

Impact of fish farming on the distribution of phosphorus in sediments in the middle Adriatic area

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Abstract

During the last decade, intensive fish farming developed along the central Croatian coast, creating a need to study and evaluate its potential influence on unaffected sites. We considered phosphorus as an indicator of the influence of fish farming and investigated the distribution of phosphorus forms in sediment from several fish farms and marine areas of different trophic status in the middle Adriatic. Analyses of samples were performed with modified SEDEX techniques. Our results indicated that authigenic apatite phosphorus showed no significant differences among the investigated stations, while organic phosphorus concentrations reflected the trophic status of the station area. Below-cage sediment was characterized by enhanced fish debris phosphorus and low detrital apatite phosphorus concentrations, while sediment from an anthropogenically influenced bay showed the highest values of iron bound phosphorus species. Among the different P fractions, fish debris phosphorus proved to be the most sensitive indicator of the influence of fish farming on marine sediment.

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1. Introduction

During the last decade, intensive fish farming (blue fin tuna, sea bass and sea bream) developed along the central Croatian coast. This development induced interest for studying and evaluating its potential influence on unaffected sites in the mid-Adriatic. Fish farms generate a wide range of dissolved and particulate matter and their effects have been demonstrated through different parameters in sediment, such as: accumulation of phosphorus and nitrogen compounds (Hall et al., 1990; Holby and Hall., 1991; Hargrave et al., 1997), organic carbon accumulation (Hall et al., 1990; Hargrave et al., 1997), negative sediment redox potential (Hargrave et al., 1993; Pawar et al., 2001), and consequently, changed or reduced benthic communities (Mazzola et al., 1999; Kovač et al., 2001; Karakassis et al., 1999).

Phosphorus and nitrogen compounds are especially important for the productivity in the water column, considering their role as limiting nutrients in different marine areas (Tyrell, 1999; Krom et al., 2004). Enrichment of the aquatic environment with these elements is indicated by enhanced levels of their concentrations in the water column and accumulation in sediment. In contrast to nitrogen, which can be partially lost due to denitrification and anammox processes (Risgaard-Petersen et al., 2003), phosphorus remains preserved in a series of fractions as a consequence of adsorption, dissolution, or precipitation processes (Froelich et al., 1988; Benitez-Nelson, 2000; Paytan et al., 2003), and is therefore useful as an environmental impact indicator. This is in agreement with Soto and Norambuena (2004), who selected and proposed phosphorus sediment concentration as the most useful parameter to indicate the impact of fish farming, due to the low variability of natural P concentrations, as well as its relation to P content in fish food, metabolic products and to organic matter produced in the water column. Phosphorus in the sediment

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as an environmental indicator of the influence of fish farming has been used frequently in studies throughout the world (Hall et al., 1990; Holby and Hall 1991; Hargrave et al., 1997; Karakassis et al., 1999; Cancemi et al., 2003; Soto and Norambuena, 2004; Porello et al., 2005; Kassila et al., 2000; Kalantzi and Karakassis, 2006).

The most used method to determine sediment P concentrations at fish farms is that of Aspila et al. (1976), which enables determination of inorganic (IP) and total phosphorus (TP) concentrations and allows comparison across all investigated fish farming sites. In contrast, sequential extraction methods (Hieltjes and Lijklema, 1980; Ruttenberg, 1992; Kassila and Hussenot, 2004; Schenau and De Lange 2000) that distinguish different inorganic P fractions (P adsorbed on to metal oxides/hydroxides, P-bound in authigenic and detrital apatite form or fish-debris P form), have been used comparatively infrequently for fish farm marine sediments (Herut and Nishri, 2004; Kassila and Hussenot, 2004; Matijević, 2006) and freshwater pond sediments (Kassila et al., 2000).

Previous investigations of P in fish farming sediments of the middle Adriatic area, performed as part of monitoring programs of ecological conditions in sea bass/sea bream and tuna farms, showed a significant increase of TP concentrations in surface sediments in comparison to samples from “unaffected” areas (Matijević et al., 2004, 2006). These results were in accordance with results from many fish farming areas such as salmon farms in northern Europe (Holby and Hall, 1994) and Chile (Soto and Norambuena, 2004), and sea bass and sea bream farms in Cephalonia Bay, Greece (Karakassis et al., 1999), SW Corsica (Cancemi et al., 2003) and the Tyrrhenian Sea (Porello

et al., 2005), indicating enhanced TP concentrations under cages in relation to control sites.

Through the use of phosphorus as an indicator of the influence of fish farming, in this paper we aimed to investigate the distribution of various phosphorus forms in sediment samples from several fish farms and marine areas of different trophic status in the middle Adriatic. To improve methods for determination of phosphorus species, we used a combination of different SEDEX techniques (Ruttenberg, 1992; Anshutz et al., 1998 and Schenau and De Lange, 2000) with additional analytical modifications.

2. Material and methods

2.1. Sampling and analytical determinations

Sampling of sediments for phosphorus species determination was performed from 2001 to 2005 at eight tuna breeding farms (A–H) located along the middle Adriatic coast, at an anthropogenically influenced Kaštela Bay station (S1), a channel station (S2) and an open sea station (S3) (Fig. 1). At each farm site, three sampling stations were chosen: under the cage (A–H), at approximately 200 m distance from the cage (A1–H1), and reference site stations (A2–H2). Basic information such as coordinates, sampling dates, station depths and sediment characteristics are presented in Table 1.

Vertical profiles of salinity and temperature in the water column were measured using a SEABIRD-25 CTD probe, while oxygen saturation was determined by classical Winkler titration (Grasshoff, 1976). Dissolved orthophosphate was determined colorimetrically on an AutoAnalyzer-3

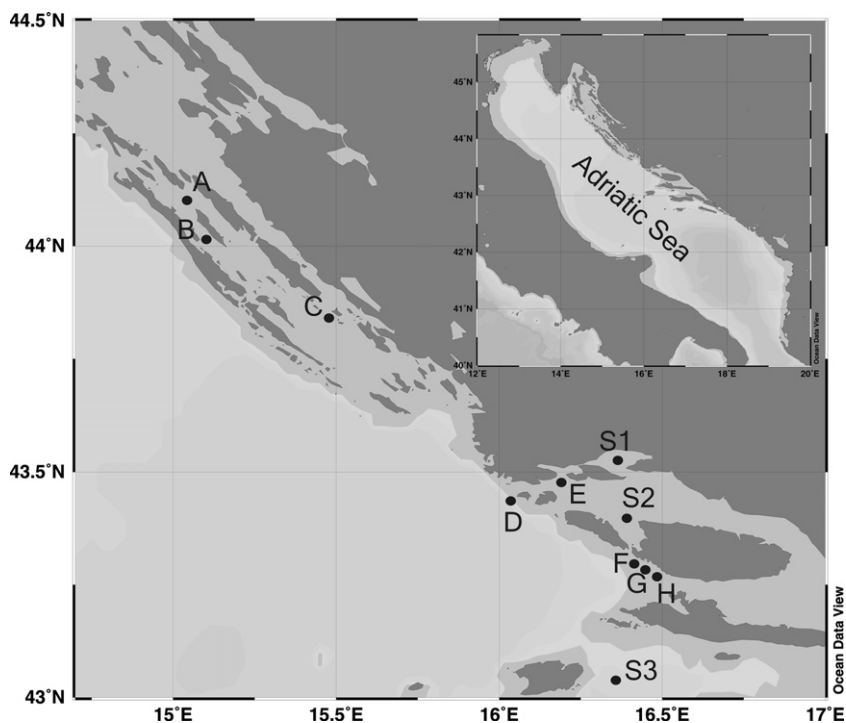


Fig. 1. Map of the investigated stations.

Table 1

Sampling dates, station coordinates, sediment type according to Folk (1954) (gM = gravelly mud; (g)mS = slightly gravelly muddy sand; gM = gravelly mud; gS = gravelly sand; mS = muddy sand; M = mud; gmS = gravelly muddy sand), organic carbon and total nitrogen content in sediment, redox potential in surface sediment layer (0–1 cm) and oxygen saturation in the bottom layer of the water column

Station	Latitude	Longitude	Depth	Sampling date	Sediment type	C-org (%)	N-tot (%)	O ₂ (%)	E _H (mV)
A	44°04,0'	15°04,1'	30	June 2001	gM	1.73	0.09	97.7	170
A1	44°04,2'	15°04,4'	20	June 2001	(g)sM	n.d.	n.d.	105.8	122
A2	43°59,5'	15°11,2'	50	June 2001	gM	2.17	0.16	96.2	87
B	44°01,9'	15°06,4'	33	June 2001	gS	2.92	0.26	95.6	89
B1	44°01,1'	15°07,0'	60	June 2001	n.d.	n.d.	n.d.	94.2	124
B2	44°01,0'	15°07,8'	66	June 2001	gM	0.90	0.06	96.2	122
C	43°50,7'	15°29,9'	26	June 2001	mS	1.41	0.18	67.3	–181
C1	43°50,8'	15°29,6'	35	June 2001	(g)mS	n.d.	n.d.	96.9	118
C2	43°49,3'	15°30,1'	70	June 2001	gM	4.96	0.04	93.3	131
D	43°26,0'	16°27,9'	21	July 2001, February 2003	(g)mS	3.39 0.27	0.22 0.40	102.3 85.2	160 –39
D1	43°27,0'	16°27,9'	65	July 2001, February 2003	mS	0.71 n.d.	0.12 n.d.	96.4 93.3	163 19
E	43°28,7'	16°10,5'	48	July 2001, February 2003	M	0.78 n.d.	0.12 n.d.	93.5 95.8	n.d. 32
E1	43°28,97'	16°09,9'	46	July 2001, February 2003	sM	1.09 n.d.	0.17 n.d.	n.d. 96.9	n.d. 62
E2	43°28,7'	16°11,0'	52	July 2001, February 2003	M	1.27 n.d.	0.17 n.d.	n.d. 94.9	n.d. 53
E3	43°27,0'	16°27,9'	65	July 2001, February 2003	M	0.75 n.d.	0.08 n.d.	n.d. 94.6	n.d. 56
F	43°18,4'	16°28,2'	12	December 2001, May 2002, March 2003	sG	1.29 0.54 0.61	0.18 0.03 0.06	101.0 104.7 86.2	16 –19 31
F1	43°18,1'	16°27,9'	37	December 2001, May 2002, March 2003	mS	1.13 0.71 0.57	0.26 0.04 0.07	96.2 107.2 86.8	50 –30 30
F2	43°178,7'	16°28'	55	December 2001, May 2002, March 2003	gM	0.92 0.41 0.35	0.11 0.01 0.04	91.8 101.8 88.6	50 –3 9
F3	43°17,5'	16°27,9'	78	December 2001, May 2002, March 2003	sM	1.26 0.39 0.36	0.17 0.01 0.05	102.5 105.7 87.6	53 41 54
G	43°17,4'	16°28,9'	45	June 2003, April 2004, January 2005	gS	0.72 n.d. 9.07	0.19 n.d. 1.98	90.0 91.2 97.3	–141 9 74
G1	43°17,5'	16°28,7'	48	June 2003, April 2004, January 2005	gmS	0.89 n.d. 5.46	0.34 n.d. 0.79	92.7 91 97.7	12 n.d. 175
G2	43°16,7'	16°28,7'	81	June 2003, April 2004, January 2005	(g)sM	0.51 n.d. 1.33	0.13 n.d. 0.22	86.6 81.5 93.4	–1 62 108
H	43°16,9'	16°29,6'	53	September 2001 June 2003, April 2004, January 2005	gS	n.d. 0.83 n.d. 4.29	n.d. 0.13 n.d. 2.61	107.9 95.9 95.2 84.8	158 28 61 122
H1	43°16,9'	16°29,5'	46	September 2001 June 2003, April 2004, January 2005	(g)mS	n.d. 0.75 n.d. 1.56	n.d. 0.11 n.d. 0.26	105.2 94.1 93.5 89.5	141 –11 n.d. 128
S1	43°31,0'	16°22,0	37	2001–2002	M	0.99–1.35	0.02–0.07	78.8–107.5	70
S2	43°25,6'	16°23,9	52	2001–2002	M	0.91–2.2	n.d.	91.6–98.9	42
S3	43°00,0'	16°20,0	102	2001–2002	gmS	0.22–1.23	0–0.02	87.7–98.9	45

n.d., not determined.

according to Grasshoff (1976), as well as total phosphorus, after UV oxidation. An international intercalibration exercise on nutrients in seawater (Quasimeme, 2002) showed that the results of the analytical procedures used differed

not more than 7.2% from assigned values for orthophosphate and 7.3% for total dissolved phosphorus.

Sediment samples were collected in duplicates by SCUBA-diver (depth < 50 m) or gravity corer (depth >

50 m) using transparent plastic liners (i.d. = 6.5 cm). Granulometric composition of the sediment samples was determined by sieving (>63 μm) and hydrometric method (<63 μm), and sediment type was classified according to Folk (1954). Carbonate content was determined according to Loring and Rantala (1992), while organic matter content was determined by H_2O_2 treatment of the samples at 450 °C for 6 h. The loss of weight after this treatment was assumed to be due to the organic matter content (Vdović et al., 1991; Bogner et al., 2005). Sediment organic carbon and total nitrogen contents were determined using CHNS-O analyzer (EA 1110, CE instruments).

For measurements of phosphorus sediment concentration, each sample was divided into slices (1 cm thick), frozen, freeze-dried, and stored in clean plastic bags until analysis. Sediment phosphorus concentrations were measured on a Shimadzu UV–Vis Spectrophotometer according to Aspila et al. (1976) using modified SEDEX methods (Ruttenberg, 1992; Schenau and De Lange, 2000; Anshutz et al., 1998) (Table 2), after extraction of 0.175 g (d. wt.) sediment sample. The extraction volume for all steps shown in Table 2 was 35 cm^3 . Results for P species concentrations are presented as spatial and temporal average values, i.e. average concentration for the first two centimeters of surface sediment layer during the investigated periods. Standard sediment materials PACS-2 (Canadian Institute for National Measurement Standards NRC-CNRC), and BCR-684 (European Commission Community Bureau) were used for method evaluation.

Total iron oxides in the sediment were determined in the extractant solution obtained after the citrate–dithionite–bicarbonate (CDB) buffer extraction of sediment (Step 2 in Table 2). CDB agent is specific for both amorphous and crystalline iron oxides, which are the most reactive iron forms in sediment, consisting mainly of Fe(III)OOH (Canfield et al., 1992). An aliquot of solution (5 cm^3), after the

extraction of sediment samples with CDB, was diluted to 25 cm^3 and analyzed for Fe by flame atomic absorption spectrophotometry (F-AAS).

2.2. Analytical modifications of SEDEX method

2.2.1. Biogenic apatite phosphorus fraction (P–FD)

According to Schenau and De Lange (2001), this fraction is extracted with 2 M ammonium chloride solution and diluted with deionised water prior to determination. However, samples analysed as recommended showed an unstable complex colour and absorbance readings. We noted that the problem was caused by an inappropriate pH, so we introduced chloroacetic acid solution (pH 2.6) instead of deionised water to obtain an optimal pH (0.9–1) for phosphomolybdate blue complex formation (Grasshoff, 1976). The absorbance measured in 5–10 min intervals is shown in Fig. 2a.

2.2.2. Iron bound phosphorus fraction (P–Fe)

This fraction is, according to Watanabe and Olsen (1962) and Ruttenberg (1992), extracted with citrate–dithionite buffer solution (CDB) and isobutanol. Problems occurred with blue colour development and separation of the isobutanol layer, which were caused by inappropriate pH for phosphomolybdate blue complex formation. Anshutz et al. (1998) solved this problem using reagents with modified ratios of ammonium heptamolybdate and sulfuric acid. Unfortunately, their method included a CDB extraction step at elevated temperature (85–90 °C), which did not allow further sediment extraction for authigenic apatite and detrital bound phosphorus determination. Therefore, we applied a combination of 16 h CDB extraction time at room temperature as proposed by Ruttenberg (1992) and reagents proposed by Anshutz et al. (1998). This combined method yielded good reproducibility and linear-

Table 2

Sequential extraction scheme details (modification of methods according to Ruttenberg, 1992; Anshutz et al., 1998; Schenau and De Lange, 2000) applied on to 0.175 g of sediment (d. wt.), methods repeatability (RSD) and literature references

Step	Phosphorus fraction	Extractant	Time	RSD%	References
1	Biogenic apatite bound phosphorus (P–FD)	Eight extractions in 2 M NH_4Cl (brought to pH 7)	4 h, 20 °C	5.1	Schenau and De Lange (2000)
2 ^a	Iron bound phosphorus (P–Fe)	1 \times 35 cm^3 0.15 M Na-citrate, 0.5 M NaHCO_3 (pH 7.6) and 1.575 g $\text{Na}_2\text{S}_2\text{O}_4$	16 h, 20 °C	6.7– 15.6	Anshutz et al. (1998)
3	Authigenic apatite phosphorus (P–AUT)	1 \times 35 cm^3 1 M NaAc i HAC (pH 4)	16 h, 20 °C	4.0	Ruttenberg (1992)
4		WASH: 1 \times 35 cm^3 2 M NH_4Cl (brought to pH 7)	2 h, 20 °C		
5		WASH: 1 \times 35 cm^3 deionised H_2O	2 h, 20 °C		
6	Detrital apatite bound phosphorus (P–DET)	1 \times 35 cm^3 1 M HCl	16 h, 20 °C	5.9	Aspila et al. (1976)
7	Total phosphorus (TP) (separate weight after 2 h ignition at 550 °C)	1 \times 35 cm^3 1 M HCl	16 h, 20 °C	1.7	Aspila et al. (1976)
8	Inorganic P (IP) (separate weight)	1 \times 35 cm^3 1 M HCl	16 h, 20 °C	3.2	Aspila et al. (1976)

^a According to Ruttenberg (1992), after extraction step 2 (CDB buffer solution) wash in 2 M NH_4Cl and deionized water is needed. However, since orthophosphate concentration analysis showed very low concentrations, we skipped the washes as recommended by Slomp et al. (1996).

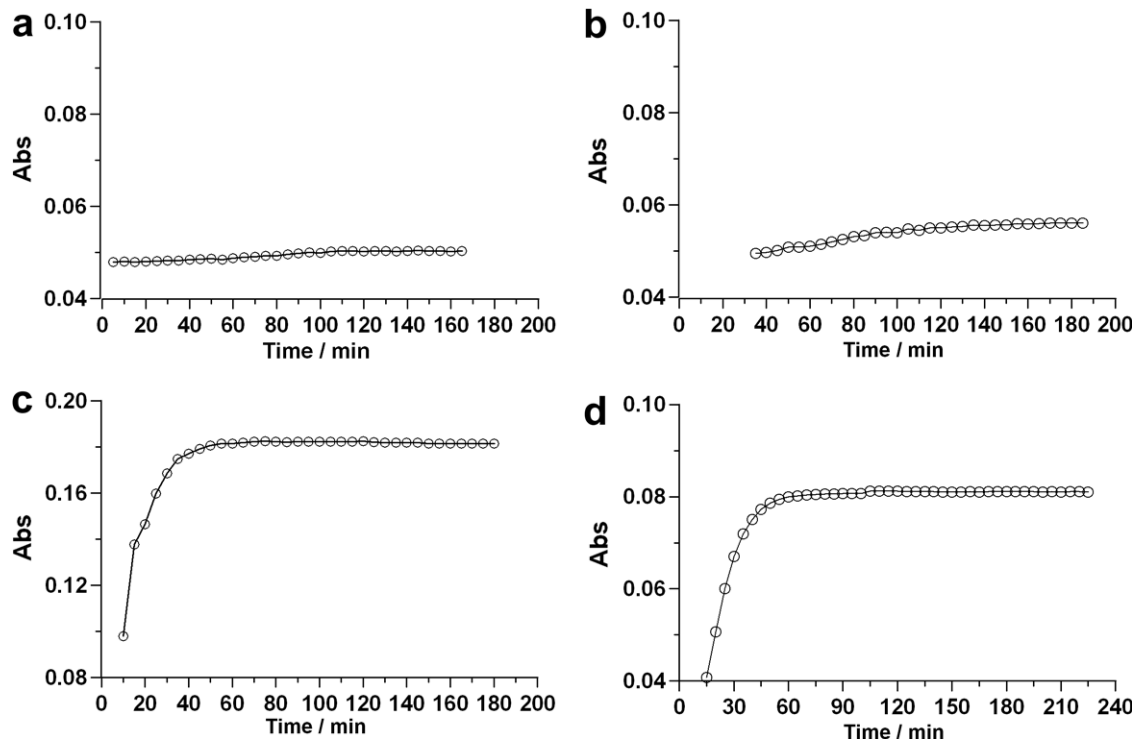


Fig. 2. Absorbance vs time curves for samples extracted with 2 M ammonium chloride solution (a), citrate dithionate buffer solution (b), 1 M sodium acetate solution (c), and 1 M chloride acid solution (d).

ity of the calibration curve. Tests with P-spiked sediment material gave significantly better results than the original method (Anshutz et al., 1998). The absorbance vs. time curve for this combined method is shown in Fig. 2b.

2.2.3. Authigenic apatite bound phosphorus fraction (P-AUT)

Determination of this fraction is based on sediment extraction with sodium acetate/acetic acid buffer solution (NaAc-HAc), acidification of the extractant and addition of the reagent (Ruttenberg, 1992). Following this procedure, we noted that the formation of colloids and an inappropriate pH for optimal phosphomolybdate blue colour development were problematic. This was solved by acidification of the extractant with sulfuric acid (1 cm³ conc. H₂SO₄ + 24 cm³ NaAc-HAc buffer) and centrifugation for 10 min at 2400 rpm after the addition of colour reagents. The absorbance measured in 5–10 min intervals is shown in Fig. 2c.

2.2.4. Detrital apatite phosphorus fraction (P-DET) and total phosphorus (TP)

P-DET and TP are phosphorus fractions extracted in 1 M HCl (Ruttenberg, 1992; Aspila et al., 1976), whose analytical determination takes place in extracted samples diluted with deionised water. During extraction we detected similar problems as for the P-FD fraction extraction. Therefore, we also introduced a solution of chloroacetic acid (pH 2.6) instead of deionised water, which, after colour reagent addition, gave an optimal reaction pH

(0.9–1) and improved signal stability. The absorbance measured in 5–10 min intervals is shown in Fig. 2d.

3. Results and discussion

3.1. Hydrographic characteristics, orthophosphate and organic phosphorus concentrations in the bottom layer of the water column

During the investigation, the temperature of the bottom layer ranged from 11.7 to 18.8 °C at the farming sites and from 11.23 to 17.26 °C, 11.61 to 16.11 °C and 13.17 to 16.47 °C at the S1, S2 and S3 stations, respectively (Table 1). Considering the temperature as one of the major parameters that influences diagenetic processes, we hold that the observed temperature differences between cage sites and the S1, S2 and S3 stations are probably too small to affect the distribution of P forms in sediment. Due to low freshwater discharges in this part of the Adriatic, the salinity of the bottom layer was permanently high (>37.7) at all stations, with a maximum of 39.2 recorded at the open sea station (S3) in 2002. The bottom layer of the water column was well oxygenated for most of the fish farming sites, with oxygen saturation ranging from 92.4% to 105.8%, as well as the bottom layers of the S1, S2 and S3 stations where ranges were 78.8–107.5%, 91.6–98.9%, and 87.7–98.9%, respectively (Table 1).

Orthophosphate concentrations at stations under the tuna cages were between 0.01 and 3.09 mmol m⁻³ (Fig. 3). Enhancements were observed under cages A, C

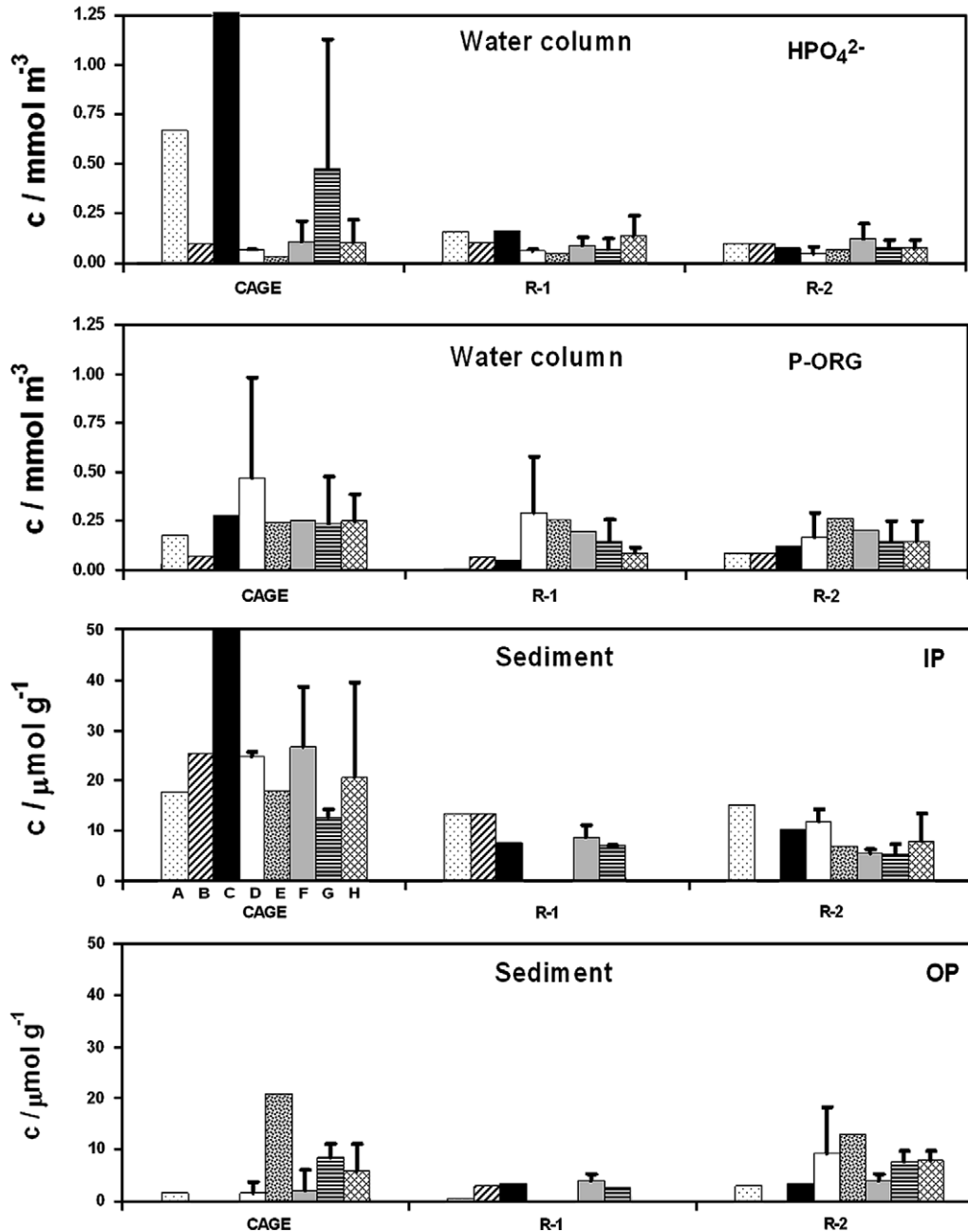


Fig. 3. Orthophosphate (HPO_4^{2-}) and organic phosphorus concentrations (P-ORG) in the bottom layer of the water column and sediment inorganic and organic phosphorus concentration (IP, OP) at tuna farms during investigated periods.

and G with values up to 47 times higher in relation to the unaffected stations, and up to 15 times higher than eutrophic areas of the middle Adriatic (Viličić, 1989). Concentrations of organic phosphorus at farm sites were also higher in relation to reference stations, but still within the range determined for unaffected areas of the middle Adriatic ($0.27 \pm 0.46 \text{ mmol m}^{-3}$) (Zore-Armanda et al., 1991, Oceanographic Referral Database, Institute of Oceanography and Fisheries Split, www.izor.hr).

Up to six fold increases in orthophosphate concentrations under tuna cages were detected in the water column from the Gaeta Gulf in the Tyrrhenian Sea (La Rosa

et al., 2002), while Pitta et al. (1999) reported five times higher orthophosphate concentrations in relation to reference sites at one Greek tuna farm. Maldonado et al. (2005) detected subtle difference between Spanish farms and control sites, while Wu et al. (1994) found increases in nutrient concentrations only in farms with poor tidal flushing and a high density of fish in the cages. According to Karakassis et al. (2001), orthophosphate and nitrogen concentrations differ diurnally with higher concentrations under the cages during maximum daily feeding in relation to reference sites. Pitta et al. (2006) found increased afternoon orthophosphate concentrations in

comparison to the morning at three different farms in the Mediterranean.

Generally, results of numerous investigations of the influence of fish farming on water column variables showed that there is no systematic negative effect (Pitta et al., 1999; La Rosa et al., 2002; Soto and Norambuena, 2004). A possible explanation of occasional increases in orthophosphate concentrations is the presence of strong currents in fish farming areas (Kalantzi and Karakassis, 2006), or rapid passing of orthophosphate through the food web (Soto and Norambuena, 2004).

3.2. Sediment phosphorus concentrations

3.2.1. Total, inorganic and organic phosphorus

Total phosphorus concentrations (TP) in the sediment of the investigated farms determined using the method of Aspila et al. (1976) ranged from 19 to 135 $\mu\text{mol g}^{-1}$ (d. wt.), which is up to five times higher in relation to the middle Adriatic area sediment (3–37 $\mu\text{mol g}^{-1}$) (Barić et al., 2002). Inorganic phosphorus sediment concentrations under the cages ranged from 5 to 135 $\mu\text{mol g}^{-1}$, and were accompanied by enhanced orthophosphate concentrations in the bottom layer of the water column in most of the sites (Fig. 3). Simultaneously, sediment organic phosphorus at most sites remained within the natural range of 0–21 $\mu\text{mol g}^{-1}$.

Enhancement of sediment phosphorus concentrations at fish farms has been demonstrated previously. P concentrations in sediment beneath a salmon farm in Chile (150–230 $\mu\text{mol g}^{-1}$) were six to nine times higher in relation to control sites (Soto and Norambuena, 2004). In the sediment beneath sea bass and sea bream farms in SW Corsica ($61 \pm 14 \mu\text{mol g}^{-1}$) and Cephalonia Bay, Greece (129–193 $\mu\text{mol g}^{-1}$), TP was three and six times higher than at control sites (Cancemi et al., 2003; Karakassis et al., 1999). Similarly, TP concentrations under farms in the Tyrrhenian Sea (24–36 $\mu\text{mol g}^{-1}$) were up to two times higher compared to unaffected sites (Porello et al., 2005).

3.2.2. Fractions of phosphorus

While the Aspila method is widely applied and concentrations are easily comparable for fish farm sediments on a worldwide scale, SEDEX methods (Hieltjes and Lijklema, 1980; Ruttenberg, 1992; Kassila and Hussenet, 2004; Schenau and De Lange 2000), that distinguish inorganic P fractions, have only been used for a small number of fish farm marine sediments (Herut and Nishri, 2004; Kassila and Hussenet, 2004; Matijević, 2006) and freshwater pond sediments (Kassila et al., 2000).

The sediment P species concentrations (P–FD, P–Fe, P–DET, P–AUT and P–ORG) investigated using modified SEDEX method on sediment of two tuna farms (G, H) and marine areas of different trophic status (S1, S2, S3) in the middle Adriatic are shown in Fig. 4.

3.2.2.1. P–FD. The fish debris P fraction includes phosphorus bound in biogenic apatite originating from hard parts

of fish material (fish bones and teeth) and very small amounts of P loosely adsorbed on to mineral surfaces and carbonates (Schenau and De Lange, 2000). According to these authors, eight extractions in 2 M NH_4Cl solution are needed to ensure complete dissolution of P bound on to fish bones in carbonate sediments.

P–FD extraction efficiency during eight extractions in 2 M NH_4Cl in sediment samples from tuna farms (G and H) and three marine areas of different trophic status (S1, S2 and S3) are given in Fig. 5. For most of the stations, the major proportion of P–FD was extracted in the first as well as in the third and fourth extraction steps (Fig. 5). Similar pattern of P–FD distribution during extractions was also found in carbonate sediment samples from Arabian Sea by Schenau and De Lange (2000), who showed that the extraction efficiency strongly depends on calcium concentration, i.e. P–FD increased once the calcium concentration in solution dropped.

P–FD concentrations at investigated sites in the middle Adriatic ranged from 0.76 to 10.48 $\mu\text{mol g}^{-1}$ (d. wt.), with the highest average values recorded for stations under tuna cages G and H (6.33 ± 2.80 and $4.8 \pm 2.60 \mu\text{mol g}^{-1}$, respectively) (Fig. 4). P–FD concentrations in fish farm sediments were 2.3 times higher than at the S1, S2 and S3 stations, which was expected considering the origin of the P–FD fraction (fish bones and hard parts of skeletons of dead fishes settled on the seabed under tuna cages).

The portion of P–FD fraction in TP at fish farms (35%) was higher in relation to sediment from the S1 (15%), S2 (18%), and S3 (19%) stations (Table 3). These results are in accordance with rare results for P–FD in marine sediments of the Arabian Sea (Schenau and De Lange, 2000, 2001), where the P–FD portion in TP was between 25% and 45%. However, they contrast with results for the apatite P-fraction in fish farm sediments obtained with other extraction methods. In the sediment of Eliat Bay, Israel, Herut and Nishri (2004) applied the P fractionation method according to Hieltjes and Lijklema (1980) and determined that 85–90% of TP was the Ca-bound P form. Comparison of three different analytical methods on the same sediment from a sea bass farm in the French Atlantic (Kassila and Hussenet, 2004) showed that 74% of Ca-bound P in TP was determined according to Hieltjes and Lijklema (1980), 62% using the SEDEX method according to Ruttenberg (1992), and 51% was obtained with the EDTA method proposed by Kassila and Hussenet (2004). The high percentages of apatite Ca-bound P in TP of these fish farm sediments imply non-selectivity of the applied methods, which disables distinction between biogenic and authigenic apatite P species provided through the eight NH_4Cl step extraction procedure of Schenau and De Lange (2000) applied in this paper.

3.2.2.2. P–Fe. The phosphorus fraction bound to iron oxyhydroxides was analyzed according to the method of Anshutz et al. (1998). Although this extraction step represents the most critical point in the sequential analysis of P

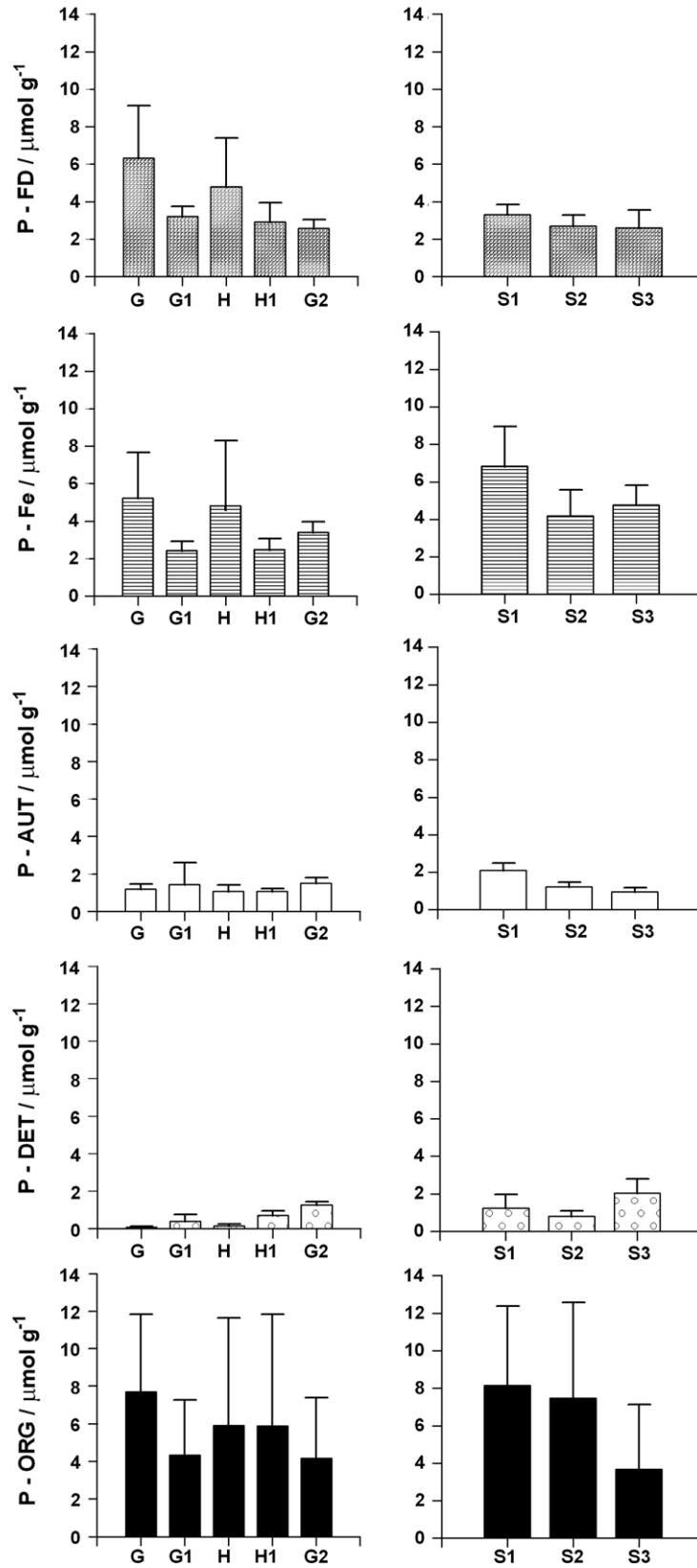


Fig. 4. Concentration ($AV \pm SD$) of P species (P-FD, P-Fe, P-AUT, P-DET and P-ORG), in sediment layer (0–2 cm) of two tuna farms (G, G1, G2, H, and H1) and marine areas of different trophic status (S1, S2, S3).

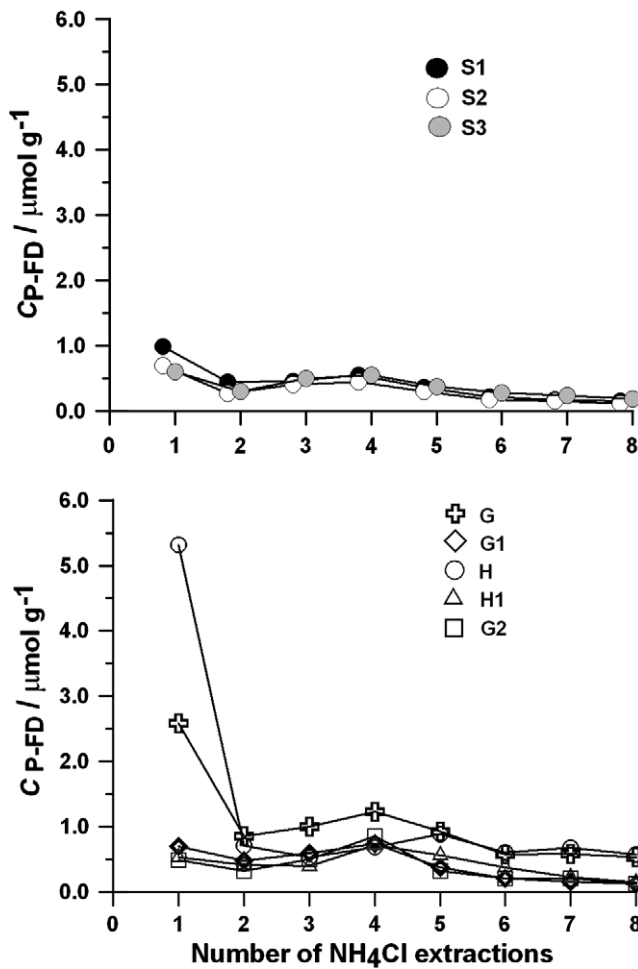


Fig. 5. P-FD concentrations during eight extractions in 2 M NH_4Cl in sediment samples of two tuna farms (G, G1, G2, H and H1) and three marine areas of different trophic status (S1, S2 and S3).

in sediment due to low repeatability (RSD 6.7–15.6%) (Table 2), it is the only method which separates the P fraction exclusively bound to iron oxyhydroxides. In contrast, the widely applied and modified method according to Williams et al. (1976) determines the P fraction bound to Al and Fe oxides and hydroxides.

P-Fe concentrations in the investigated samples ranged from 1.21 to 11.91 $\mu\text{mol g}^{-1}$ (d. wt.), with the highest average value recorded at S1 station in the eutrophic Kaštela Bay ($6.50 \pm 2.11 \mu\text{mol g}^{-1}$) and the lowest at the tuna farm

reference station G1 ($2.43 \pm 0.48 \mu\text{mol g}^{-1}$) (Fig. 4). P-Fe concentrations in sediment under the cages (H: $5.22 \pm 2.44 \mu\text{mol g}^{-1}$; G: $4.59 \pm 3.70 \mu\text{mol g}^{-1}$) were 1.5–2 times enhanced in relation to their reference stations (H1, G1, G2).

Since data from the literature for the P-Fe fraction differs greatly, depending on station location and sediment type (Jensen et al., 1995; Slomp et al., 1996; Anshutz et al., 1998; Andrieux and Aminot, 1997; Schenau and De Lange, 2001; Lopez, 2004), consideration should be given to the P-Fe/TP ratio rather than to concentrations. The average P-Fe portion in TP for middle Adriatic sediments ranged from 12.9% to 40.1% with P-Fe as the most important inorganic P form (Table 3). Similar results have been found elsewhere in the world. Jensen et al. (1995) found 34–50% of P-Fe in TP in the 0–1 cm sediment layer for the clayey sediment of Aarhus Bay, Denmark, but also identified a seasonal correlation of P-Fe with orthophosphate fluxes at the sediment/water interface. In the surface sediment layer of sandy and silty sediment in the North Atlantic, the proportion of P-Fe form in TP was 25–30%; this had a key role in authigenic apatite formation (Slomp et al., 1996). Investigations of silty clay sediment at the Canadian and Portuguese continental margins demonstrated 25% P-Fe in TP, with a decreasing trend towards deeper sediment depth (Anshutz et al., 1998). In sandy sediment of the Bay of Seine (Andrieux and Aminot, 1997), the P-Fe portion (determined with Al oxides) was 3–27%, while in carbonate sediments of the Arabian Sea, Schenau and De Lange (2001) found the portion ranged from 6.4% to 22.4%. Lopez (2004) identified P-Fe in clayey sediment from Minorca as the main inorganic P fraction, with 16.6% in TP.

Generally, the P-Fe portion of TP in sediment varies greatly and there is no general trend in relation to sediment type. We found similar results for the middle Adriatic sediments (Figs. 4 and 6, Table 3). According to some authors (Jensen et al., 1998; Petersson and Istvanovics, 1988), the P-Fe form could be overestimated in carbonate sediments due to dissolution of Ca-bound P during the CDB-extraction step. Unlike them, Lopez (2004) found this extractant always related to iron in solutions, and not with calcium.

3.2.2.3. P-AUT. Concentrations of P bound on authigenic apatite (P-AUT) ranged from 0.57 $\mu\text{mol g}^{-1}$ (S3) to 2.68 $\mu\text{mol g}^{-1}$ (d. wt.) (S1). Average P-AUT concentrations

Table 3
Portion of sediment phosphorus fraction in total sediment phosphorus concentration (AV \pm SD) at monitored stations

Station	P-FD/TP (%)	P-Fe/TP (%)	P-AUT/TP (%)	P-DET/TP (%)	P-ORG/TP (%)
S1	15.1 \pm 1.8	32.4 \pm 10.0	9.7 \pm 2.1	6.3 \pm 4.3	35.4 \pm 13.9
S2	17.7 \pm 5.3	27.4 \pm 12.1	7.8 \pm 1.7	5.4 \pm 3.1	41.0 \pm 18.7
S3	19.4 \pm 4.0	40.1 \pm 11.2	7.5 \pm 2.4	18.0 \pm 7.0	18.7 \pm 14.5
G	34.3 \pm 22.2	33.7 \pm 9.4	5.6 \pm 1.3	0.4 \pm 0.3	34.7 \pm 15.5
G1	27.3 \pm 5.4	23.1 \pm 6.2	12.4 \pm 11.3	3.0 \pm 1.9	34.4 \pm 14.9
H	35 \pm 22.7	37 \pm 11.7	5.9 \pm 4.7	0.6 \pm 0.2	27.1 \pm 8.2
H1	25.4 \pm 8.1	27.1 \pm 8.1	9.3 \pm 3.4	7.0 \pm 4.1	38.5 \pm 18.8
G2	22.7 \pm 5.5	29.6 \pm 5.5	12.3 \pm 3.7	10.7 \pm 3.5	32.7 \pm 16.1

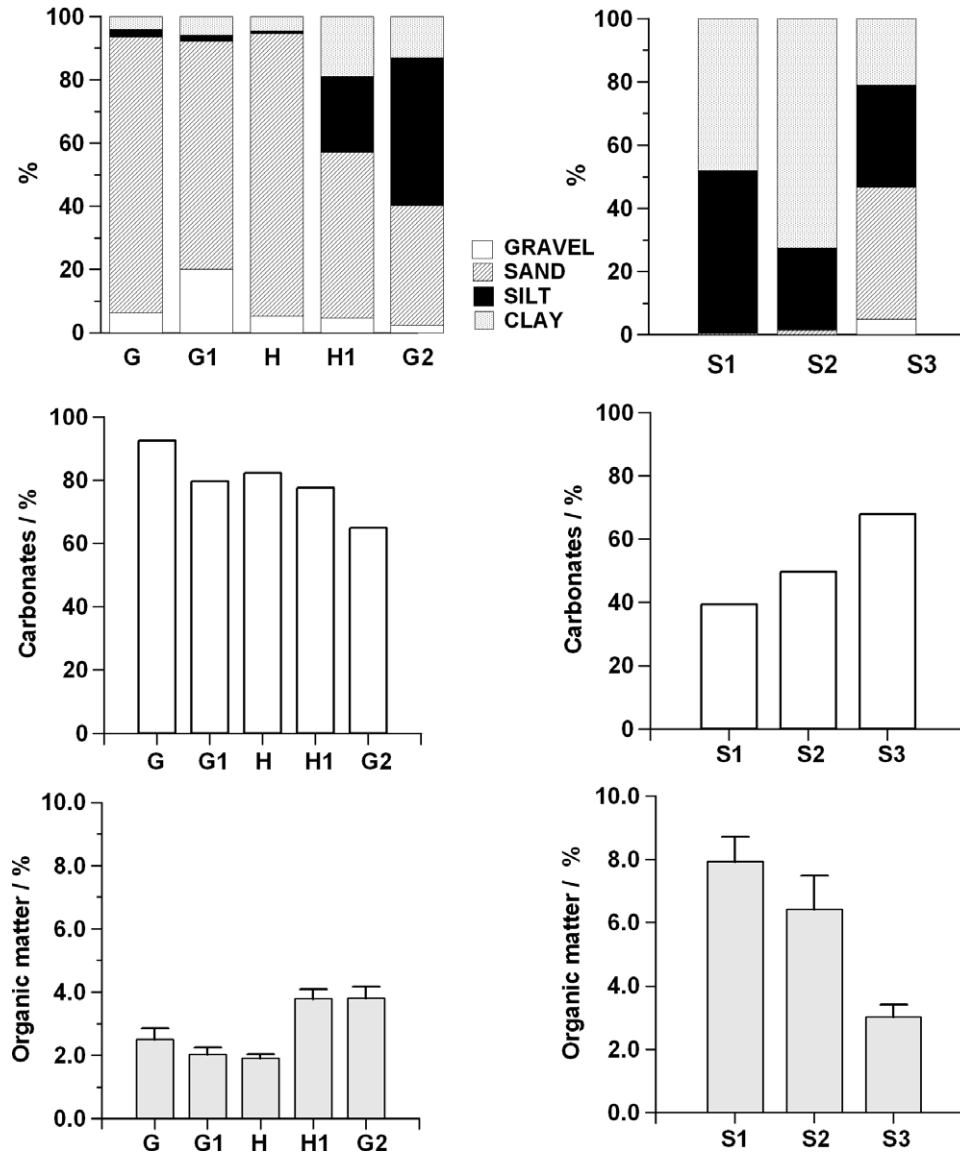


Fig. 6. Granulometric composition, organic matter content and carbonate content at tuna farm stations (G, G1, G2, H, and H1) and S1, S2 and S3 stations.

in cage sediments G and H (1.17 ± 0.3 ; $1.06 \pm 0.4 \mu\text{mol g}^{-1}$) (Fig. 4) were within the range of concentrations recorded for sediment from station S3, with a similar sandy sediment type.

The P-AUT portion of TP ranged from 5.6% to 12.4% (Table 3), with the lowest portion in sediment under the cages, and the highest at reference stations (H1, G2). In relation to data for different world areas, our results belong to a group with a low proportion of this fraction in TP. In carbonate sediments of the Bermuda Islands (Jensen et al., 1998) and Crete (Eijsink et al., 2000), the P-AUT portion in TP was 63–78% and constituted the main P form, while in clayey sediment of Aarhus Bay (Jensen et al., 1995) it was present at 30–40%. In sandy and silty sediments of the North Atlantic, P-AUT in TP ranged between 47% and 53% (Slomp et al., 1996), while, according to sequential P

analysis of clayey sediment from Minorca (Lopez, 2004), P-AUT ranged from 12% to 20% of TP. According to Schenau and De Lange (2001), the P-AUT portion in TP in carbonate sediments of the Arabian Sea was between 10% and 12%.

Most previous results for P-AUT were obtained using sequential analysis steps (NaAc in acetic acid buffer solution as extractant) without prior distinction of the biogenic apatite sediment P component P-FD. Therefore, we suggest that the higher portions of P-AUT in TP above-mentioned in this work almost certainly present both apatite components. This assumption is supported by the similarity of our data on P-AUT portions in TP (Table 3) determined at tuna farms in the middle Adriatic and the results obtained by Schenau and De Lange (2001), who applied the same method for distinction of the biogenic from the authigenic apatite form.

3.2.2.4. P-DET. P bound in detrital apatite (P-DET) ranged from 0.04 to 3.44 $\mu\text{mol g}^{-1}$ d. wt., with the highest average value at station S3 ($2.03 \pm 0.75 \mu\text{mol g}^{-1}$) and the lowest value at cage station H ($0.79 \pm 0.12 \mu\text{mol g}^{-1}$) (Fig. 4). The high concentration at S3 is probably a consequence of enhanced input of detrital phosphorus present mainly in marine sediment under strong riverine influence, i.e. terrestrial material which contains spherical detrital particles with smooth edges and a small specific area (Ruttenberg, 1992). Although the P-DET portion in TP varied over a very wide range (0.4–18%) (Table 3), P-DET was the smallest fraction in TP from the middle Adriatic sediment. According to values reported in the literature for carbonate sediments near Crete (Eijsink et al., 2000), P-DET in TP was only 4%. In silty sediment of the Es Grau Lagoon, Majorca (Lopez, 2004) its portion was 8–10%, while in sediments from the Arabian Sea it was 9–23% (Schenau and De Lange, 2001), with the highest values recorded in deep sea sediments. The highest proportion of P-DET in TP determined for the S3 station (Table 3) can be explained with terrestrial input from the river Neretva, while the extremely low proportions of P-DET in TP for cage stations (G, H) are probably caused by settling of large amounts of biogenic material (fish particles) in sediments.

3.2.2.5. P-ORG. Organic phosphorus (P-ORG) is the most delicate fraction for determination among all analysed P fractions due to its heterogenic nature and continuous transformations in the degradation processes during sinking and burial in sediments (Ruttenberg, 1992).

P-ORG_{ASP} concentrations were determined after Aspila et al. (1976), and P-ORG_{SED} was calculated as the difference between TP and the sum of the inorganic P forms determined by SEDEX (P-FD, P-Fe, P-AUT and P-DET). The average discrepancy between P-ORG_{ASP} and P-ORG_{SED} concentrations for all investigated stations was 28.9% (Matijević, 2006), which is in accordance with differences reported in the literature (Slomp et al., 1996; Ruttenberg, 1992; Lopez, 2004; De Lange, 1992). In this paper, we present data on P-ORG_{SED} (Fig. 5, Table 3) rather than P-ORG_{ASP} because the method of Aspila et al. (1976) is known to overestimate organic P due to post-ashing dissolution of inorganic P in 1 M HCl (Ruttenberg, 1992).

P-ORG_{SED} concentrations at all investigated stations ranged from 0.1 to 13 $\mu\text{mol g}^{-1}$. Average values for stations of higher clay content in sediment (S1 and S2) were similar (8.2 ± 4.4 and $7.5 \pm 4.9 \mu\text{mol g}^{-1}$), while the lowest value was determined for the sandy sediment station S3 (3.7 ± 3.4) (Figs. 4 and 6). Higher P-ORG concentrations in fine-sized sediments (Fig. 6) are correlated with higher sediment organic matter content, as previously reported for middle Adriatic sediments (Barić et al., 2002; Matijević, 2006). P-ORG average values for the G1 and G2 stations were similar to the S3 station, probably due to a similar sandy sediment composition, while average P-ORG_{SED} concentrations for sandy sediments under the cages (H, G) were up to two times higher than at the G1 and G2

reference stations (Fig. 4, Fig. 6). The reason is probably higher organic material input at the fish farm sites which induced higher organic P concentrations, as was found in eutrophicated bay sediments of the middle Adriatic area (Barić et al., 2002).

Similar to organic P concentrations, the ratio of organic P in TP (Table 3) was also highest in sediments at the S1 and S2 stations, and under the cages (H, G), and ranged from 34.7% to 41%. Levels were similar to the silty and clay-like sediments of Aarhus Bay (Jensen et al., 1995) or North Atlantic sediments (Slomp et al., 1996) with 35% of P-ORG in TP. The lowest proportion of P-ORG in TP (18.7%) was found in the carbonate sediment of station S3, which is in accordance with data from the literature for carbonate sediments of the Bermuda islands (Jensen et al., 1998) with 22% of P-ORG, or Minorca, where the OP portion was 25% (Lopez, 2004).

3.3. Sediment vertical profiles of P fraction concentrations

Vertical profiles of different P fractions in sediment (Fig. 7) showed enhanced concentrations of P-Fe, P-FD and P-ORG in sediment of cage stations (G, H) in relation to reference stations (G1, H1, and H2). The enhancement was found in the first centimetres of sediment, where concentrations were 2–5 times higher in relation to deeper layers. In contrast, concentrations of authigenic apatite and detrital apatite P forms were highest in sediment at reference stations (H1, G1, and G2), which can be explained with either terrestrial input or with internal transformation from other P forms. P-AUT and P-DET concentrations are not directly influenced by processes in the water column and, according to their seasonal variations or sediment vertical distribution, they are the most inert P species (Ruttenberg, 1992; Jensen et al., 1995).

Vertical profiles of Fe(III)OOH (Fig. 7) contained up to five times higher concentrations at reference stations (G2 and H1) in relation to cage stations (G and H), probably due to the lower sedimentation rate of organic matter (see sediment organic carbon content in Table 2), and more positive redox potential at the G2 and H1 stations (Matijević et al., 2006). Statistically significant correlations between Fe(III)OOH and P-Fe concentrations were found for cage stations (G, H) ($R = 0.728$, $n = 10$, $p < 0.05$) and for reference stations ($R = 0.763$, $n = 21$, $p < 0.01$). Considering the correlations, decreased Fe(III)OOH concentrations in sediment under the cages (G and H) are presumably the result of reduction processes and transition of Fe^{3+} to Fe^{2+} at lower redox potentials. According to Jensen et al. (1995), orthophosphate adsorption/desorption processes on iron oxyhydroxides are the main factors controlling orthophosphate concentrations at the sediment/water interface.

3.4. Statistical data analysis

Results were analyzed using the Statistica software module for Cluster analysis, applying the Euclidian distance

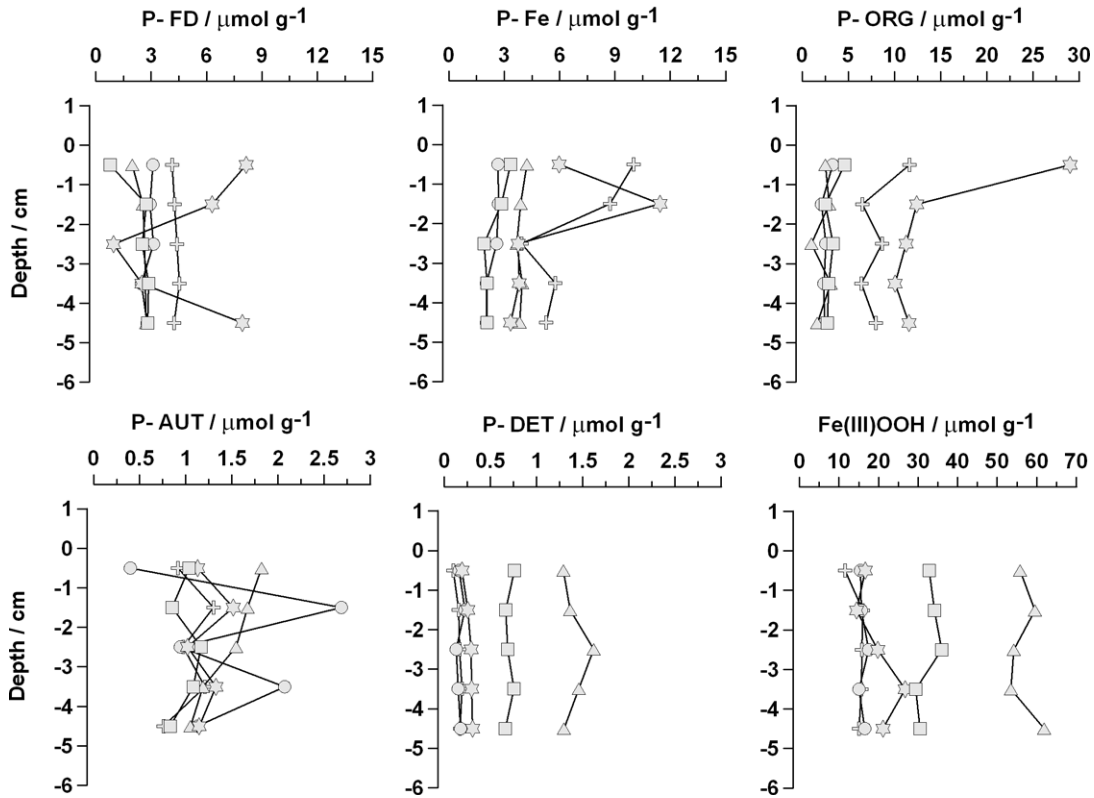


Fig. 7. Sediment vertical profiles of P-FD, P-Fe, P-AUT, P-DET, P-ORG and Fe(III)OOH concentrations at stations G, G1, G2, H and H1 in January 2005.

method (Fig. 8). Due to the different investigation periods, for this analysis we used only the data from mutual sampling in June 2002 at the G, G1, G2, H, H1, S1, S2 and S3 stations, and only for the top sediment layer (0–1 cm). Parameters used for analysis were: P-FD, P-Fe, P-AUT, P-DET and P-ORG_(ASP) concentrations in sediment, orthophosphate concentrations in the pore water, redox-potential, sediment Fe(III)OOH concentrations, granulometric con-

tent, organic matter and carbonate content. Cluster analysis distinguished two clusters of stations: a group with stations H, G, G1, H2 and S3, characterized by coarse sized sediment and lower organic matter content, and a group of fine sized sediment and higher organic matter content (S1, S2) (Fig. 8). In addition, within the group of stations with coarse sized sediment we found a difference between stations under the cages (H, G) and their reference stations (G1, H2), as well as in relation to the open sea station S3.

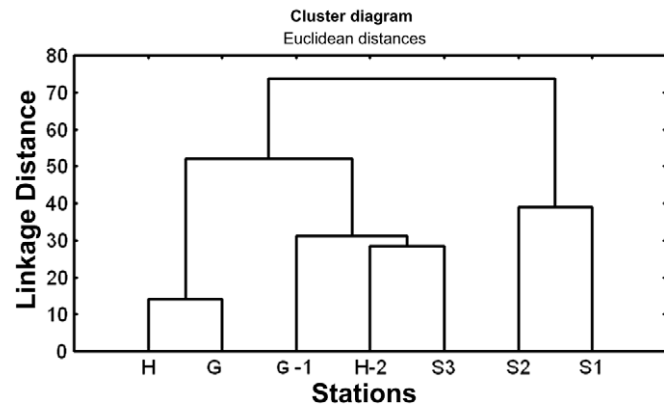


Fig. 8. Cluster analysis of measured parameters: P-FD, P-Fe, P-AUT, P-DET, P-ORG_(ASP) concentrations in sediment layer (0–1 cm), orthophosphate concentrations in the pore water, redox-potential, sediment Fe(III)OOH concentrations, granulometric content, organic matter and carbonate content at farm stations (G, G1, G2, H, H1) and S1, S2 and S3 stations.

4. Conclusions

Modified SEDEX techniques proposed in this paper proved to be useful for determination of sediment phosphorus species concentrations (P in biogenic apatite or fish debris P; P bound on to iron oxides/hydroxides; authigenic apatite P; detrital apatite P and organic P) in areas of different trophic status in the middle Adriatic area (bay, channel, open sea and fish farming stations). The application of modified analytical methods revealed advantages to present sequential techniques, especially in the distinction of biogenic apatite in the fish debris P fraction which is particularly important in sediment beneath fish farms.

The results obtained for P fractionation in the surface sediment layer (0–1 cm depth) indicated that iron bound P and organic P concentrations reflect the trophic status of the station area. Fish debris P concentrations from sediments below fish farm cages were enhanced in relation to

the unaffected sites investigated. This increment is a direct consequence of settlement of the biogenic apatite incorporated in fish skeletons on the seabed under the cages. Phosphorus bound in biogenic apatite is the most represented in the fish debris P fraction, demonstrating that this P fraction is the most sensitive indicator of the influence of fish farming on marine sediments.

The vertical distribution of P species in sediment confirmed the influence of fish farming through enhancement of fish debris, iron bound P and organic P under fish farm cages, while the correlation between iron bound P and Fe(III)OOH sediment concentration is probably a consequence of adsorption/desorption processes in the deeper sediment layer. Authigenic and detrital apatite P concentrations were not influenced by fish farming and can be explained by either terrestrial inputs or by internal transformation from other P forms.

Statistical analysis results obtained using sediment P species concentrations, redox-potential, Fe(III)OOH concentrations, granulometric, organic matter and carbonate content as well as orthophosphate pore water concentrations at stations with different trophic status in the middle Adriatic area indicate that sediment type is the leading factor in sediment P species distribution.

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