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## Distribution of phosphorus species in below-cage sediments at the tuna farms in the middle Adriatic Sea (Croatia)

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*The phosphorus species in sediments from two tuna farms located in semi-enclosed bays at island of Brač, (Croatia), were examined. Investigations were performed at two sites inside the breeding area on each farm and at control site in adjacent bay with no tuna farm. Measurements included concentrations of organic phosphorus (OP) and inorganic P species: P in biogenic apatite (P-FD), P adsorbed onto iron-oxy/hydroxides (P-Fe), P in authigenic apatite (P-AUT) and P in detrital apatite (P-DET). Regarding the previous analyses of phosphorus in middle Adriatic sediment, additional extraction step in determination of OP was applied. Granulometric composition, organic matter (OM), organic carbon (C-ORG), total nitrogen (N-TOT), carbonate content (CA) and sediment redox potential were also determined to investigate interactions between P species and specified physico-chemical characteristics of sediment.*

*Results proved total phosphorus (TP) sediment concentrations below the tuna cages enhanced up to 6 times in relation to the sites inside the farm area and to the control site. Sequential analyses of inorganic and organic P fractions indicated elevated concentrations of P-FD, P-AUT and P-Fe in below-cage sediments compared to other investigated sediments. Calculated portions of each fraction in the TP of all stations were as follows: P-FD (41%), P-Fe (32%), P-AUT (25%), P-ORG (1%) and P-DET (1%). In relation to previously obtained results for the middle Adriatic sediments under the anthropogenic impact, higher P-FD and P-AUT portion, as well as lower portion of P-ORG was revealed. Altered percentages between P-AUT and P-ORG are result of applying the different technique in sediment extraction regarding the earlier analyses. Sediment vertical profiles of P-FD, P-Fe and P-AUT pointed to elevated concentrations in surface layer (0-1cm) in below-cage sediments that can be assigned to fish farming influence. P-DET vertical profiles reflected the most inert nature of detrital apatite P species, while P-ORG profiles probably indicated fast degradation of P in organic matter. Redox potential in below-cage sediments was permanently more negative than at referential site indicating enhanced input of organic material from fish farm and its decay in anoxic conditions. Accordingly, molar C-ORG/TP and C-ORG/N-TOT ratios were significantly lower in below-cage sediments compared to referential sediments due to increased input of phosphorus and nitrogen compounds as well as to fast degradation of organic material settled in sediments.*

*Spearman correlation analysis of all investigated parameters provided linkage between P forms, N-TOT, fine-grained particles and OM that arises from increased nitrogen and phosphorus input in the organic material from the water column. The absence of usual correlation between C-ORG and OM is due to relatively rapid removal of carbon from sediments under the cages.*

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**Key words:** Adriatic Sea, tuna farming, phosphorus, sediment, SEDEX method

## INTRODUCTION

The farming of marine fish in Croatia mainly consisting of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and Atlantic bluefin tuna (*Thunnus thynnus*) increased from <1500 t in 1996 in total production to 8570 tonnes in 2010 (www.fao.org/fisherie/countrysector). This expansion has led to enhanced concern for the environmental integrity of the Croatian coastal areas from the public and the scientific community, particularly regarding the ecological influences of fish farming. Fish farms generate a wide range of dissolved and particulate matter and their effects have been demonstrated through different parameters in sediment, such as: phosphorus, nitrogen and organic carbon accumulation (HALL *et al.*, 1990; HOLBY & HALL, 1991; HARGRAVE *et al.*, 1997; CARROL *et al.*, 2003), 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; KALANTZI & KARAKASSIS, 2006; HOLMER *et al.*, 2007; VITA & MARIN, 2007; YUCEL-GIER *et al.*, 2007; VIDOVIĆ *et al.*, 2009; PAPPAGEORGIOU *et al.*, 2010). In contrast to nitrogen, which can be partially lost due to denitrification and anammox processes (RISGAARD-PETERSEN *et al.*, 2003), phosphorus (P) 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; FAUL *et al.*, 2005), and is therefore useful as an environmental impact indicator. Phosphorus in the sediment as an indicator of the fish farming influence has been used frequently in studies throughout the world (HALL *et al.*, 1990; HOLBY & HALL, 1991; HARGRAVE *et al.*, 1997; KARAKASSIS *et al.*, 1999; CANCEMI *et al.*, 2003; SOTO & NORAMBUENA, 2004; PORELLO *et al.*, 2005; KASSILA *et al.*, 2000; YUCEL-GIER *et al.*, 2007). Sediment P content was also used, among the other parameters, in meta-analysis and modeling studies of the effects impacts of fish farming on benthic chemistry (KALANTZI & KARAKASSIS, 2006; GILES, 2008; MAYOR & SLOAN, 2011).

Investigations of P in “fish farm sediments”

of the middle Adriatic area at sea bass/sea bream and tuna farms showed a significant increase of total P in surface sediments layers (MATIJEVIĆ *et al.*, 2004, 2006, 2008a, VIDOVIĆ *et al.*, 2009). More detailed investigations of phosphorus in sediment point to enhancement of inorganic P concentrations under the cages, while organic P was mainly within the range of previously determined values for the middle Adriatic sediments (MATIJEVIĆ *et al.* 2009a). Sequential analysis of inorganic P species in sediments (P in biogenic, authigenic and detrital apatite, P bound onto iron-oxyhydroxides) showed an increased portion of biogenic apatite P and decreased detrital apatite P at the farm stations with respect to the P pool at the reference station as a direct consequence of fish farming.

In this paper we aimed to determine the concentrations of organic phosphorus (OP) and inorganic P species: P in biogenic apatite (P-FD), P adsorbed onto iron-oxy/hydroxides (P-Fe), P in authigenic apatite (P-AUT) and P in detrital apatite (P-DET) in sediments from two tuna farms in the middle Adriatic related to control site with no fish farm activity. Regarding the previous analyses of phosphorus in middle Adriatic sediments, additional extraction step in determination of OP was applied. Characteristics of vertical profiles of organic and inorganic P forms in sediment cores were also examined. To identify potential geochemical changes in below-cage sediments in relation to „unaffected site“, we investigated interactions between physico-chemical characteristics of sediment (sediment type, carbonates, organic matter content, organic carbon, total nitrogen and redox potential) and P forms.

## MATERIAL AND METHODS

The study was carried out at two bluefin tuna farms that are located at island of Brač, Croatia, middle Adriatic in the semi-enclosed Grška Bay and Smrka Bay (Fig. 1). These tuna farms were established in 2001 and have an annual production of about 450 tones with average breeding cycles of 20 months. Measurements and samplings were performed in December 2007 and September 2008 at two locations inside the

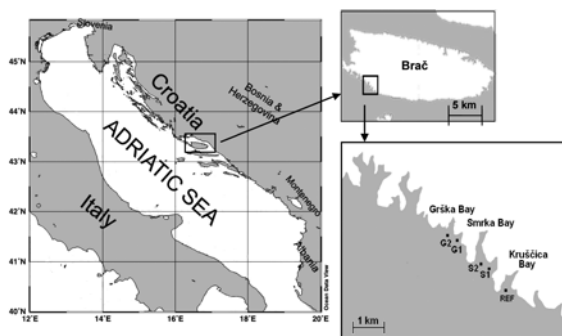


Fig. 1. Location of sampling sites

breeding area; directly below the cage (G1, S1) and at sites 200 meters distanced from the cages (G2, S2). The control site (REF) was located in nearby Krušćica Bay 1 kilometer distanced away with no fish farm inside (Fig. 1).

Sediment samples were collected by SCUBA-divers using plastic tubes which were inserted into the sediment to preserve an undisturbed core. Redox-potential was measured in sediment core „in situ” by vertical penetration of Pt electrode connected to voltmeter Metrohm E-605 with Ag/AgCl reference electrode. Calibration was performed in the quinhydrone buffer solutions (pH 4 and pH 7) prepared according to Metrohm, Ag Herissau, Switzerland. For measurements of sediment phosphorus concentration, each sample was divided into slices (1 cm thick), frozen and freeze dried until the laboratory analysis. Phosphorus concentrations were measured according to modified SEDEX method (MATIJEVIĆ *et al.*, 2008a). Determined inorganic phosphorus species were: P in biogenic apatite or “fish debris” P-FD, authigenic apatite phosphorus P-AUT, detrital apatite phosphorus P-DET, phosphorus adsorbed on iron-oxihydroxides P-Fe. Regarding the previous analyses of phosphorus in middle Adriatic sediment,

additional extraction step in determination of OP was applied such as suggested by RUTTENBERG (1992). Standard reference marine sediment for P concentration determination was used (PACS-2, NRC-CNRC).

Sediment organic carbon and total nitrogen contents were determined using a CHNS-O Carlo Erba analyzer (EA 1110, CE instruments). Before analysis, freeze-dried sediment samples were prepared according to UJIHÉ *et al.* (2001) by acidification of the sediments with HCl to remove carbonates. Granulometric composition of the sediment samples was determined by sieving ( $>63 \mu\text{m}$ ) and the hydrometric method ( $<63 \mu\text{m}$ ), while sediment type was classified according to SHEPARD (1954). Organic matter content was determined gravimetrically according to VDOVIĆ *et al.* (1991).

Non-parametric Spearman rank order correlations were used to assess the relationships between granulometric and chemical parameters. The analysis were performed using the statistical package StatSoft Inc. (2000) STATISTICA (<http://www.statsoft.com>).

## RESULTS AND DISCUSSION

Analysis of granulometric fractions (gravel, sand, silt and clay) in sediments at investigated stations are presented on box-whisker graphs in Fig. 2. Sand was prevailing at all sites ( $79 \pm 8\%$ ), while minor fractions were gravel ( $9 \pm 7\%$ ), silt and clay with  $5 \pm 4\%$  and  $7 \pm 5\%$ , respectively. Higher portions of gravel and sand particles are obvious in sediments at fish farm sites related to REF site that can be result of enhanced input of coarser material due to different environmental conditions during the sedimentation.

Table 1. Geographic position, station depths, sediment type (Shepard's classification) organic matter (OM), carbonate (CA), organic carbon (C-ORG) and total nitrogen (N-TOT) content in sediments at G1, G2, S1, S2 and REF sites during December 2007 and September 2008

Station	$\Phi$	$\lambda$	Depth (m)	OM (%)	CA (%)	Sediment type
S1	43° 16,944	16° 29,762	43	2.40±0.58	85.7±5.0	Sand
S2	43° 17,031	16° 29,213	55	2.02±0.16	80.8±8.9	Sand
G1	43° 17,345	16° 28,984	45	2.59±0.51	86.1±10.3	Sand
G2	43° 17,453	16° 28,712	55	1.96±0.33	83.2±4.9	Sand
REF	43° 16,615	16° 30,254	54	2.66±0.26	81.8±1.9	Silty sand

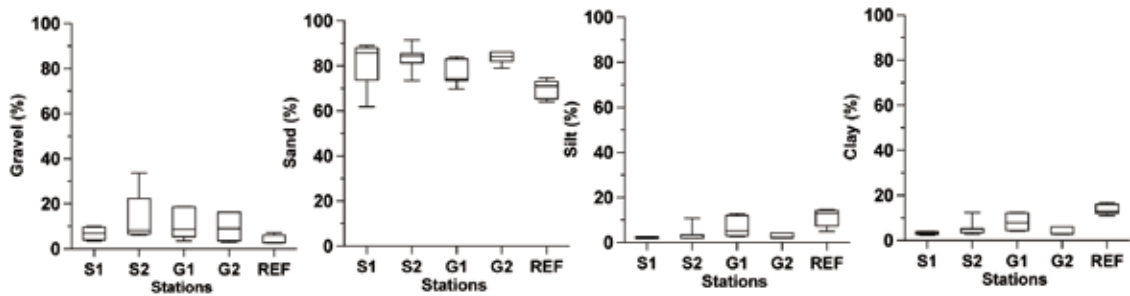


Fig. 2. Box whisker plots of granulometric fractions (gravel, sand, silt and clay) in the sediments at G1, G2, S1, S2 and REF sites

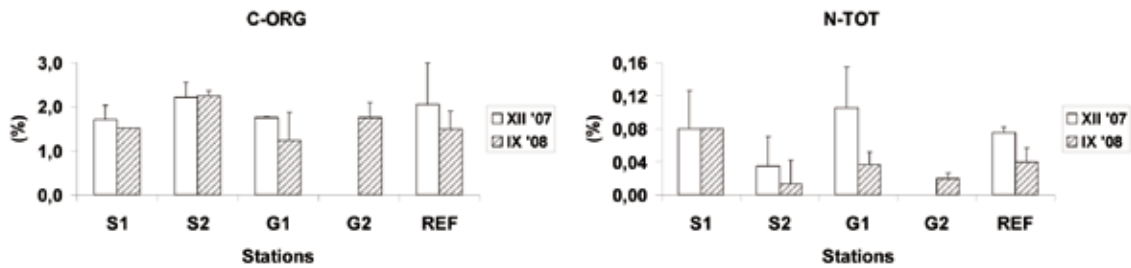


Fig. 3. Sediment organic carbon and total nitrogen content (average values  $\pm$ STD) at stations S1, S2, G1, G2, and REF during December 2007 and September 2008

According to Shepard's classification (SHEPARD, 1954), prevalent sediment type at investigated stations was sand (in 23 subsamples), while silty sand was determined only in 3 subsamples in sediments at REF station (Table 1).

Carbonate content (CA) in sediments varied between 65 and 96% at S2 and G1 station, respectively, whereas average values indicated relatively equal CA at all stations (Table 1). This is in accordance with sand as prevailing granulometric size fraction and previous investigations of middle Adriatic sediments where CA higher than 60% were determined in shallower coastal areas and were most abundant in the coarse-grained sediment fraction (MATIJEVIĆ *et al.*, 2009b).

The organic matter content (OM) ranged from 1.5 to 3.3% at G2 and G1 station, respectively. The highest OM was at REF site (Table 1), where highest portion of silt and clay particles in sediment were determined (Fig. 2). These results agree well with the published data for OM in the middle Adriatic coastal sediments (average value:  $3.8 \pm 2.4\%$ ) where a positive correlation exists between organic matter and portion of fine-sized particles in sediments (BOGNER *et al.*, 2005). Due to this association, OM content at

investigated Grška and Smrka Bay sediments probably represents natural sediment characteristics. According to obtained results in Greek fish farm sediments (MANTZAVRAKOS *et al.* 2007) no significant differences in OM distribution between the cage and control sites were also reported, while KARAKASSIS *et al.* (1998) reported enhancement of OM below cages.

The organic carbon content (C-ORG) ranged from 0.87 to 2.72% at G1 and REF site, respectively (Fig. 3, 4), that was in the range of values found in sediments under the fish farming impact in the Adriatic Sea (0.7-10.13%) as published according to NAJDEK *et al.* (2007) and MATIJEVIĆ *et al.* (2006).

There was no significant difference in C-ORG content between sites under the cages in relation to sites distanced from cages and to referential site (Fig. 3).

On the contrary, N-TOT content was enhanced in below-cage sediments compared to other stations (Fig. 3, 4), while N-TOT range (0.01-0.15%) was in accordance to values determined for middle Adriatic sediments "undisturbed" by fish farming (MATIJEVIĆ *et al.*, 2008a). Vertical sediment profiles reflected unequal distribution with maximum N-TOT content in

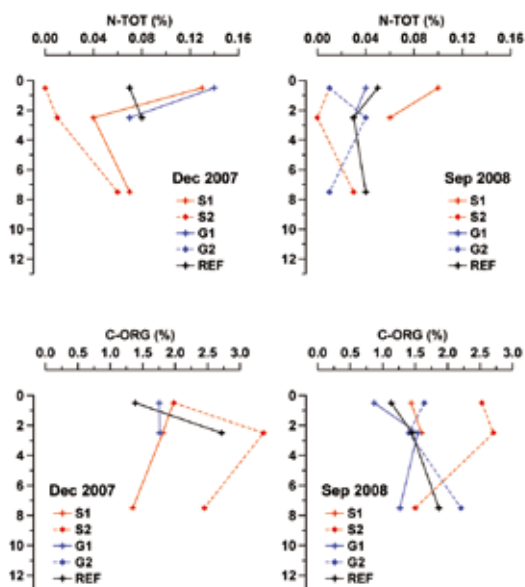


Fig. 4. Vertical profiles of organic carbon and total nitrogen content in sediments at stations G1, G2, S1, S2 and REF during December 2007 and September 2008

surface layer of below-cage sediments (Fig. 4).

Increased N-TOT content in surface sediment layer is reported earlier for the same fish farm in Grška Bay (N-TOT range: 0.07-0.95%) (MATIJEVIĆ *et al.*, 2006), that is in accordance with enrichment reported for some world fish farm areas (KARAKASSIS *et al.*, 2000, KOVAČ *et al.*, 2004).

Organic carbon and total nitrogen proved to be useful variables in meta-analysis and examinations of trends in fish farm benthic impacts made on results obtained for large number of world studies (KALANTZI & KARAKASSIS 2006; GILES 2008). In these studies C-ORG content in fish farm sediments was in wide range (0.2-26.1%), while N-TOT ranged between 0.01 and 3.6%. GILES (2008) also suggested sediment enrichment state considering C-ORG and N-TOT values (low, moderate, high and very high), within that our results fall into the category of moderately and low impacted sediments by fish farming.

Sediment redox potential  $E_H$  during both sampling periods was in highest ranges at sites under the cages; at S1 station (-391 mV to 40 mV) and at G1 station (-330 mV to 93 mV) (Fig. 5). These minimum values belong to lowest  $E_H$  recorded for the fish farm sites at the middle

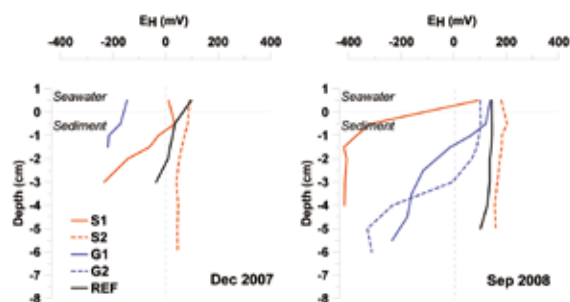


Fig. 5. Vertical profiles of sediment redox-potential at stations G1, G2, S1, S2 and REF during December 2007 and September 2008

Adriatic area (MATIJEVIĆ *et al.*, 2006). Extremely negative potentials indicate high concentrations of sulphide ions ( $S^{2-}$ ) as well as degradation of organic matter in the absence of oxygen (MATIJEVIĆ *et al.*, 2007). Additionally, visual monitoring of below-cage sediments proved purple surface layer from settled remains of fish food and excretes while characteristic smell of  $H_2S$  spread from the sediment cores. Lower  $E_H$  ranges were in sediments at S2 site and at REF site that were not in the fish farm zone.

The main difference in  $E_H$  profiles between the sites was redox transition depth. During December 2007 in below cage sediments G1 and S1 negative  $E_H$  was even in the bottom seawater, or in surface sediment layer (0.5-1 cm), while in September 2008 redox transition depth at G1 was deeper (1.5-2 cm). Vertical  $E_H$  profiles at REF site in December 2007 revealed redox transition depth in the sediment layer at depth 3-4 cm, while during September 2008 complete  $E_H$  profile was positive and relatively uniform (Fig. 5).

Similar variations in depth of redox-cline in grain-sized sediments in middle Adriatic occurs as part of seasonal oscillations of this parameter (MATIJEVIĆ *et al.*, 2007). However, extremely negative potentials even in the bottom water and in surface sediment layers under the cages at tuna farms in the middle Adriatic, can be attributed to organic matter input caused by the fish farming (MATIJEVIĆ *et al.*, 2006).

Total phosphorus concentrations (TP) in sediments (calculated as sum of inorganic and organic P species) ranged between 6.5 and 161.9

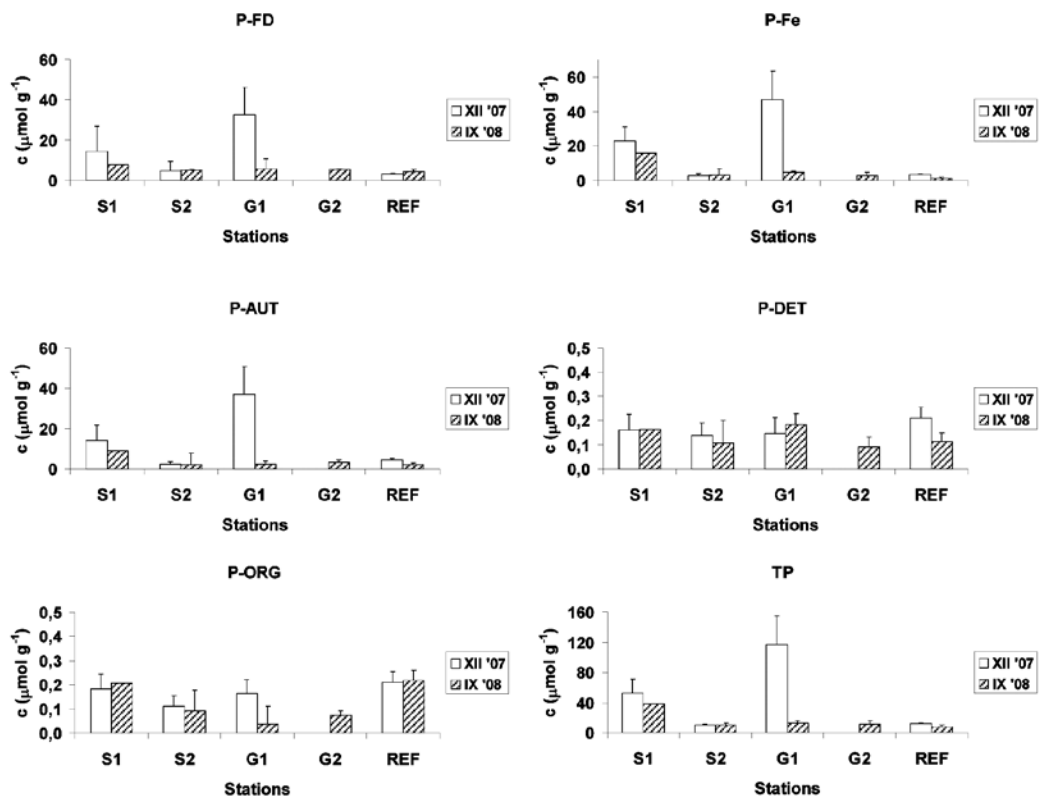


Fig. 6. Concentrations of inorganic P species (P bound in fish debris FD-P, P adsorbed in iron oxides/hydroxides Fe-P, detrital apatite phosphorus DET-P, authigenic apatite phosphorus AUT-P) and organic P (P-ORG) in sediment samples at investigated sites (average value $\pm$ standard deviation)

$\mu\text{mol g}^{-1}$  at REF (September 2008) and at G1 site (December 2007), respectively. Average TP concentrations are up to five times higher in below-cage sediments than at other investigated sites (Fig. 6). Extremely elevated TP in sediment at G1 is probably consequence of local organic matter in sample that is in accordance with negative  $E_H$  (Fig. 5), while significantly lower value determined in September 2008 can be assigned to relocation of tuna cages around the breeding area.

TP concentration range in sediments under the cages is even higher than the values reported for fish farms at the middle Adriatic area ( $19\text{--}135 \mu\text{mol g}^{-1}$ ), while TP in sediments of other investigated sites belongs to ranges determined for open sea sediments ( $13.5\pm 3.5 \mu\text{mol g}^{-1}$ ) (MATIJEVIĆ *et al.*, 2008a). TP enhancement in below-cage sediments in relation to unaffected sites also agrees well with results published for salmon, sea bass and sea bream farms (HOLBY & HALL, 1994; SOTO & NORAMBUENA, 2004; KARAKASSIS *et al.*, 1999;

CANCEMI *et al.*, 2003; PORELLO *et al.*, 2005, VIDOVIĆ *et al.*, 2009).

The fish debris P fraction (P-FD) comprises phosphorus bound in biogenic apatite originating from hard parts of fish tissues (fish bones and teeth) and small amounts of P loosely adsorbed on to mineral surfaces and carbonates (SCHENAU and DE LANGE, 2000). Concentrations of P-FD in sediments at investigated sites ranged from 1 to  $49.1 \mu\text{mol g}^{-1}$ , at REF and G1 site, respectively (Fig. 6). In sediments at S2 and G2, as well as for G1 in September 2008, P-FD concentrations were 15-20% higher compared to values determined at REF station. P-FD ranges in below-cage sediments reported here are significantly higher than published results for tuna farms in the Middle Adriatic ( $6.3\pm 2.8 \mu\text{mol g}^{-1}$ ) (MATIJEVIĆ *et al.*, 2006), while concentrations at REF site belong to range determined in open sea sediments ( $0.7\text{--}4.2 \mu\text{mol g}^{-1}$ ) (MATIJEVIĆ *et al.*, 2008a).

Elevated P-FD values and high standard deviations in sediments under the cages com-

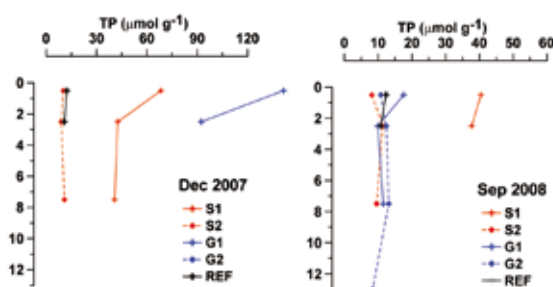


Fig. 7. Vertical profiles of total phosphorus concentrations (TP) in sediment samples at G1, G2, S1, S2 and REF site during December 2007 and September 2008 (average value for each sediment depth)

pared to REF site are expected considering the origin of this fraction (“fish debris P”) that proved as sensitive indicator of the fish farm influence on the marine sediment (MATIJEVIĆ *et al.*, 2006, 2008a).

Average portion of P-FD fraction in TP was 41% that is higher than the portions calculated for sediments in the Middle Adriatic (P-FD/TP: 6-35%). According to previous investigations P-FD portions are usually higher in grain-size sediments of biogenic origin (MATIJEVIĆ *et al.*, 2008a).

The phosphorus fraction bound to iron oxyhydroxides (P-Fe) ranged between 0.9 and 67.5  $\mu\text{mol g}^{-1}$  at REF and G1 station, respectively (Fig. 6). In sediments at S2 and G2 sites P-Fe concentrations were in range of values at REF site, while slightly higher values were at G1 in September 2008.

P-Fe determined in sediments under the cages are up to 5 times higher than the values determined in Adriatic sediments under the anthropogenic influence (1.2-11.2  $\mu\text{mol g}^{-1}$ ) that also included sediments in eutrophicated bays and fish farms. Average portion of P-Fe in TP is 32% at investigated sites that is in agreement to portions reported for middle Adriatic sediments (13-40%) (MATIJEVIĆ *et al.*, 2008a). According to the same authors P-Fe was the prevailing species in IP pool of the middle Adriatic sediments and it was positively correlated with sediment redox-potential and concentrations of iron in oxyhydroxides. These relationships also verified the P-Fe importance in adsorption/desorption processes in sediment P cycle as stated by JENSEN *et al.* (1995).

Concentrations of P bound in authigenic apatite (P-AUT) ranged between 0.7 and 51.2  $\mu\text{mol g}^{-1}$ , at G1 (December 2007) and S2 site (December 2007), respectively (Fig. 6). Given values for sediments under the cages (G1, S1) are significantly higher than the average for sediments in the middle Adriatic (0.6-3.0  $\mu\text{mol g}^{-1}$ ) reported by MATIJEVIĆ *et al.* (2008a).

Average portion of P-AUT fraction was 25% that is increased compared to previous results for middle Adriatic sediments with P-AUT abundance range of 6-13%. Elevated average P-AUT/TP calculated for investigated sites reported in this paper can be assigned to internal transformation of P-ORG into P-AUT in sediment revealed by applying different technique of extraction of P-ORG fraction (see P-ORG paragraph). Namely, linkage between P-AUT formation from P-ORG released into the pore water as a result of organic matter degradation was established in the sediment of stations in the middle Adriatic area (MATIJEVIĆ *et al.*, 2009b), as well as for the sediments at Mississippi Delta and Long Island (RUTTENBERG and BERNER 1993). Furthermore, investigations of oceanic particulate P and P in sediments, emphasized transformation of P-ORG into P-AUT immediately after the arrival at the sediment surface without required burial process (FAUL *et al.*, 2005).

Concentration of P bound in detrital apatite (P-DET) ranged between 0.03 and 0.27  $\mu\text{mol g}^{-1}$ , at G2, (September 2008) and REF site (December 2007), respectively (Fig. 6). These values belong within range of P-DET determined in sediments at the Middle Adriatic area (0.04-3.44  $\mu\text{mol g}^{-1}$ ) with the highest P-DET at stations under the river influence (MATIJEVIĆ *et al.* 2008a). Detrital phosphorus is present mainly in marine sediment under strong river influence, i.e. terrestrial material which contains spherical detrital particles with smooth edges and a small specific area (RUTTENBERG, 1992).

P-DET in sediments at investigated sites in Grška and Smrka Bay is least abundant fraction in TP with average portion of 0.9%. That is in the range of earlier determined P-DET/TP for the middle Adriatic sediments (0.4-18%) (MATIJEVIĆ *et al.*, 2009a).

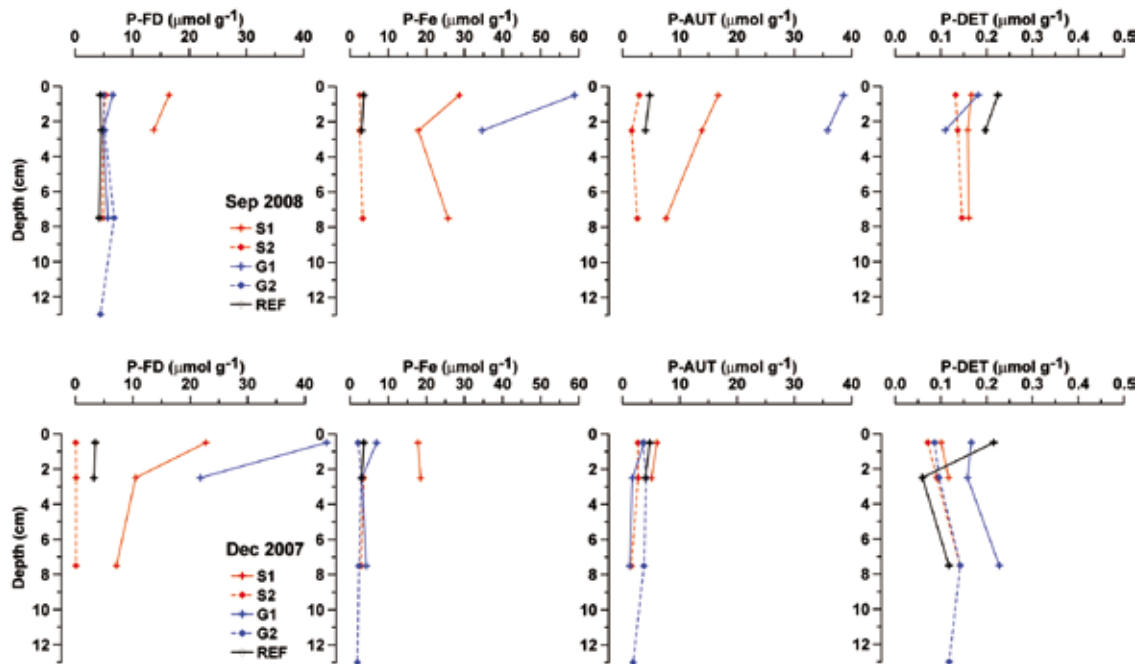


Fig 8. Vertical profiles of inorganic P species concentrations (P-FD, P-Fe, P-DE, P-AUT and organic P (P-ORG) in sediment samples at investigated sites (average value for each sediment depth)

P-ORG concentrations reported for middle Adriatic sediments (MATIJEVIĆ *et al.*, 2006; 2008a,b; 2009a,b) were calculated as the difference between TP separately determined and sum of inorganic P species (P-FD, P-Fe, P-AUT and P-DET). In this paper P-ORG fraction is determined using different technique that includes the ignition of the sediment residue after the extractions of inorganic fractions as recommended by RUTTENBERG (1992).

P-ORG ranged from 0.01 to 0.8  $\mu\text{mol g}^{-1}$ , determined at REF station in December 2007 (Fig. 6). Lower values were in sediments under the cages (S1 and G1) as well as in sediments at sites 200 meters distanced from the cages. P-ORG concentrations (determined as previously explained) in coarse-grained sediments at the middle Adriatic ranged between 0.1 and 11  $\mu\text{mol g}^{-1}$ , (MATIJEVIĆ *et al.* 2009a). Average abundance of P-ORG in TP was 1% that significantly differed than P-ORG/TP in previously cited papers (range: 18-41%), clarified as consequence of transformation of P-ORG into P-AUT.

Vertical profiles of TP concentrations reflect unequal distribution in sediments under the cages related to other investigated sites (Fig. 7).

Significantly enhanced TP concentrations in surface sediment layers (0-2 cm) at S1 and G1 site during both sampling periods are indication of enhanced input of P in sediment under the cages, in relation to relatively uniform TP distribution at S2, G2 and REF sites.

Previous investigations of P in fish farming sediments of the middle Adriatic area, also indicated TP up to five times higher in surface sediments compared to samples from “unaffected” areas (MATIJEVIĆ *et al.*, 2004, 2006, 2008a, 2009a, VIDOVIĆ *et al.*, 2009).

The influence of more intensive P input from the cages is reflected in vertical distribution of inorganic P species P-FD, P-Fe and P-AUT that showed enhancement in surface sediment layers at sites under the cages (S1 and G1) compared to other sites with uniform distribution (Fig. 8).

Similar enhanced P-FD and P-Fe concentrations in first centimeters of sediment (2-5 times higher related to deeper layers) was found at tuna farms in the middle Adriatic (MATIJEVIĆ *et al.*, 2008b), while P-AUT and P-DET forms were elevated in sediments at control sites.

Increase of P-Fe obvious in deeper sediment layer at S1 station in December 2007 (Fig. 8)



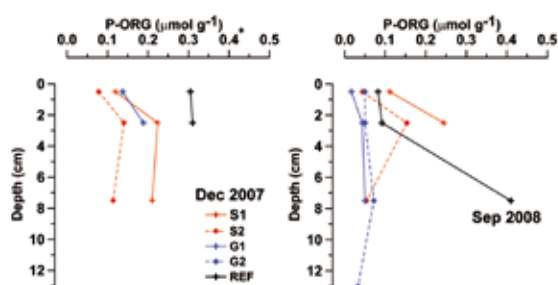


Fig. 9. Vertical profiles of organic P (P-ORG) in sediment samples at investigated sites (average value for each sediment depth)

can be assigned to adsorption/desorption processes of  $\text{HPO}_4^{2-}$  from iron oxy-hydroxides in sediments. According to literature, releasing or binding of orthophosphate at the mineral surfaces are functions of sediment redox potential (STUMM & MORGAN, 1996; GOMEZ *et al.* 1999). Similar vertical profiles with sharp decrease in P-Fe at depth of redox-cline followed by increase in Fe concentrations were found in middle Adriatic sediments (MATIJEVIĆ *et al.*, 2007).

Profiles of detrital apatite P show elevated concentrations in surface sediment layers at REF site with relative equal distribution at other sites. P-DET in sediments usually presents the most inert P species as a result of sedimentation with no precipitation reactions (RUTTENBERG, 1992).

P-ORG vertical profiles (Fig. 9) show enhanced concentrations in deeper sediment layers for most of the sites, similar to vertical profiles of C-ORG (Fig. 4) that can be explained with relative fast degradation of organic matter in surface sediment layers below the cages.

Molar ratios of organic carbon, total phosphorus and total nitrogen (C-ORG/TP and C-ORG/N-TOT) were also calculated in order to investigate potential fish farming impact on the origin of organic matter in sediments. However, due to the particular importance of surface sediment in processes of accumulation and degradation of organic matter in sediments (MATIJEVIĆ *et al.*, 2008b), in Table 2 are presented ratios of biogenic elements in sediment layer at 0-1cm depth.

C-ORG and TP ratio (C-ORG/TP) in investigated sediments ranged from 12 to 259, with significantly lower values in sediments under the cages (Table 2). According to DOLENEC *et al.* (1998); ALGEO & INGALL (2007); C-ORG/TP <200 indicates significantly higher P content in relation to carbon that can be either due to good preservation of phosphorus forms (that implicates oxic conditions in sediments), either to elevated input of P from the water column. Considering the anoxic conditions in sediments under the cages (Fig. 5), low C-ORG/TP is certainly consequence of P input through the material from the fish farm and fast degradation of organic carbon.

C-ORG/N-TOT ratios were also lower in sediments under the cages related to other investigated sites indicated higher input of nitrogen species that can be attributed to fish excretions and degradation of their metabolic products.

The obtained results were analyzed by using Spearman correlation method in Statistica 6 Software module (Table 3). Parameters used for analysis were: P-FD, P-Fe, P-AUT, P-DET and P-ORG concentrations in sediment, redox-potential, granulometric composition, organic matter, carbonate, organic carbon and total nitrogen content in sediments.

Positive correlations were established between all P forms except P-DET that is the most inert P species in sediment. OM was positively correlated with fine-grained particles (silt and clay) as it was reported for sediments at the Middle Adriatic (MATIJEVIĆ *et al.* 2008b), while negative correlation between C-ORG and fine sized particles was not in agreement with results attained by the same authors. The absence of usual correlation between C-ORG and OM is due to relatively rapid removal of carbon

Table 2. Molar ratios of organic carbon and total phosphorus (C-ORG/TP) and organic carbon and total nitrogen (C-ORG/N-TOT) in surface sediment at investigated sites

	C-ORG/TP		C-ORG/N-TOT	
	XII 2007	IX 2008	XII 2007	IX 2008
S1	19.8	34.3	17.7	16.8
S2	166.1	258.9		294.9
G1	12.1	41.3	14.6	25.4
G2		126.7		191.7
REF	96.1	105.0	23.1	26.5

Table 3. Spearman correlation coefficients between granulometric composition (gravel, sand, silt, and clay), organic matter (OM), carbonate (CA), total, inorganic and organic phosphorus concentration (TP, P-FD, P-Fe, P-AUT, P-DET and P-ORG) in sediments at all investigated stations (Statistically important correlation  $p < 0.01$ : bold letters),  $p < 0.05$ : italic)

	P-FD	P-ORG	P-DET	P-Fe	P-AUT	TP	Gravel	Sand	Silt	Clay	OM	CA	C-ORG
P-ORG	-0.06												
P-DET	-0.06	0.18											
P-Fe	<b>0.76</b>	<i>0.37</i>	0.11										
P-AUT	<b>0.61</b>	<b>0.48</b>	0.15	<b>0.72</b>									
TP	<b>0.78</b>	<i>0.38</i>	0.12	<b>0.92</b>	<b>0.88</b>								
Gravel	0.18	-0.05	0.00	0.15	0.17	0.15							
Sand	<i>0.37</i>	0.07	-0.06	<i>0.34</i>	0.26	<i>0.30</i>	-0.08						
Silt	-0.12	-0.02	0.05	-0.13	-0.11	-0.12	<b>-0.58</b>	<b>-0.47</b>					
Clay	-0.18	0.11	0.22	-0.05	0.01	-0.04	<b>-0.58</b>	<b>-0.45</b>	<b>0.94</b>				
OM	0.18	0.23	0.18	0.23	<i>0.35</i>	0.25	-0.36	-0.14	<b>0.61</b>	<b>0.62</b>			
CA	0.01	<i>-0.32</i>	-0.01	-0.01	-0.17	0.00	-0.13	-0.17	0.12	0.13	<b>-0.40</b>		
C-ORG	-0.13	0.21	0.00	-0.11	-0.01	-0.12	0.06	0.27	<i>-0.34</i>	<i>-0.35</i>	-0.03	<b>-0.45</b>	
N-TOT	0.28	<b>0.42</b>	<i>0.34</i>	<b>0.55</b>	<b>0.56</b>	<b>0.53</b>	-0.01	-0.06	<i>0.32</i>	<b>0.44</b>	<b>0.55</b>	-0.12	-0.29

from organic matter in sediments under the cages. Positive correlation between N-TOT, all P forms, OM and fine sized particles arise from enhanced input of nitrogen and phosphorus in the organic material from the water column or from slower remineralization of nitrogen species in sediments.

## CONCLUSIONS

The concentrations of total phosphorus (TP) in sediment below the tuna cages were enhanced up to 6 times in relation to the sites inside the farm area and to the control site.

Sequential analyses of inorganic and organic P fractions indicated to elevated concentrations of P-FD, P-AUT and P-Fe in below-cage sediments compared to other investigated sites. Calculated portions of each P fraction in the TP revealed higher P-FD and P-AUT portion, as well as lower P-ORG portion compared to previously obtained results for the middle Adriatic sediments under the anthropogenic impact. Altered abundances between P-AUT and P-ORG are result of applying the different technique in sediment extraction regarding the earlier analyses.

Redox potential in below-cage sediments

was permanently more negative than at referential site indicating enhanced input of organic material from fish farm and its decay in anoxic conditions.

Sediment vertical profiles of P-FD, P-Fe, P-AUT fraction pointed to elevated concentrations in surface layer (0-1cm), that can be assigned to fish farming influence. P-DET vertical profiles reflected the most inert nature of detrital apatite P, while P-ORG profiles probably indicated fast degradation of organic material settled below the cages.

Redox potential in below-cage sediments was permanently more negative than at referential site indicating enhanced input of organic material from fish farm and its decay in anoxic conditions.

Accordingly, molar C-ORG/TP and C-ORG/N-TOT ratios were significantly lower in below-cage sediments compared to REF site due to increased input of phosphorus and nitrogen compounds as well as to fast degradation of organic material settled in sediments.

Spearman correlation analysis of all investigated parameters provided linkage between P forms, N-TOT, fine-grained particles and OM that arises from increased nitrogen and phosphorus input in the organic material from the

water column. The absence of usual correlation between C-ORG and OM is due to relatively rapid removal of carbon from sediments under the cages.

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## Raspodjela oblika fosfora u sedimentima ispod uzgajališta tuna u srednjem Jadranu (Hrvatska)

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### SAŽETAK

U ovom su radu istraživani oblici fosfora u sedimentu sa dva uzgajališta tuna smještenih u uvalama Grška i Smrka (otok Brač). Istraživanja su provedena u svakoj uvali na dvije postaje unutar zone uzgoja kao i na kontrolnoj postaji smještenoj u susjednoj uvali bez uzgajališta ribe. Mjerenja su uključivala koncentracije organskog fosfora (OP) i različitih oblika anorganskog fosfora: fosfor vezan u biogenom (P-FD), autigenom (P-AUT) i detritusnom apatitu (P-DET), te fosfor vezan na okside i hidrokside željeza (P-Fe). U odnosu na prethodne analize fosfora u sedimentu Jadrana, primijenjena je dodatna ekstrakcija u određivanju OP. U sedimentu su određeni i granulometrijski sastav, sadržaj organske tvari (OM), karbonata, (CA), organskog ugljika (C-ORG), i ukupnog dušika (N-TOT), kao i redoks potencijal da bi se ispitala povezanost između oblika fosfora i navedenih fizikalno kemijskih parametara.

Rezultati su pokazali da su koncentracije ukupnog fosfora (TP) u sedimentu ispod kaveza ribe povišene i do 6 puta u odnosu na postaje u zoni uzgajališta i na kontrolnu postaju. Sekvencijalna analiza anorganskog fosfora ukazala je na povišene koncentracije P-FD, P-AUT i P-Fe ispod kaveza u usporedbi s ostalim sedimentima. Izračunati udjeli svakog oblika fosfora u TP na svim postajama su bili: P-FD (41%), P-Fe (32%), P-AUT (25%), P-ORG (1%) i P-DET (1%). U usporedbi sa prethodnim rezultatima istraživanja sedimenata pod antropogenim utjecajem u srednjem Jadranu, ustanovljeni su viši udjeli P-FD i P-AUT frakcije, kao i niži udjeli P-ORG. Izmijenjeni odnosi udjela P-AUT i P-ORG su rezultat primjene različite tehnike u ekstrakciji sedimenta. Iz vertikalnih su profila P-FD, P-Fe i P-AUT oblika bile vidljive povišene koncentracije u površinskom sloju (0-1cm) sedimenta ispod kaveza koje se mogu pripisati utjecaju rada uzgajališta. Redoks-potencijal sedimenta ispod kaveza je bio konstantno negativniji nego na kontrolnoj postaji što upućuje na pojačani unos organske tvari sa uzgajališta i njenu razgradnju u anoksičnim uvjetima. Molarni omjeri C-ORG/TP i C-ORG/N-TOT su bili značajno niži u sedimentu ispod kaveza u odnosu na kontrolnu postaju zbog pojačanog unosa P i dušikovih vrsta kao i zbog brze razgradnje organske tvari unesene u sediment.

Korelacijskom Spearman analizom svih istraživanih parametara je ustanovljena povezanost između oblika P, N-TOT, sitno-zrnatog sedimenta i sadržaja organske tvari koja se može objasniti povišenim unosom dušika i fosfora u organskoj tvari iz vodenog stupca. Odsutnost uobičajene korelacije između C-ORG i OM je zbog relativno brzog uklanjanja ugljika iz sedimenta ispod kaveza.

**Ključne riječi:** Jadransko more, uzgajalište ribe, fosfor, sediment, SEDEX metoda