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Fish farming and anti-fouling paints: a potential source of Cu and Zn in farmed fish

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ABSTRACT: The accumulation of copper and zinc, the basic components of anti-fouling paints, was examined in cultured sea bass *Dicentrarchus labrax* and sea bream *Sparus aurata*. Samplings were carried out at 4 eastern Mediterranean fish farms. Two of the farms used nets treated with anti-fouling paints, and 2 used untreated nets. At each farm samples of sediment and fish tissue (muscle, liver and gills) were analysed for heavy metal concentrations. The results showed that while total copper and zinc concentrations in sediments were quite similar in samples collected from the 4 farms, the extractable copper concentration in sediment from farms using anti-fouling treatment was 2 to 3 times higher than from those using untreated nets. Statistical analysis revealed no significant differences for either metal between sediment samples from farms using anti-fouling treatment and those which did not, except for extractable copper concentrations. However, analysis of the biological samples showed that copper concentrations in muscle tissue were lower in samples from farms using untreated nets, with the highest copper concentrations being observed in fish livers from farms using anti-fouling paints. General linear model results indicated significant differences between heavy metal concentrations in fish samples from farms using anti-fouling paints and those which did not. Furthermore, 1-way ANOVA indicated that these differences were confined to liver tissue for zinc, whereas significant differences for copper were seen in all tissues (except for gills of sea bream) for both species studied. These results indicate that the use of anti-fouling paints may be a potential source of metal accumulation in cultured fish.

KEY WORDS: Aquaculture · Anti-fouling · Copper · Zinc · Fish · Eastern Mediterranean

INTRODUCTION

Biofouling can be defined as the growth of unwanted organisms on the surfaces of man-made structures immersed in the sea (WHOI 1952). It is widely accepted that biofouling in the aquaculture industry is an expensive problem (Enright 1993, Hodson et al. 1997, Braithwaite et al. 2007). It occurs in all oceans and at all depths; however, its character and magnitude vary markedly with physical and biological factors (Benson et al. 1973). For the aquaculturist the effects of biofouling are largely detrimental (Braithwaite et al. 2007). Biofouling growth on fish cages has 3 main negative effects: the restriction of water exchange, disease risk and cage deformation, and structural fatigue due to the extra weight imposed by fouling (Fitridge et al. 2012). The most common way to prevent or delay biofouling in European finfish mariculture is to coat the submerged structures and net-cages with anti-fouling paints (Cotou et al. 2012). With the gradual elimination of triorganotin-based formulations (e.g. tributyltin [TBT]), copper has become the principal biocidal component of most anti-fouling paints, usually in the form of copper oxide (Cu_2O) (Yebra et al. 2004). Zinc is also a common component in anti-fouling paints, and has the potential to cause adverse effects (Ytreberg et al. 2010). It comes in the form of zinc pyrithione, and it is

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considered to be one of the most popular surrogate anti-fouling biocides (Yebra et al. 2004, Bao et al. 2008).

However, in an industry selling a food product, the use of copper and zinc as anti-fouling compounds is undesirable from both health and marketing perspectives. These metals are listed under the EU Dangerous Substances Directive, which recognises their toxicity to aquatic organisms and long-term adverse effects on the environment, and as such their release into the environment requires control (67/548/EEC). The extensive use of copper for anti-fouling purposes has raised concerns that the treatment may have an adverse effect on natural or farmed organisms in the environment (Børufsen Solberg et al. 2002). In general, high copper concentrations have been observed in sediments near salmon farming facilities that use anti-fouling treatments (Debourg et al. 1993, Burridge et al. 1999, Chou et al. 2002, Brooks & Mahnken 2003, Dean et al. 2007). In addition, a number of authors have reported that copper from treated nets could have lethal or sub-lethal effects on farmed fish (Anderson et al. 1995, Bellas et al. 2001, Burridge & Zitko 2002) and could affect the immediate immune defence mechanism of the exposed fish (Cotou et al. 2012).

In the eastern Mediterranean only 1 preliminary study has investigated the impacts of anti-fouling paints on the concentrations of heavy metals in different tissues of cultured fish (Fig. 1; Castritsi-Catharios et al. 2013). However, studies on the influence of anti-fouling paints on the concentrations of heavy metals in farmed fish from sites which use treated nets in comparison to farmed fish from those using untreated nets have not yet been done. This study aims to partly fill the gap in this scientific knowledge by providing baseline information on the influence of anti-fouling paints on heavy metal accumulation in several tissues of 2 different species (*Sparus aurata*, *Dicentrarchus labrax*) of farmed fish in the eastern Mediterranean.

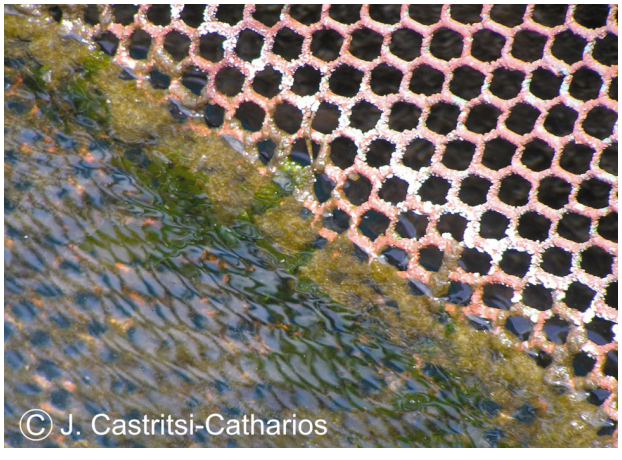

Fig. 1. Photograph (taken from the archives of Dr. Castritsi-Catharios) showing the extensive use of anti-fouling paints (red-coloured net) at fish farms

MATERIALS AND METHODS

Sampling strategy

Samplings were carried out during the summer of 2012 at 4 fish farms located in the eastern Mediterranean (Fig. 2). Two of the farms were located in the Aegean Sea (AEG1 & AEG2), and 2, in the Ionian Sea (ION1 & ION2). The studied farms were anonymous as a condition of the co-operation with the farmers. AEG1 and ION1 used nets treated with anti-fouling paints, whereas AEG2 and ION2 utilised untreated nets. These are henceforth referred as AF and NAF farms, respectively. The maximum depth of the cages at the farms was 10 m, and they were located in shallow coastal areas where depths ranged from 30 to 40 m. Water temperature was between 16.8 and 23.2°C. Salinity values ranged from 36.5 to 38.0 psu, and pH ranged from 7.75 to 8.13. The average value

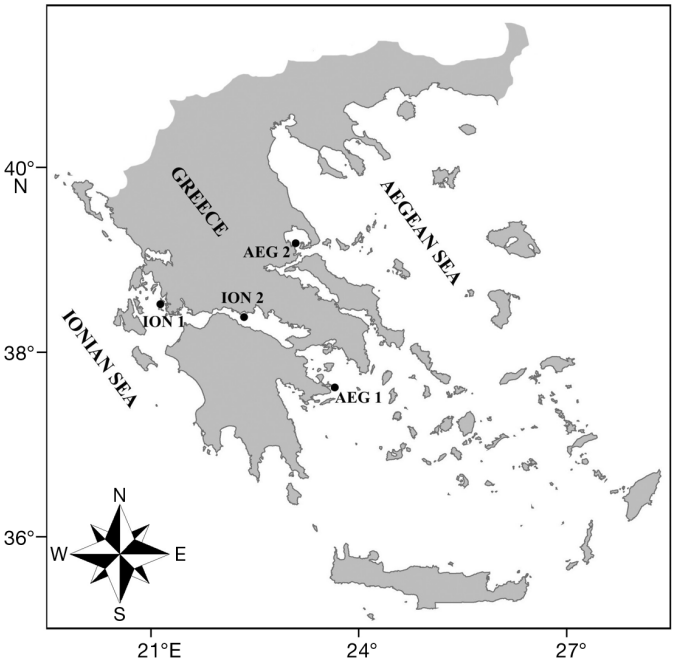

Fig. 2. Locations of the fish farms studied (AEG1 & ION1 used nets treated with anti-fouling paints, whereas AEG2 & ION2 used untreated nets)

for current speed was approximately 3.5 to 4 cm s^{-1}. The areas studied were located far from other sources of pollution (estuaries, industry, edible oil presses, agriculture, pesticides, etc.).

For the sediment analysis, 3 replicates of sediment core samples were collected by scuba divers at the centre of each fish farm (0 m), while a reference station with a similar substratum type and at a comparable depth was established 500 m away from each fish farm (n = 24). Samples were collected from the surface layer of the sediments (0 to 2 cm; Washington State Department of Ecology 2008).

Analytical methods

Grain-size analysis was performed at each sampling station using standard sieving and settling procedures (Buchanan 1971). To characterise the sediment samples in terms of particle size distribution (texture), 3 fractions were collected (sand: 2000–63 µm, silt: 63–2 µm, and clay: <2 µm) in pre-weighed containers, then dried and weighed. Sediment grain size was determined according to the method of Gray & Elliott (2009). All sediment samples were composed mainly of sand (68.3 to 79.4%). All the analyses were carried out on the <63 µm fraction, as this fraction minimises the grain-size dependence of metal concentrations (Luoma & Rainbow 2008). The dried samples were sifted through a 2 mm sieve. The fraction that passed through this sieve was then sifted through a 63 µm sieve. The total and extractable concentrations of metals were determined for all sediment samples. The total concentration of metals is generally considered insufficient for an adequate assessment of their environmental impact; therfore, it is necessary to make an estimate of the potentially bioavailable proportion (Luoma & Rainbow 2008). 'Bioavailable' is defined as the amount of metal that can be exchanged with organisms and assimilated into their tissues (Hendozko et al. 2010). The method in which sediment samples are leached with 0.5 N HCl (extractable method) is capable of identifying anthropogenic fingerprints in sediments (Chester & Voutsinou 1981) and enables the bioavailable fraction of the element associated with the sediment particles to be determined (Bryan & Langston 1992, Szefer et al. 1995, 1999). Hence, in the present study we have considered both methods (total and extractable) for an accurate measurement of metal concentrations in sediment samples.

For the biological analysis, totals of 20 cultured sea bream (*Sparus aurata*) and 20 cultured sea bass (*Dicentrarchus labrax*) were supplied by the 4 fish farms in the study (5 individuals of each species from each fish farm). To avoid any possible variability due to the growth stage of the fish, the specimens chosen were of approximately the same age and size (commercial size; differences in weight between fish groups were not significant). Immediately after collection, the fish were killed on ice and transferred to the laboratory. Total length, fork length and total weight were recorded for each specimen. Metals in muscle, liver and gills were analysed separately for each tissue and fish (n = 120). The fish were dissected using a pre-cleaned, stainless steel knife, and approximately 0.5 g of each tissue was sampled.

Both sediment and biological samples were stored in labelled, zip-lock bags at −20°C until laboratory analysis was carried out. All samples were handled very carefully in order to avoid cross-contamination.

Chemical analysis

Element concentrations of heavy metals copper (Cu) and zinc (Zn) in sediment samples were determined using the method described in USEPA Method 3051a (USEPA 2007) for microwave-assisted acid digestion of sediments, sludges, soils and oils. Cu and Zn concentrations in biological tissues were determined using the method described in USEPA Method 3052 (USEPA 1996) for microwave-assisted acid digestion of siliceous and organically based matrices. Acid-cleaned Teflon vessels and a closed, high-pressure microwave system (Multiwave 3000, Anton Paar) were used for the digestion. Each sediment sample was measured 3 times. The matrix modifier used for metal analysis was magnesium nitrate hydrate matrix modifier [$Mg(NO_3)_2$] $6H_2O$. For a 5 µl matrix modifier addition, 1.75 g of magnesium nitrate hydrate matrix modifier (10.5%) was used. Standards, as well as blanks and samples, were prepared daily, using commercial materials (Fifteen Element A/S STD [Water Wtr Poll]), and they were used to check for matrix effects by running a standard additional method. A deuterium background correction was applied to determinations using graphite furnace atomic absorption spectrometry. A standard was run for every 10 samples analysed. Quality control measures for biological tissues included the use of a blank every 7th sample and internationally certified reference materials (CRMs National Research Council of Canada; Joint Research Centre of the European Commission). Elemental concentrations were expressed in milligrams per kilogram dry

weight. All labware used was acid washed in 10% HNO_3 (1.44 N). High-purity reagents were used for sediment and biological sample digestion, as well as for blanks and calibration curve standards. The accuracy of measurements was examined by preparing and analysing a standard solution with a known concentration (2 ppb), which was then run as a sample. The accuracy of the analytical procedure was acceptable (85.70 ± 3.60%). An additional procedure was used to certify the accuracy of the method (recovery was 95.30 ± 4.70%). For the assessment of precision, 3 different samples of sediment and 3 of biological tissues were analysed 6 times each. The detection limits (LOD) for the procedure were calculated by multiplying the standard deviation of the blanks by 3.

For sediment samples, 9 ml of concentrated HNO_3 and 3 ml of concentrated HCl were added to 0.5 ± 0.01 g of sediment sample. The vessels were sealed and transferred to the microwave system where they remained at >180°C for >10 min. After digestion, samples were diluted with ultra-pure water in 50 ml volumetric flasks. To ascertain the proportion of readily extractable forms of the metals in the total metal concentration, samples (about 2 g) were leached for 16 h with 0.5 N HCl (Agemian & Chau 1976). All samples were then stored in polypropylene sample bottles at 4°C until analysis. Cu and Zn concentrations were analysed with flame atomic absorption spectrometry using a Perkin Elmer 3300 AAS.

For biological samples, 9 ml of concentrated HNO_3 were added to 0.5 ± 0.01 g of sample. The vessels were sealed and transferred to the microwave system where they remained at >180°C for >10 min. After digestion, samples were diluted with ultrapure water into 25 ml volumetric flasks. Samples were then stored in polypropylene sample bottles at 4°C until analysis. Quantitative determinations of Cu were carried out with a graphite furnace atomic absorption spectrometer, while for Zn they were carried out with a flame atomic absorption spectrometer, using standard addition methods.

Statistical analysis

Data for statistical analysis were evaluated for normal distribution by employing the Anderson-Darling test for normality and homogeneity of variance by assessing residual plots and employing Bartlett's (Snedecor & Cochran 1989) and Levene's tests (Levene 1960). One-way ANOVA was used to determine differences in metal concentrations between the sediment samples from 0 and 500 m sampling stations (separately for the AF and NAF farms), and between the AF and NAF farms (0 m) for the total and extractable concentration methods (since no significant differences were found within groups). Furthermore, the general linear model (GLM) was used to determine statistically significant differences between the biological samples from the fish farms studied (response vs. farm, response vs. tissues and response vs. farm × tissue). Afterwards, differences between level means per farm were examined using Tukey's multiple comparisons of means, in order to find out if there were significant differences between the biological samples from AF and NAF farms, respectively. Thereafter, since no significant differences were found, 1-way ANOVA was carried out for comparison between the data from the 2 AF farms (AEG1 & ION1) and the 2 NAF farms (AEG2 & ION2), separately for each tissue, in order to determine the source of differences between tissue samples. From each of the 4 fish farms the study was supplied with 5 individuals each of 2 different species to analyse, and 3 tissues were extracted from each individual. In total there were 20 samples for each species and tissue; thus, thereafter df = 19 for each species. All statistical tests were performed using the SPSS (V. 11.0) software package with a significance level of $\alpha = 0.05$.

RESULTS

Sediment data

Mean Cu and Zn total metal concentrations (HNO_3-HCl), mean extractable metal concentrations (0.5 N HCl) and the percentage ratio of extractable to total metal concentrations in the sediment samples at the 4 fish farms studied are presented in Table 1. In almost all cases, the maximum mean concentration of the 2 heavy metals was observed at sampling stations from AF farms for both total and extractable concentration methods. The maximum mean total Cu concentration at AF farms was 98.73 mg kg^{-1} (AEG1), while at NAF farms the mean concentration was 85.03 mg kg^{-1} (AEG2). The maximum mean extractable Cu concentration at AF farms was 15.89 mg kg^{-1} (AEG1) and 7.73 mg kg^{-1} at NAF farms (AEG2). In addition, the maximum mean total Zn concentration observed in sediment samples was 166.47 mg kg^{-1} at AF farms (AEG1) and 137.07 mg kg^{-1} at NAF farms (AEG2), while the mean extractable Zn concentration was 23.37 mg kg^{-1} (AEG1) and 9.81 mg kg^{-1} (AEG2).

The maximum mean ratio of extractable to total metal concentrations in the sediment samples was

Table 1. Mean (±SD) copper (Cu) and zinc (zn) total metal concentrations (HNO_3-HCl) and extractable metal concentrations (0.5 N HCl) (in mg kg^{-1} dry wt), and percentage ratio (%) of extractable to total metal concentrations in the sediment samples at the 4 fish farms studied, which use nets either with or without anti-fouling paint

Farm	Distance from farm (m)	Method	Cu	Zn
Anti-fouling				
AEG1	0	Total	90.67 ± 2.15	159.40 ± 0.98
		Extractable	15.89 ± 0.56	23.37 ± 1.63
		%	17.53	14.66
	500	Total	98.73 ± 0.98	166.47 ± 2.23
		Extractable	13.27 ± 2.33	21.75 ± 1.23
		%	13.44	13.07
ION1	0	Total	88.53 ± 2.44	146.00 ± 4.25
		Extractable	15.80 ± 0.47	19.48 ± 0.45
		%	17.85	13.34
	500	Total	85.17 ± 1.47	146.60 ± 0.71
		Extractable	14.98 ± 1.37	19.32 ± 1.35
		%	17.59	13.19
No anti-fouling				
AEG2	0	Total	85.03 ± 2.21	130.80 ± 0.95
		Extractable	7.73 ± 0.07	9.81 ± 0.46
		%	9.09	7.51
	500	Total	82.00 ± 0.36	137.07 ± 1.07
		Extractable	6.04 ± 0.12	9.28 ± 0.32
		%	7.37	6.77
ION2	0	Total	71.43 ± 1.06	129.63 ± 1.02
		Extractable	5.84 ± 0.57	9.00 ± 0.36
		%	8.18	6.94
	500	Total	80.00 ± 0.26	122.80 ± 2.40
		Extractable	4.95 ± 0.91	7.97 ± 0.33
		%	6.19	6.49

17.85% for Cu (ION1) and 14.66% for Zn (AEG1), and both of these ratios were recorded at AF farms (Table 1).

The only cases in which 1-way ANOVA indicated a significant difference were between the extractable Cu concentrations at 0 and 500 m sampling stations at AF farms ($F = 0.037$, $p < 0.05$) and at 0 m stations at AF and NAF fish farms ($F = 121.98$, $p < 0.05$).

Biological data

The morphometric data from the fish studied, which were collected from the AEG1 & ION1 (AF) and AEG2 & ION2 (NAF) farms, are given in Table 2. In almost all cases (except for Zn in the gills of sea bass), the concentrations of heavy metals were lower in samples collected from farms using untreated nets. Furthermore, the lowest mean concentration of Cu was detected in the gills of both species (*Sparus aurata*, *Dicentrarchus labrax*) studied at NAF farms, while that of Zn was found in the liver. For both species studied, the maximum concentrations of heavy metals were detected in the livers of fish collected from farms utilising anti-fouling paints. For sea bream, the maximum concentrations of Cu and Zn were 26.00 and 333.20 mg kg^{-1}, respectively, while for sea bass these concentrations were 28.50 and 435.30 mg kg^{-1}. Furthermore, the mean concentrations of Zn in

Table 2. *Sparus aurata and Dicentrarchus labrax*. Morphometric data of farmed fish collected from the 4 fish farms studied (see Fig. 2). Data are means (±SD), with ranges in parentheses (n = 5 fish per farm and species). AF: ant-fouling paint; NAF: no anti-fouling paint; TL: total length; FL: fork length; TW: total weight

Net type	Farm	TL (cm)	FL (cm)	TW (kg)
Sea bream				
AF	AEG1	25.04 ± 0.77 (24.10–25.90)	22.92 ± 0.71 (22.10–23.70)	252.30 ± 35.52 (219.60–299.30)
AF	ION1	28.48 ± 0.93 (27.70–30.10)	26.26 ± 0.95 (25.60–27.90)	403.10 ± 47.02 (362.30–478.00)
NAF	AEG2	28.12 ± 1.01 (27.00–29.60)	25.60 ± 0.97 (24.60–27.00)	388.30 ± 39.50 (331.80–440.10)
NAF	ION2	27.28 ± 0.90 (26.10–28.40)	24.90 ± 0.73 (23.90–25.70)	345.70 ± 37.11 (297.10–394.00)
Sea bass				
AF	AEG1	33.56 ± 1.71 (31.30–36.00)	31.80 ± 1.54 (29.80–34.00)	492.90 ± 71.54 (412.90–594.50)
AF	ION1	27.90 ± 0.81 (26.80–28.80)	26.38 ± 0.85 (25.20–27.30)	305.98 ± 12.94 (287.00–320.00)
NAF	AEG2	30.60 ± 1.78 (28.50–3.60)	29.06 ± 1.76 (27.00–30.90)	378.30 ± 72.71 (294.70–462.80)
NAF	ION2	34.00 ± 1.31 (32.50–35.60)	32.04 ± 1.21 (30.70–33.40)	463.30 ± 48.25 (404.50–529.30)

Table 3. *Sparus aurata and Dicentrarchus labrax*. Heavy metal concentrations (mg kg^{-1} dry wt) in farmed fish tissues at the 4 fish farms studied (see Fig. 2). Data are means (±SD), with ranges in parentheses (n = 5 fish per farm and species)

Farm	Tissue	Sea bream		Sea bass	
		Cu	Zn	Cu	Zn
Anti-fouling					
AEG1	Muscle	14.40 ± 1.92 (12.00–16.50)	28.89 ± 14.67 (5.45–42.25)	11.30 ± 2.31 (8.00–13.50)	29.36 ± 9.72 (13.20–37.30)
	Liver	21.90 ± 2.48 (19.50–26.00)	189.70 ± 62.50 (108.50–252.60)	23.80 ± 2.93 (21.00–28.50)	338.63 ± 75.20 (265.10–435.30)
	Gills	9.90 ± 1.43 (8.00–11.50)	38.42 ± 9.33 (28.45–49.20)	10.00 ± 3.10 (6.00–13.50)	20.02 ± 9.23 (12.05–33.45)
ION1	Muscle	13.60 ± 1.89 (10.50–15.50)	34.82 ± 6.04 (29.20–41.30)	11.80 ± 3.75 (7.00–16.00)	38.62 ± 5.55 (33.10–47.50)
	Liver	19.60 ± 3.13 (15.50–23.00)	269.70 ± 48.70 (201.20–333.20)	21.60 ± 3.75 (18.50–28.00)	253.02 ± 79.14 (138.30–340.10)
	Gills	7.50 ± 1.17 (5.50–8.50)	28.83 ± 12.99 (7.20–41.65)	12.60 ± 3.53 (9.00–18.00)	16.46 ± 7.62 (6.95–24.30)
No anti-fouling					
AEG2	Muscle	8.20 ± 2.14 (5.50–11.00)	31.61 ± 5.31 (24.90–39.75)	9.80 ± 2.05 (6.50–12.00)	31.96 ± 1.98 (29.70–34.05)
	Liver	13.40 ± 1.20 (12.00–15.00)	13.96 ± 1.85 (11.40–15.50)	13.90 ± 3.27 (9.50–18.00)	15.80 ± 5.70 (7.90–20.00)
	Gills	8.20 ± 2.41 (6.00–12.00)	33.82 ± 4.74 (29.20–41.20)	5.30 ± 1.26 (3.50–6.50)	12.02 ± 4.27 (8.80–19.30)
ION2	Muscle	9.50 ± 1.70 (8.00–12.00)	28.82 ± 6.21 (19.30–36.50)	7.00 ± 1.70 (5.00–9.50)	24.91 ± 2.21 (22.35–27.85)
	Liver	11.80 ± 2.20 (8.50–14.00)	18.89 ± 16.39 (5.35–42.00)	11.40 ± 2.46 (8.50–14.50)	15.03 ± 7.95 (10.70–29.15)
	Gills	5.50 ± 1.46 (3.50–7.50)	29.60 ± 3.84 (25.15–41.65)	8.30 ± 2.25 (5.00–10.50)	30.21 ± 3.49 (25.60–34.95)

the liver of sea bream and sea bass were, respectively, 14 and 19 times higher in AF farms than in NAF farms. Heavy metal concentrations in most gill samples were found to be similar in both species studied at both AF and NAF farms (Table 3).

GLM results indicated significant differences between heavy metal concentrations in the biological samples of the 4 studied fish farms (Table 4). Tukey's multiple comparisons of the means revealed by the use of this model showed that heavy metal concentrations in fish were significantly different ($p < 0.001$) among all farms, except between farms AEG1 & ION1 (AF) and farms AEG2 & ION2 (NAF) ($p > 0.05$). Furthermore, 1-way ANOVA showed that differences revealed by the GLM were confined to liver tissue for Zn, while significant differences for Cu were indicated in all tissues (except for the gills of sea bream) for both of the studied species (Table 5).

Table 4. *Sparus aurata and Dicentrarchus labrax*. General linear model results (*F*-values) examining the differences (significant at $p < 0.05$) between heavy metal concentrations in the biological samples from the AEG1 & ION1 and AEG2 & ION2 farms for both species studied. df = 59. ***$p < 0.001$

Variables	Cu	Zn
Sea bream		
Farm	34.41***	46.42***
Tissue	99.51***	92.84***
Farm × Tissue	5.16***	44.82***
Sea bass		
Farm	22.31***	45.10***
Tissue	56.93***	110.64***
Farm × Tissue	4.57***	44.44***

Table 5. *Sparus aurata and Dicentrarchus labrax*. One-way ANOVA results (*F*-values) examining the source of the differences (significant at $p < 0.05$) that were revealed by the general linear model. ns: non-significant. df = 19. *$p < 0.05$, ***$p < 0.001$

Variables	Cu	Zn
Sea bream		
Muscle	36.99***	0.18
Liver	55.80***	96.82***
Gills	3.94	0.23
Sea bass		
Muscle	7.08*	3.17
Liver	49.10***	106.58***
Gills	11.82*	0.48

DISCUSSION

In this study, the total concentrations of Cu and Zn in the sediment at sampling stations directly beneath the fish cages (0 m) were similar to those at the reference stations (500 m). The highest total concentrations of both Cu and Zn appeared at farm AEG1 (AF). Statistical analysis showed significant differences between the samples from the AF and NAF farms only in the case of extractable Cu concentration. In addition, 1-way ANOVA revealed significant differences in ex-

tractable Cu concentration between sampling stations beneath the fish cages (0 m) and the reference stations (500 m) for each AF farm (AEG1 & ION1). This may be attributed to the fact that paints produced for aquaculture can contain 40% Cu by weight (Braithwaite & McEvoy 2005), and Zn is often used in combination with Cu, thereby increasing the overall toxicity of the formulation, and facilitating the leaching process (Watermann et al. 2005). Furthermore, this result indicates that Cu inputs were higher, and, consequently, sediment enrichment of this element would be expected near farms using anti-fouling paints. This is in agreement with the hypothesis that the sedimentation of uneaten feed and faeces could be a source of Zn, whereas most of the Cu concentration originates from a different source (i.e. anti-fouling paints), which varies widely according to the frequency of net maintenance with antifouling paints and the hydrodynamics at each farm (Uotila 1991, Chou et al. 2002, Belias et al. 2003, Dean et al. 2007, Basaran et al. 2010, Wu & Yang 2010, Kalantzi et al. 2013b). Several previous studies have reported elevated concentrations of elements such as Cu and Zn, which are associated with anti-fouling use, in marine surface sediments (Debourg et al. 1993, Burridge et al. 1999, Børufsen Solberg et al. 2002, Chou et al. 2002, Parker & Aube 2002, Brooks & Mahnken 2003).

The statistical analysis in our study showed significant differences between heavy metal concentrations in the biological samples from AF and NAF farms, indicating that the use of anti-fouling paints, as is current aquaculture practice for the painting of nets, is a significant source of chemical pollution in cultured fish. In contrast, other scientists have concluded that metal concentrations in fish exposed to treated and untreated nets were not significantly different (Peterson et al. 1991, Børufsen Solberg et al. 2002, Cotou et al. 2012).

Børufsen Solberg et al. (2002) reported that, from a nutritional point of view, the use of anti-fouling paints on nets did not affect the quality of seafood products either within or around the fish cages. This is in agreement with the results of this study, since the concentrations of Cu and Zn in muscle tissue from both species studied did not exceed the maximum limits for food consumption (30 and 100 mg kg^{-1} dry wt, respectively; FAO 1983, Papagiannis et al. 2004).

The maximum concentrations of heavy metals were detected in liver tissue of sea bream and sea bass from AF farms, which is in agreement with other studies that also found the highest levels of Cu and Zn in the liver (Børufsen Solberg et al. 2002, Castritsi-Catharios et al. 2013). Furthermore, mean concentrations of Zn in the livers of both species were 19 and 14 times higher in AF farms than in NAF farms, respectively. The accumulation of elements in different fish tissues depends on the function of each tissue, the uptake route, the physiology of the fish species and behavioural factors such as habitat use and feeding habits, as well as the degree of contamination (Alam et al. 2002). Metals and trace elements chiefly accumulate in metabolically active tissues (Saha et al. 2006, Adhikari et al. 2009). The liver has been shown to be the main storage tissue for metals (Alam et al. 2002, Coğun et al. 2006, Ferreira et al. 2008). It is well known that a great deal of metallothionein induction occurs in the liver tissue of fish (Canli & Atli 2003). The differences between metal concentrations in the different tissues may be a result of their differing capacity to induce metal-binding proteins such as metallothioneins (Canli & Atli 2003), while the differences between metal concentrations in the tissues from AF and NAF farms could be due to the use of anti-fouling paint.

The results from the present study showed that Cu concentrations were significantly higher in all tissues of the 2 studied species from AF farms, except for the gills of sea bream. The leaching rate of Cu from anti-fouling paints varies according to the structure of the paint formulation and environmental conditions (Valkirs et al. 2003, Schiff et al. 2004). The environmental bioavailability, biodistribution to various parts of the organism and bioaccumulation of Cu are dramatically influenced by physicochemical parameters such as pH and salinity (Guardiola et al. 2012). These parameters were similar at all the studied fish farms, the only difference between them being the use of nets treated with anti-fouling paints. In almost all cases, heavy metal concentrations were seen to be either similar or lower in gill tissue than in muscle tissue from the 2 studied species, and, in some cases, metal concentrations in gill samples from AF farms were similar to those from NAF farms, with no significant differences found. Similarly, other scientists have analysed Cu concentrations in the gills of fish from net pen aquaculture, and have found no accumulation (Burridge et al. 2010). However, previous studies have demonstrated that heavy metal accumulation is higher in gill tissue than in muscle tissue (Canli & Atli 2003, Dural et al. 2006, Coban et al. 2009, Castritsi-Catharios et al. 2013, Kalantzi et al. 2013a). 'Species' appears to be one of the most important factors for the accumulation of elements in fish tissues (Kalantzi et al. 2013a). This may be attributed to physiological differences between species; a

suggestion underlined by the fact that, although the 2 farmed species utilise similar feeding habits, they differ in analyte uptake.

CONCLUSIONS

The highest metal concentrations of extractable Cu were found in the sediment samples collected under the cages of the fish farms that used nets treated with anti-fouling paint. These concentrations were 2 to 3 times higher than at farms using untreated nets. In addition, heavy metal concentrations were almost always lower in the biological samples collected from farms using untreated nets. The highest metal concentrations appeared in the liver tissue of both studied species (*Sparus aurata*, *Dicentrarchus labrax*). In no case did the concentrations of Cu and Zn in the muscle tissue of either studied species from AF farms exceed the maximum limits allowed for food. All the aforementioned data indicate that the use of anti-fouling paints may be a potential source of metal accumulation in cultured fish. Nevertheless, further studies controlling for the various factors involved are required in order to draw firm conclusions.

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