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Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments

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ABSTRACT

Farmed fish and wild fish aggregating in the vicinity of four Mediterranean fish farms with different environmental conditions were sampled. Levels of metals (including As and Se) were measured in the muscle, liver, gills, bone and intestine. The wild fish from sites with anoxic substrata accumulate metals (including As and Se) from the ambient habitat in their gills whereas those from sites with oxic substrata concentrate these elements through their diet in their intestine. Tissues of wild fish aggregating around farm cages accumulate a greater number of these elements and with higher concentrations than farmed fish. Habitat, diet, ecological needs, fat content of fish, and protein expression may play an important role in these element differences between fish species. Fe in flathead grey mullet, As in surmullet, rainbow wrasse, grey gurnard and picarel and Hg in bogue may pose a risk for human health. Farmed and wild fish are good sources of P, K, Cr and Se while flathead grey mullet, picarel and comber are excellent sources of Ca and Se.

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1. Introduction

Aquaculture is a fast expanding industry that has local environmental impacts. Deposits of uneaten fish feed and faeces on sediments under and in the vicinity of fish farms reduce redox and particle size and increase sulphides and organic content (Karakassis, Tsapakis, & Hatziyanni, 1998). These environmental conditions around fish farms increase the abundance of deposit-feeding invertebrates, demersal fish and mobile carnivores (Debruyn, Trudel, Eyding, Harding, McNally, & Mountain, 2006). In the Mediterranean, abundant and diverse fish assemblages thrive beneath the cage farms (Dempster, Sanchez-Jerez, Bayle-Sempere, Giménez-Casalduero, & Valle, 2002; Smith et al., 2003) and may buffer the ecosystem mitigating environmental degradation (Bayle-Sempere et al., 2013). The biology and physiology of wild fish may be affected as they become farm effluent feeders, feeding on commercial pellets and subsequently altering their fat deposition and fatty acid composition (Grigorakis & Rigos, 2011).

Wild fish assemblages around fish farms are often exposed to conditions that are unusual for the Mediterranean, such as the hypoxia in farms established at shallow, poorly flushed sites with reducing sediments (Karakassis et al., 1998). It is known that the behaviour of some metals in the marine environment changes when redox is altered, occasionally resulting in changes in bioavailability. Around organically enriched, highly reducing fish farms, the bioavailability of metals is likely to be low due to the presence of metal-binding phases in sediments, such as organic material and sulphides (Dean, Shimmield, & Black, 2007; Kalantzi et al., 2013). However, metals bound to the sediment might pose a threat to aquatic biota either through leaching into the water-column by direct contact (Chen & Chen, 1999) or through consumption of contaminated benthic organisms (Roach, Maher, & Krikowa, 2008).

Fish contain long-chain, $n-3$ polyunsaturated fatty acids (LC $n-3$ PUFA) in their lipids such as eicosapentaenoic acid (EPA or 20:5$n-3$) and docosahexaenoic acid (DHA or 22:6$n-3$) as well as micro- and macro-nutrients that play a vital role in human nutrition, disease prevention and health promotion (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002; Szlinder-Richert, Usydus, Malesa-Ciecwierz, Polak-Juszczak, & Ruczynska, 2011). However, fish can accumulate several toxic contaminants such as metals and persistent organic contaminants (e.g. PAHs, PCBs) and consequently fish consumption may pose a risk to human health through the food chain transfer (Uysal, Emre, & Köse, 2008). Contaminant accumulation in fish is controlled by uptake through ingestion of

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suspended particulate matter and food, ion exchange of dissolved metals across lipophilic membranes such as gills, absorption on tissues and membrane surfaces, and removal through excretory processes in the gut and gills (Alam et al., 2002; Burger et al., 2002; Ikem & Egilla, 2008).

There is an increasing awareness of the elemental differences between farmed and wild fish (Fallah, Saei-Dehkordi, Nematollahi, & Jafari, 2011; Foran et al., 2004; Padula, Daughtry, & Nowak, 2008; Yildiz, 2008). However, as far as we know, there are no published data comparing metals and elements in tissues between farmed and wild fish aggregations around cages. Recreational and commercial fishing in the vicinity of fish farms is a common phenomenon in Greece. Therefore, it seems that metal and other element determination in farmed and wild fish around fish farms is important in human health risk assessment. Metal accumulation in different fish species may be affected by many factors such as size, sexual maturity, seasonal changes, feeding habits, trophic level, water quality and environmental contamination (Adhikari, Ghosh, Rai, & Ayyappan, 2009; Canli & Atli, 2003; Saha, Sarkar, & Bhattacharya, 2006).

The overall aim of this study was to investigate whether sediment geochemistry plays an important role in the bioavailability of a range of elements, making them less available to farmed fish but more available to deposit-feeding wild fish that inhabit the environment surrounding the fish cages. Towards this end, the concentrations of 28 metals (including As and Se) were measured in fish tissues of wild fish assemblages thriving beneath cages of farms established in sites with oxic and anoxic substrata. A comparison between farmed and wild fish assemblages was also made. To assess the human health risks, element concentrations were compared with established limits. The nutritional value of micro- and macro-nutrients of farmed and wild fish was also evaluated, according to recommended daily allowances in foodstuffs.

2. Materials and methods

2.1. Study areas and samples collection

Farmed seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus auratus*) were collected from two fish farms in two different sites in the Aegean Sea (AEG1 and AEG2) and two in the Ionian Sea (ION1 and ION2), Greece. Both species are commercially grown in marine fish cages in these farms but, when the samples were taken, gilthead seabream was not available at ION1. Wild specimens were also collected around the cages, using nets operated by scuba divers or by local fishermen. The sampled specimens of farmed and wild fish were 9 and 20 in AEG1, 10 and 12 in AEG2, 4 and 5 in ION1 and 8 and 5 in ION2 respectively (Table 1) Time and date of harvest was the same for both farmed and wild fish in each site but different between sites. The physical and geochemical characteristics of sampling areas were recorded (Table 2). Sampling farms are anonymous in this report because the fish farmers agreed to cooperate in the study on condition that their identities were not revealed. The farms in AEG1 and ION1 are located in shallow exposed straits ca 200–300 m from shore. The farm in AEG2 lies in a semi-exposed area and the fourth fish farm in ION2 is located in a shallow, enclosed bay.

Immediately after collection, fish were killed in ice and transferred to the laboratory. After species identification total length and body weight were recorded for each specimen (Table 1). Fish were dissected using a pre-cleaned, stainless steel knife and approximately 5 g of each tissue of interest (muscle, liver, gills, bone, intestine) were sampled. Previous research found that using metal knifes for scales and otoliths removal did not contaminate samples (Adey, 2007; Gordon & Swan, 2002). Triplicate sediment core samples (4.5 cm inner diameter) were collected by scuba divers under the cages (0 m) as well as at 5 and 10 m from the edge of the cages downstream in the residual current direction, in order to measure the environmental variables of the sediment surface layer (0–2 cm) of fish farms. Both fish and sediment samples were stored in labelled, zip-lock bags at −20 °C until laboratory analysis. All samples were handled very carefully in order to avoid cross-contamination.

2.2. Environmental variables

Depth, water temperature and salinity were measured *in situ*. Sediment grain size (median grain size, MD and silt – clay%) was determined according to the method of Gray and Elliott (2009). Redox potential (Eh) was measured at the water–sediment interface and sediment subsurface by means of a WTW electrode, standardised with Zobell's solution (Zobell, 1946) and corrected to standard hydrogen electrode potential. Following the protocol from Brooks (2001), sulphide concentration in the top 5 cm of sediment was determined in each sample using an Orion™ advanced portable ISE/pH/mV/ORP/temperature metre with an *Ionplus* Silver/sulphide electrode (Thermo Fisher Scientific, Winsford, United Kingdom) (Table 2).

All sediment samples were freeze-dried until constant weight and finely ground before sub-sampling. Organic material (loss on ignition, LOI) was determined as labile organic matter (LOM) and refractory organic matter (ROM) (Loh, 2005). Total organic carbon (TOC) and nitrogen (TON) in the sediment samples were determined by means of a CHN Elemental Analyzer (Perkin Elmer 2400; Massachusetts, US), according to Tung and Tanner (2003). The separation of organic from inorganic forms of carbon followed the method reviewed in Verardo, Froelich, and McIntyre (1990). Following the procedure of Holmer et al. (2007), sedimentation rates were estimated by deploying sediment traps under the fish cages for approximately 48 h (Table 2).

2.3. Chemical analysis

Metal (including As and Se) concentrations, hereinafter referred to as analytes, were determined using a modification of the method described by the USEPA Method 3052 (1996) for microwave-assisted acid digestion of siliceous and organically based matrices. All samples were freeze-dried to constant weight, homogenised and stored under a dry atmosphere until digestion. For total dissolution, 5 ml of conc. HNO_3 were added to 0.261 ± 0.07 g (dry weight) of tissue sample in acid-cleaned Teflon vessels. After pre-digestion for an hour in a sandbath, vessels were sealed and placed in a closed, high pressure, microwave system (Multiwave 3000, Anton Paar, Austria). After digestion, samples were diluted with ultrapure water into 50 ml volumetric flasks and stored in polypropylene sample bottles at 4 °C until further analysis.

For the measurement of analyte concentrations in the sample digests, an Inductively Coupled Plasma – Mass Spectrometer (ICP–MS, Thermo Fischer Scientific, Winsford, United Kingdom; Plasma lab software) was used, according to USEPA Method 6020A (2007). Each sample was analysed in triplicate. An optimal sample dilution factor of x600 was chosen for samples. The instrument was calibrated using standard solutions for the six-point calibration curve, which were prepared by diluting certified primary standard stock solutions with ultrapure water and 2% high purity HNO_3. An internal standard containing Indium and Bismuth (10 μg/L) was added to each sample and calibration standard. A standard was run for every 10 samples analysed. Analyte concentrations were expressed in wet weight. All labware used was acid washed in 10% HNO_3. Analytical grade reagents were used for

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