experienced the same temperature variations in synchrony.

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