Pollution from Fish Farming Activities in the Adriatic Sea

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Contents

Abstract Fish farming (sea bream and sea bass, as well as bluefin tuna) production in Croatia exhibited a large increment from $<1{,}500$ t per year in 1997 to $>14{,}000$ t per year in 2018. This expansion has led to enhanced concern for the environmental integrity of the coastal areas from the public and the scientific community, particularly regarding ecological impact of fish farming. To reduce pollution caused by these activities in Croatia, monitoring of physical, chemical and biological parameters in the water column and sediments was introduced. According to Croatian legislative, monitoring activities are based on Environmental Impact Assessment studies (EIAs) for particular fish farm facility. These monitoring programs are not unified considering the parameters to be monitored as well as criteria or standards applied. Similar situation of different monitoring practice of environmental impact of fish farming based on EIAs can be found on European and Mediterranean level.

In this chapter an overview of physicochemical parameters monitored at Croatian fish farms is given. On the example of one sea bass and sea bream farm, methodology of sampling and analysis of the water column and sediments is presented as

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well as the obtained results. Analysed parameters from the water column were: oxygen concentration, pH value, dissolved phosphorus (inorganic and organic), inorganic nitrogen (sum of nitrates, nitrites and ammonia), organic nitrogen concentrations. Parameters determined in sediments were : phosphorus, organic carbon, total nitrogen content and sediment redox potential. According to the presented results parameters in sediments such as phosphorus concentration and redox potential proved as very good indicators of fish farm generated pollution, while from the water column, dissolved inorganic nitrogen form ammonia and organic phosphorus indicated changes caused by farming activities. Parameters proposed to be monitored in sediment represent the organic matter content (carbon, nitrogen and phosphorus) and the degree of its degradation (redox potential). Their analyses are not expensive and they are feasible to obtain. Majority of parameters monitored in the water column are already included in the water quality analysis that are monitored for the commitments of European directives (WFD, MSFD) or Mediterranean plans (IMAP), and their interpretation can be obtained in accordance with the existing threshold values.

Brief overview of Montenegrin mariculture activities, legislation, monitoring and perspectives is also given to compare with Croatian experiences. It can be summarized that presented Croatian monitoring design of physicochemical parameters in water column and particularly in sediments can be applicable example of future monitoring practice on the Montenegrin fish farm facilities.

Keywords Adriatic Sea, Fish farming, Nutrients, Pollution, Sediment, Water column

1 Introduction

Aquaculture in Croatia is very important economic activity with long tradition. It is playing an important role in Croatian fisheries with the share of aquaculture in the total fishery in Croatia of 18.6% (in 2017) that is close to the EU average of 20.4% (http://www.fao.org/fishery/countrysector/naso_croatia).

Farming of aquatic organisms in Republic of Croatia includes marine and freshwater aquaculture. In 2018 total aquaculture production was 18,067 t and the marine fish production had the largest share (84% in total) (Fig. 1).

Marine aquaculture includes farming of finfish, pelagic fish and shellfish (Fig. 2). Marine finfish farming in Croatia is dominated by European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) with the production of these two species exceeding 11,000 t in 2018, and Atlantic bluefin tuna (*Thunnus thynnus*) with >3,220 t) (http://www.fao.org/fishery/countrysector/naso_croatia). Shellfish farming comprises farming of Mediterranean mussel (*Mytilus galloprovincialis*) and European flat oyster (*Ostrea edulis*) whose production in 2018 was 900 t, i.e. 5% in total marine production (https://ribarstvo.mps.hr/default.aspx?id=14). According

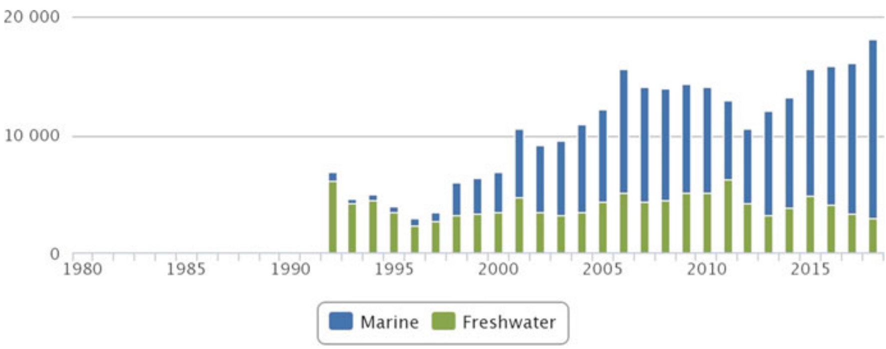

Fig. 1 Aquaculture production by the culture environment in the Republic of Croatia (Source: https.www.fao.org/fishery/countrysector/naso_croatia)

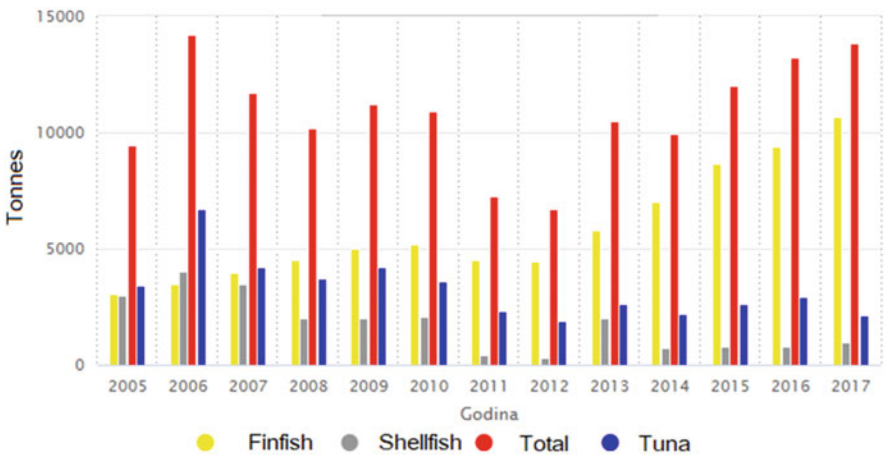

Fig. 2 The production of finfish (gilthead sea bream and sea bass), shellfish and tuna in Croatia for the 2005–2017 period (http://baltazar.izor.hr/azopub/bindex)

to National Strategic Plan for Aquaculture Development of Republic of Croatia (NSPA) in strategic objectives and priorities it was planned to reach the number of 10,000 t of marine fish (excluding tuna, until 2020, and for tunas 3,000 t) [1].

Croatia pioneered commercial marine aquaculture with one of the first and largest hatcheries for European sea bass in the early 1980s. During the 1990s war activities interrupted its development and it took a long time to consolidate. Marine aquaculture has completely recovered and now represents the fastest growing part of total aquaculture sector in Croatia. Production involves a closed farming cycle, where the first phases take place in a hatchery and then in the floating cages at sea.

Farming of Atlantic bluefin tuna (*Thunnus thynnus*) in Croatia has started in the 1990s. Production is based on capture of wild tunas (8–10 kg in size) and their subsequent farming to the market size (30 kg and larger). If the captured tuna have acceptable size at capture, the cage farming may last only a few months, but if this is not the case, these smaller captured fish may be kept in cages for up to 2 years. Tuna is fed with small pelagic ("blue") fish, which is rich in fat content. Due to the low utilization rate of this feed, large quantities of food is needed, which may sometimes lead to unwanted impact on environment.

Annual production of Atlantic bluefin tuna has been stagnating in the last few years due to the restriction measures in tuna fishery managed by ICCAT (International Commission for the Conservation of Atlantic Bluefin tuna). Atlantic bluefin tuna represent on average nearly 25% of the volume of marine aquaculture production. Almost the entire tuna production is placed on the Japanese market.

Shellfish farming has the longest tradition in Croatia. There are written evidences about oysters farming in the sixteenth century in Mali Ston Bay. Farming includes Mediterranean mussel (*Mytilus galloprovincialis*) and European flat oyster (*Ostrea edulis*), using traditional farming technology of floating parks. All shellfish production areas are continuously monitored by the State (Monitoring of water quality).

According to the Ministry of Agriculture, Directorate of Fisheries, in 2017, the total number of aquaculture production centres was 204, including both marine (158 farms) and freshwater (46 farms).

The farms are mostly microscale enterprises, particularly family-owned farms. There are 35 companies registered for finfish farming on a total of 63 locations at sea, while 9 locations are licensed for polyculture (farming of fish and shellfish). There are also 4 land-based hatcheries. Tuna farming takes place in floating cages at sea, in Split-Dalmatia County and dominantly in Zadar County. There are 4 companies registered for tuna farming on a total of 10 locations. Oysters are in general farmed in the area of Malostonski Bay and Malo More, while mussels are mostly farmed in the area of western coast of Istria, area of the river Krka estuary, and in Novigrad Sea. There are 124 registered shellfish farmers on a total of 280 locations (http://www.fao.org/fishery/countrysector/naso_croatia).

The public awareness of the aquaculture in Croatia has experienced significant transformation; from acceptable "eco-production" activity, at the beginning, to activity with negative anthropogenic impact on the marine environment, nowadays. The sources of possible negative impact are: generated waste (especially caused by feeding), the appearance of pests and diseases, genetic pollution caused by the escape of cultivated fish and their interbreeding with wild fish populations, visual "pollution" of the area, unwanted odours, etc. The intensity of negative impact depends on the farm size, the type and the efficiency of feeding; the substrate (bottom) type, the sea depth, the intensity and volume of water mass exchange.

2 State of Knowledge About Environmental Impact of Fish Farming

Since mariculture production becomes more intensive, it requires new sites for cultivation and this consequently results in conflict with other users of the coast. Therefore, targeted research with the aim to assess the impact of fish farming on the marine environment is necessary [2].

Great expansion of fish farm activities in Croatia has led to enhanced concern for the environmental integrity of the Croatian coastal areas from the public and the scientific community, particularly regarding its ecological influences.

Fish farming releases a variety of waste into the marine environment including nutrients, organic matter as well as pharmaceutical products, which can have undesirable impacts [3]. According to Karakassis et al. (2005), there is a small risk of hypernutrification at large spatial scales in the Mediterranean, while potential changes in the water column occur at short spatial scales [4]. The effects of fish farming, monitored in the immediate vicinity of the fish cages (small spatial scales), on the nutrients and chlorophyll-a concentrations in the water column in most cases were not significant [5–7].

On the contrary, degradation of the seabed beneath and around the fish cages is the most widely documented effect of fish farming. These changes were demonstrated through disturbances of different parameters such as a negative sediment redox-potential [8, 9], accumulation of organic carbon, phosphorus and nitrogen compounds [10–15] and accordingly changed or reduced benthic communities [16–22].

Targeted researches of fish farm impacts were also focused on the efficiency of feeding and food composition in order to improve the growth of cultured organisms and reduce the negative environmental impact [23, 24]. The impact of farms on the nearby seagrass communities in the Mediterranean was also researched [18, 25]. Slišković et al. (2010) described the populations of bio-fouling communities on farming installations in the eastern Adriatic [26]. The fate of the organic matter accumulated under farming installations [27, 28], the regeneration of bottom system after the removal of cages [29, 30] and genetic comparisons of wild and cultured populations of fish [31, 32] were the objects of numerous research studies.

3 Legislative Regulation of Fish Farming Activities

The greatest number of regulations that directly impact how the activity of aquaculture is performed at EU level comes from the field of environmental protection. The key regulations include the Water Framework Directive (WFD), (2000/60/EC), the Directive on the Conservation of Natural Habitats and Wild Flora and Fauna (Council Directive 92/43/EEC), the Directive on Conservation of Wild Birds (Directive 2009/147/EC 15) (together creating framework of establishing Natura 2000

Network), the Environmental Impact Assessment Directive (EIA Directive 2014/52/EU), the Marine Strategy Framework Directive (MSFD), (2008/56/EC), the Directive on Alien and Locally Absent Species in Aquaculture (EC) No 708/2007 and the Strategic Environmental Assessment Directive (SEA) (2001/42/EC).

From the point of view of aquaculture, the WFD and MSFD refer primarily to issues of water quality. The objective of the MSFD is to safeguard the ecological standards in the maritime environment. The EIA and SEA directives regulate the issues of size and shape of spatial interventions that require impact assessments, that is, create the framework for performing environmental impact assessments when preparing strategic plans for development of a specific activity.

3.1 Monitoring of the Impact of Mariculture on the Environment

Republic of Croatia implements the Plan for Monitoring sea and shellfish quality in production areas and areas of reintroduction of live shellfish, which is in accordance with the Regulation (EC) (No 854/2004) determining specific rules for the organization of official controls on products of animal origin intended for human consumption.

Monitoring the impact of fish farming on the environment is regulated by the Croatian Law on Protection of the Environment (OG 82/94 and OG 128/99) [33, 34] and the Ordinance amending the Ordinance on assessment environmental impact (OG 59/00 and OG 136/04) [35, 36]. All marine fish and shellfish farms of annual production more than 50 tons, and all marine hatcheries with an annual production of more than 500,000 fish juveniles, are obliged to prepare an EIA study, which prescribes the monitoring of the impact of farming on the environment for specific area.

Unfortunately, this monitoring plan is not unified, nor are the indicators defined follow, neither the criteria nor the required standards. Consequently, monitoring of marine fish farms is inconsistent considering the physical, chemical and biological parameters to be monitored. Similar experiences of monitoring of environmental impact of fish farming based on EIAs can be found on European and Mediterranean level.

Aquaculture is according to Land Based Protocol (LBS) included in the list of sectors of activity which are to be primarily considered when setting priorities for the preparation of action plans, programmes and measures for the elimination of the pollution from land-based sources and activities. (Both the HELCOM and OSPAR as well as relevant FAO and GFCM Decisions and Guidelines provide measures to address pollution from aquaculture.) However, there are no relevant provisions in the existing Mediterranean Regional Plans. Developing of technical guidelines and management standards to tackle inputs of nutrients and contaminants from aquaculture is taken into consideration during preparing new UNEP Mediterranean Regional Plans (https://wedocs.unep.org/handle/20.500.11822/26540?show=ful).

As a part of Possible Elements of the UNEP/MAP Regional Plan on Aquaculture Management the objective of the Regional Plan is "to minimize water pollution caused or induced by aquaculture sector". Inside the Proposed measures to be taken, one of the proposed measures is to *Control discharges through monitoring of:*

– Sediments: phosphorus, carbon and nitrogen content, redox potential.
– Water column: oxygen, nutrients (inorganic nitrogen and phosphorus), total nitrogen and phosphorus, chlorophyll-a, TRIX index, etc.

Except for the above-mentioned discrepancy in monitoring of fish farms in Croatia, monitoring of fish farms on Croatian farms that are prescribed with EIAs mostly matches with the proposed Control of discharges and monitored parameters of future UNEP/MAP's Regional Plan on Aquaculture Management to minimize water pollution by aquaculture sector.

So, this chapter will give an overview of physicochemical parameters monitored at Croatian fish farms with the emphasis on parameters that proved as good indicators of fish farming impact. Methodology of sampling and analysis will be presented as well as interpretation of results obtained for one sea bass and sea bream farm in the Adriatic Sea area.

Presented design of the monitoring can also be applicable and useful example of monitoring practice on the Montenegrin fish farm facilities.

4 Overview of the Physicochemical Parameters Monitored in the Water Column and Sediment at Fish Farm

4.1 Sampling and Analysis

Republic of Croatia implements the Monitoring Plan to estimate fish farm impact on physical and chemical parameters through analysis of seawater and sediment at the fish farm area as well as on the area of similar hydro morphologic characteristics without fish farm (control site). (Parallel with these investigations, at the same sites the composition of benthic communities and the physical impact of fish farming on the seabed as well as changes in the composition of fish communities around the fish farm were examined.)

Sampling was obtained at the Adriatic seabass and sea bream farm located in the bay on 3 sites inside it, approximately 200 m distanced away: K1 – cage (44°37.977'N and 14°27.174′E), 12 m depth; K2 (44°38.004'N and 14°26.981′E, 38 m depth; and K3 (44°38.003'N and 14°26.823′E), 39 m depth. Neighbouring bay without fish farm inside was chosen for control location with site K-REF (44°37.349'N and 14°27.890′E), 21 m deep.

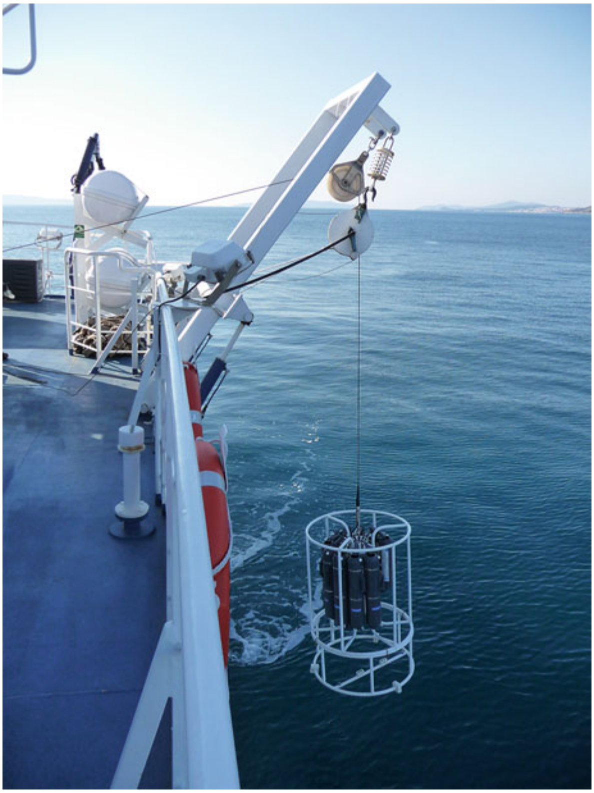

Fig. 3 Seawater samplers – carousel of Niskin bottles (Photo by S. Muslim)

Sampling of seawater and sediments at investigated sites was obtained from oceanographic research vessel “Bios Dva” from the Institute of Oceanography and Fisheries (IOF) in Split, Croatia. Seawater was sampled using a carousel of Niskin bottles, which can be triggered to close at selected depth for water collections (Fig. 3). Sampling was obtained at standard oceanographic levels (0, 5, 10, 20, 30, 50 m), depending on the depth of the water column at the site.

Measured parameters in seawater samples were: oxygen saturation, pH value, concentrations of dissolved inorganic nitrogen species (nitrates NO_3^-, nitrites NO_2^-, ammonia NH_4^+), orthophosphates (HPO_4^{2-}) and orthosilicates (SiO_4^{4-}) as well as dissolved organic phosphorus and nitrogen concentration. (Dissolved inorganic nitrogen, total dissolved phosphorus, oxygen and chl-a concentrations are included in the calculation of TRIX index [37].

Most of these parameters are included in the water quality analysis that is monitored according to the Water Framework Directive 2000/60/EU demands, or they are incorporated into monitoring for the MSFD (2008/56/EU) (Descriptor 5, Eutrophication), or IMAP’s Ecological objective 5 (Eutrophication).

Determination of oxygen saturation in seawater samples were obtained by using standard oceanographic Winkler method according to Grasshof (1976) [38]. pH value was measured with combined glass electrode on pH-meter Sartorius PB-11.

Concentrations of nutrients (ammonia and orthophosphates) were determined immediately after the collection of samples without freezing on board laboratory according to Grasshof (1976) [38] on spectrophotometer Shimadzu UV Mini 1240. For analysis of other nutrients (nitrates, nitrites, orthosilicates, organic phosphorus and organic nitrogen), seawater samples were frozen (on −18°C) and brought to Laboratory of Chemical Oceanography and Sedimentology at the IOF, Split. Analysis of nutrient concentrations was obtained colorimetrically on Auto Analyzer Seal Analytical according to Grasshof (1976) [38] and Operating Manuel of the instrument.

In order to estimate fish farm impact on geochemical characteristics of sediment, phosphorus, organic carbon and total nitrogen content in sampled sediments were determined. According to previous investigations of sediments under the fish farms, ratio of phosphorus, carbon and nitrogen was largely changed in relation to reference sites undisturbed by the mariculture [39, 40]. Phosphorus proved as good indicator of fish farm impact through elevated concentrations in most of the investigated sediments of European and world fish farms [6, 12, 29, 41–43].

Investigations of „cage sediments "in the Adriatic indicated increased concentrations of certain inorganic phosphorus forms that were attributed to remains of uneaten fish food as a direct influence of fish farming [14, 42].

Sampling of marine sediment was obtained by gravity corer from the deck of research vessel "Bios Dva", while sediment core was divided on subsamples each 1 cm thick.

At the sampling site below the cage, sediment was sampled by autonomic diver who took the sample by inserting the plastic tube of corer into the seafloor. Immediately after the sampling, diver emerged the sample at the surface in the vertical position to preserve the undisturbed sediment core (Fig. 4).

Sediment was divided into subsamples 1 cm thick, frozen (−18°C) and brought to Laboratory of Chemical Oceanography and Sedimentology of IOF – Split, Croatia for further analysis. For analysis of phosphorus concentration in sediment, samples were freeze-dried, grounded and sieved on the sieve ($\Phi < 250$ μm). Extraction of inorganic and total phosphorus was obtained according to Aspila et al. (1976) [44]. Orthophosphate concentration in extracts was determined on spectrophotometer UV MINI 1240 Shimadzu according to Grasshoff (1976) [38]. Standard marine sediment reference material PACS-2 (NRC-CNRC) was used for method evaluation.

Redox-potential in undisturbed sediment cores (Fig. 5) was measured „in situ" by vertical penetration of Pt electrode with Ag/AgCl reference electrode, with quinhydrone buffer solutions in pH = 4 and pH = 7 prepared according to Metrohm (Ag Herisau, Switzerland).

For determination of organic carbon and total nitrogen in sediments, freeze-dried sample was treated with hydrochloric acid to remove carbonates [45]. Analysis of element contents in sediments was obtained on CHNS-O analyzer (Perkin Elmer 2,400 Series II).

Fig. 4 Sediment sampling by autonomic divers with plastic tubes of corer (Photo by S. Matijević)

4.2 Results of Monitoring in the Water Column and Sediment

4.2.1 Oxygen in the Water Column

Oxygen solubility in the natural waters runs according to Henry's law, like other atmospheric gasses. In the state of equilibrium oxygen concentration is proportional to its partial pressure in the atmosphere. The main processes that can disturb this balance are primary production of organic matter (photosynthesis) that increases oxygen content and respiration or heterotrophic degradation (oxidation) of organic matter that decreases oxygen content. Oxygen is very sensitive indicator of intensity of bio-chemical processes and besides temperature and salinity is the most common chemical constituent determined in the water column. According to ecological definition, oxygen concentration (in mL/L), that is considered as critical for the life of benthic organisms is 0.7–2 mL/L and presents hypoxic conditions, while values $\leq$0.7 mL/L implicate anoxia.

Oxygen concentration in the water column at fish farm sites and reference site ranged from 5.10 to 6.21 mL/L (Table 1) with the lowest value determined in surface layer under the cage, and highest value in the bottom layer at K3 site located at the exit of the bay with farm inside.

Average values of O_2 concentrations indicate no significant differences between sites (Table 1). All determined O_2 concentrations are significantly higher than 2 mL/L (the value that could potentially have negative impact on the life of organisms in the marine environment), so obtained results implicate good state of oxygen at all investigated sites.

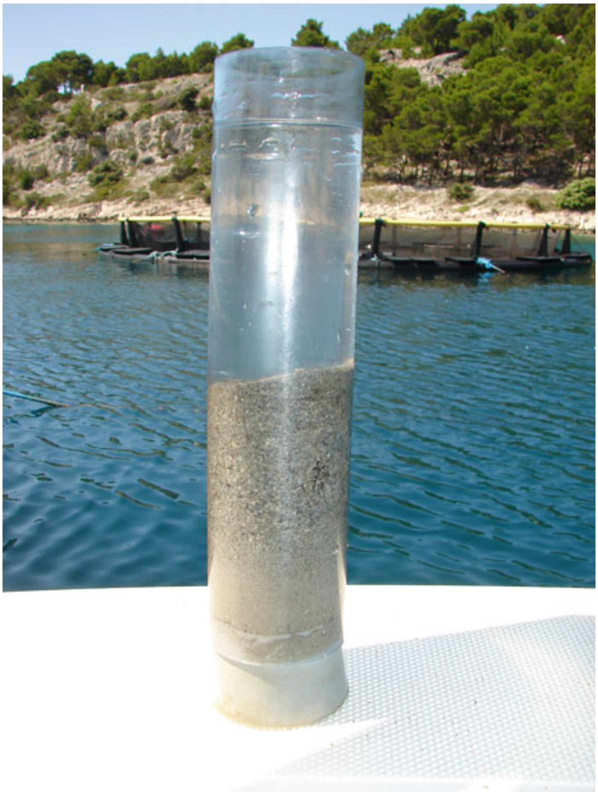

Fig. 5 Undisturbed sediment core sampled by autonomic divers. (Photo by S. Matijević)

Vertical distribution of oxygen concentrations was characterized with higher values in the middle and bottom layer. That is in accordance with distribution of temperature in the water column and the solubility – temperature dependence of gasses solubility in water (solubility of gas increase with temperature decrease in the deeper layer of the water column at investigated sites). Additionally, this distribution pattern of vertical of oxygen concentrations is in accordance with usual oxygen profiles in the Adriatic during spring season that indicates stratification of the water column (http://baltazar.izor.hr/azopub/bindex).

4.2.2 pH Value in Seawater

Seawater pH is slightly alkali due to excess of dissolved anions that is mainly influenced by marine carbon cycle. Namely, during the processes of production and degradation of organic matter in marine ecosystem CO_2 is produced (respiration) or it is removed (photosynthesis). Usual pH value for Adriatic Sea is pH $= 8.2 \pm 0.1$. During intensive photosynthesis pH can increase up to 0.2 units, while degradation of organic matter can decrease it under the pH $= 8$. Despite these

Table 1 Basic statistic parameters (range, average value and standard deviation) for all parameters investigated in the water column and sediments at investigated sites

Parameter		K1	K2	K3	REF
Water column					
O_2 (mL/L)	AV ± STD	5.36 ± 0.31	5.65 ± 0.34	5.78 ± 0.33	5.61 ± 0.33
	Range	5.10–5.70	5.30–6.14	5.42–6.21	5.31–5.99
pH		8.20 ± 0.01	8.2 ± 0.01	8.19 ± 0.01	8.19 ± 0.01
		8.19–8.21	8.18–8.21	8.18–8.19	8.18–8.19
TIN (μmol/L)		4.9 ± 3.9	0.84 ± 0.29	0.56 ± 0.24	0.69 ± 0.31
		1.12–8.96	0.64–1.34	0.28–0.94	0.40–1.07
NH_4^+ (μmol/L)		4.51 ± 3.85	0.41 ± 0.29	0.26 ± 0.27	0.41 ± 0.19
		1.12–8.96	0.23–0.93	0.05–0.72	0.28–0.68
NO_3^- (μmol/L)		0.31 ± 0.10	0.31 ± 0.06	0.22 ± 0.11	0.18 ± 0.19
		0.23–0.42	0.26–0.38	0.12–0.39	0–0.40
NO_2^- (μmol/L)		0.09 ± 0.05	0.12 ± 0.06	0.08 ± 0.01	0.09 ± 0.01
		0.05–0.15	0.01–0.16	0.07–0.11	0.09–0.10
HPO_4^{2-} (μmol/L)		0.07 ± 0.01	0.05 ± 0.01	0.058 ± 0.060	0.06 ± 0.01
		0.05–0.08	0.04–0.06	0.00–0.135	0.05–0.07
SiO_4^{4-} (μmol/L)		1.36 ± 0.07	1.51 ± 0.08	1.39 ± 0.05	1.45 ± 0.08
		1.31–1.44	1.43–1.64	1.35–1.46	1.36–1.54
N-ORG (μmol/L)		6.9 ± 0.9	8.24 ± 0.66	8.10 ± 0.89	7.99 ± 0.93
		6.35–7.89	7.36–9.19	6.79–9.06	6.63–8.68
P-ORG (μmol/L)		0.43 ± 0.32	0.06 ± 0.08	0.15 ± 0.15	0.27 ± 2.59
		0.11–0.76	0.01–0.21	0.02–0.40	0.05–0.59
Sediment					
TP (μmol/g)		17.7 ± 29.9	9.8	5.3	3.0 ± 0.4
		4.2–106.4			2.2–3.4
IP (μmol/g)		16.0 ± 30.2	6.4	1.7	0.9 ± 0.4
		2.9–106.4			0.1–1.4
OP (μmol/g)		2.6 ± 2.3	6.4	1.7	0.9 ± 0.4
		0.14–4.2			0.1–1.4
C-ORG (%)		3.27–3.44	2.62–2.94	2.57–2.71	2.32–2.92
		3.35 ± 0.08	2.80 ± 0.16	2.65 ± 0.07	2.70 ± 0.5
EH (mV)	Range	−(322) to 59	−(88) to 120	(−30) to 100	(−59) to 65

two natural processes, pH value is also impacted with industrial and effluent waters inputs as well as with freshwater sources. Due to buffering of carbonate system in seawater, these impacts are locally restricted.

pH values determined in the water column at investigated sites ranged between 8.18 and 8.21, that is in accordance with values determined for the Adriatic. From average values, no existence of significant differences between sites is obvious (Table 1). Vertical distribution of pH values indicated negative gradient from surface to bottom layer that can be a consequence of respiration in deeper layers that releases the CO_2.

4.2.3 Concentrations of Nutrients in the Water Column

Dissolved nitrogen and phosphorus salts (nutrients), with sunlight, carbon dioxide, some trace elements and vitamins are necessary for photosynthesis in the marine environment. Primary producers by photosynthesis generate amino acids, proteins and nucleic acids that are incorporated in marine food chains. After the death of organisms and organic matter sedimentation, heterotrophic degradation and remineralization occur. Products of organic matter degradation are dissolved inorganic nutrients (nitrates, nitrites, ammonia and orthophosphates) that return into the water column. Some phytoplankton species (diatoms, silicoflagellates) can also use dissolved silicate for their skeletons formation. Regeneration of silicate is taking part by chemical dissolution of settled biogenic silica (opal). These processes do not present complete biogeochemical cycles of nitrogen, phosphorus and silica. Part of these elements is permanently removed from the cycles (by sediment burial, mineral adsorption or gasification), but they can also be input by freshwater, groundwater or from the atmosphere.

Total dissolved inorganic nitrogen in natural waters exists in oxidized (nitrates, nitrites) or in reduced forms (ammonia). Due to very fast oxidation and reduction processes, here we present sum of all inorganic nitrogen forms (TIN = sum of nitrates, nitrites and ammonia). Basic statistic parameters for TIN in the water column of investigated sites in farm location (K1, K2 and K3) and nearby site (K-REF) are given in Table 1.

Concentration of total inorganic nitrogen ranged from 0.28 μmol/L (at K3 site) to 8.96 μmol/L at site K1. From average values of TIN concentrations significantly higher value in the water column at site K1 under the cage regarding other sites is evident. Average TIN concentration determined at sites K2, K3 and –K-REF (0.65–0.89 μmol/L) in relation to values established for the coastal Adriatic area belongs to group of low values. According to defined threshold values determined in accordance with WFD (2000/60/EU), for TIN in Croatian part of the Adriatic, mentioned cage values belong to “very good state” category (OG 73/2013) [46].

Vertical distribution of TIN concentrations indicated relatively unique distribution at K2, K3 and KR1 sites, except at 10 m depth layer at K2. At site K1, TIN concentrations were up to 10 times higher related to other sites at the bay with farm, as well as to reference site K-REF. Significantly elevated concentrations were in surface layer and at 5 m depth.

Vertical distribution of each nitrogen species inside TIN (nitrates, nitrites and ammonia) indicates that obtained differences in TIN are consequence of increased or decreased ammonia concentrations. High ammonia concentrations determined in the water column at K1 site belong to group of extremely high values determined for the middle Adriatic. Similar high ammonia concentrations were also obtained for the water column below the tuna cages monitored in the middle Adriatic area. Increase in NH_4^+ concentrations can be assigned to fish farm impact as potential source of ammonia salts. Similar was obtained for middle Adriatic fish farm where urea concentrations were enhanced in the upper part of the water column (0–20 m) compared to the reference sites [15]. Considering the urea's role as an intermediate species in the oxidation of compounds from organic nitrogen to ammonia, the increase was attributed to fish excretions and degradation of their metabolic products.

Concentrations of nitrates were twice higher than nitrite concentrations (Table 1), that is also in accordance with the results obtained for the water column of the Adriatic areas. Vertical distribution of NO_3^- concentrations was in accordance with its usual distribution pattern during spring season (with elevated concentrations in the bottom layer of deeper sites); while NO_2^- concentrations in the water column of all investigated sites were relatively uniform.

According to abundance of certain nitrogen species at "cage site" K1, ammonia was the most predominant species (85%), while at K2, K3 and K-REF sites its portion ranged between 45 and 62%. The abundance of nitrates at site below the cage was the lowest (12%), while at other sites it ranged from 40 to 44% (at K2 and K3), and 21% at K-REF site. Nitrate portion was 3% (at K1) and 14–18% on other investigated sites. Similar portions of inorganic nitrogen species determined for June 2015 at investigated sites are generally in agreement with natural seasonal oscillations of ratios of certain species for the coastal Adriatic area [47] with the exception of site K1.

Besides the inorganic nitrogen forms, concentrations of organic forms were also determined (Table 1). The highest average value was calculated for K2 site (8.24 ± 0.66 μmol/L).

Average values of N-ORG concentrations indicated lower values for the water column at site K1 related to other investigated sites. These results obtained for fish farm investigation belong to group of low N-ORG concentrations determined during long-term investigations of coastal Adriatic areas (range:4.8–13.8 μmol/L) [47]. N-ORG portions in total dissolved nitrogen generally exceed TIN portions (from 62% at K1 site to 93% at K3), that is in accordance with usual distribution of nitrogen forms in the Adriatic water column. Due to unknown portions of autochthonous and allochthonous fractions in organic forms of N (more complex analytical determination is needed), detailed analysis of their input or output from this marine environment is not possible.

Orthophosphate concentrations ranged between 0 and 0.135 μmol/L (in the middle and bottom layer at K3 site). HPO_4^{2-} range of concentrations is inside ranges determined during 2011/2012 period for Adriatic [47]. The obviously highest concentrations were at K3 site, while for other sites, as well as for reference site K-REF, average values were lower. These differences can be assigned to impact of circulation in the bay rather than to fish farm impact. According to defined threshold values determined in accordance with WFD (2000/60/EU), for HPO_4^{2-} in Croatian part of the Adriatic, most of these values belong to "very good state" category (OG 73/2013) [46].

Vertical profiles of orthophosphate concentrations at investigated sites indicated relatively unequal distribution. The highest values were obtained in surface and the bottom layer for deepest K3 site. Relatively unequal vertical distribution marked at other sites is in accordance with vertical profiles of HPO_4^{2-} for most of the coastal middle Adriatic locations, except for estuaries or city harbours where increased input of HPO_4^{2-} was obvious in surface layer.

Calculated portions of orthophosphates and organic phosphorus forms in total phosphorus indicated prevailing of organic form at K1 and KR1 sites (80 and 69%), while inorganic phosphorus prevailed at other sites (55 and 57%). Relatively low orthophosphates portions in total dissolved phosphorus is in accordance with average portions obtained for the middle Adriatic water column (26 ± 14%) [47].

P-ORG concentrations indicated higher values for the water column at site K1 related to other investigated sites. Similar results were obtained for tuna farms at the middle Adriatic area. According to defined threshold values determined in accordance with WFD (2000/60/EU), for P-TOT (sum of P-ORG and orthophosphate) in Croatian part of the Adriatic, most of these values belong to "very good state" category (OG 73/2013) [46], P-TOT concentrations at K1 site belong to "good state" category.

Orthosilicate concentrations (SiO_4^{4-}) were in narrow range (1.31–1.64 μmol/L) determined at K1 and K2, respectively (Table 1). These values are inside wider range of orthosilicate concentrations determined for Adriatic (0–67 μmol/L) [47]. Average values of SiO_4^{4-} indicated no significant differences between investigated sites. Vertical distribution of orthosilicate concentrations was relatively equal inside the investigated bays, with no expressed maximum in surface or bottom layer that can be a consequence of seasonal oscillations.

4.2.4 Concentrations of Phosphorus in Sediment

Dissolved phosphorus (P) from water column arrives into the sediment in organic (photosynthetically produced) forms and inorganic forms (mineral origin). During diagenesis in sediment, total phosphorus content varies and its distribution into different fractions takes part. Phosphorus in sediments can exist in several forms: phosphorus adsorbed on mineral surfaces, phosphorus in mineral precipitates (iron bound P, apatite P) and detrital P as well as P incorporated in organic matter [42]. Phosphorus in sediment proved as good indicator of fish farm impact where concentrations obtained in sediments under the cages were up to 10 times elevated regarding the reference sites with no fish farm inside [15, 42]. Methods for phosphorus determination in sediments applied in this investigation divide only two phosphorus fractions; organic P and total P that presents sum of listed inorganic P forms and organic P (TP).

Total phosphorus concentration (TP) ranged between 2.2 and 106.4 μmol g^{-1} at K3 and K1 sites, respectively (Table 1). These values are inside the range determined for sediments under the anthropogenic influence such as eutrophicated bays and fish farms in the Adriatic (19–135 μmol g^{-1}) [42]. Extremely elevated concentrations in surface layer at K1 site can be assigned to input of phosphorus compounds probably originated from settled remains of fish food and excretes.

Average TP values indicated significant differences between site under the cage and other investigated sites in the farm location with obvious negative gradient in TP concentrations from inside to the exit of the bay. This result is implication of fish farm impact on the very tight area under the cage that was also found during previous investigations of fish farm influence on the environmental parameters in the water column and sediments.

Inorganic phosphorus concentrations ranged between 1.9 and 106.4 μmol g^{-1}, which belong to group of extremely high values compared to values obtained in sediments at the middle Adriatic. Organic phosphorus range was from 0.1 to 5.8 μmol g^{-1} and was in accordance with values previously determined for the Adriatic sediments. Vertical profiles of total and inorganic phosphorus indicated extremely high concentrations in surface sediment layer (0–1 cm). However, lower values obtained for depths higher than 3 cm layer can be attributed to group of TP and IP concentrations usual for sediments that are under the anthropogenic impact. Vertical distribution also indicated that almost all phosphorus was in inorganic form, while organic P was not detected in surface layers.

In sediments at KR1 site vertical distribution of IP was relatively unique, and concentrations of organic phosphorus (OP) were significantly lower. According to previous investigations extremely high IP portion in total phosphorus (89%) was obtained only for sandy sediments under the cages of sea bass and sea bream farms in the north and middle Adriatic. Investigations of different fractions inside the sediment inorganic phosphorus pool at sea bass and sea bream farms in the middle Adriatic [14, 48] by using SEDEX analysis proved that the highest portion was for P bound to iron oxides and hydroxides. Extremely high portion of "fish debris phosphorus" fraction (that includes P bound in biogenic apatite originated from remains of fish bones and tooth settled in sediment) was obtained and it was assigned to fish farm activity. Method applied for the samples from this fish farm location enables only organic and total inorganic phosphorus concentrations, so it is not possible to determine what P fraction is responsible for disturbed ratio between IP and OP fraction in surface sediment at fish farm site.

4.2.5 Sediment Redox Potential

Sediment redox potential measurements obtained at site below cage and REF site showed wider range of values (−322 to 59 mV) (Table 1). Redox-cline was determined at K1 below the cage even in the first centimetre of depth, while at other and reference site, redox cline was deeper. These negative values of redox potential belong to lowest E_H recorded for the fish farm sites at the middle Adriatic area [14]. Extremely negative potentials indicate high concentrations of sulphide ions (S^{2-}) as well as degradation of organic matter in the absence of oxygen [48]. Additionally, visual monitoring of below-cage sediments indicated appearance of filamentous sulphur Beggiatoa bacteria that confirmed the measurement results.

4.2.6 Organic Carbon Content in Sediment

Organic carbon content in sediment (C-ORG) at investigated sites was between 2.57 and 3.44% at K-REF and K1 site, respectively. These values were 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) [22] and Matijević et al. (2006) [14]. Giles (2008) suggested sediment enrichment state considering C-ORG and N-TOT values (low, moderate, high and very high) [40], and within that, presented results fall into the category of sediments moderately enriched by fish farming impact.

5 Conclusions

An overview of physicochemical parameters from the water column and sediments monitored at Croatian fish farms is presented through investigations at one sea bass and sea bream farm. Examined parameters from the water column were: oxygen concentration, pH value, dissolved phosphorus (inorganic and organic), inorganic nitrogen (sum of nitrates, nitrites and ammonia) and organic nitrogen concentrations. Parameters determined in sediments were phosphorus, organic carbon, total nitrogen content and sediment redox potential.

According to results from the water column the key observations arose. Analysis of nutrients indicated the increased concentrations of dissolved nitrogen and organic phosphorus in the water column due to the increase of ammonia or urea (from the fish excretion and degradation of their metabolic products) and the increase of the input of organic matter (food, excreta and metabolic products).

In the sediments the extremely increase in phosphorus concentration was found, which was directly connected to the input of organic material from the farm area. Extremely negative redox potentials were detected that indicate high concentrations of sulphide ions as well as degradation of organic matter in the absence of oxygen. Additionally, the appearance of sulphur bacteria genus Beggiatoa confirmed the consumption of oxygen for the organic matter degradation in the sediment and anoxic conditions.

It is very important to highlight that all mentioned consequences of the fish farming impact were mainly localized in the area under the fish cages and decreased in value with the increasing distance from the cages.

Taking into account presented results, it can be concluded that parameters in sediments such as phosphorus concentration and redox potential proved as very good indicators of fish farm generated pollution, while from the water column, dissolved inorganic nitrogen form ammonia and organic phosphorus indicated changes caused by farming activities.

Parameters proposed to be monitored in sediment represent the organic matter content (carbon, nitrogen and phosphorus) and the degree of its degradation (redox potential). Their analyses are not expensive and they are feasible to obtain. Majority of parameters monitored in the water column are already included in the water quality analysis that are monitored for the commitments of European directives (WFD, MSFD) or Mediterranean plans (IMAP), and their interpretation can be obtained in accordance with the existing threshold values.

Furthermore, it is also very important to monitor "control site" with similar hydrographic conditions as farm location but with no farm inside, that is very useful in the interpretation of obtained monitoring results.

To compare with Croatian presented experiences, brief overview of Montenegrin mariculture activities, legislation, monitoring and perspectives is as follows.

Marine aquaculture in Montenegro comprises gilthead seabream and European seabass and shellfish farming that includes Mediterranean mussel and European flat oyster. All mariculture activities are concentrated in the Boka Kotorska Bay. There are 20 mussel farms, all using floating park systems (long-lines). Sea bream and sea bass farming started in the late nineties, and currently there are two farms using the floating cage system breeding method whose first phase taking place in the hatchery and then in floating cages in the sea [49]. The multitrophic integrated mariculture farming of mussels and fish is implemented on both farms. The annual production at fish farms was 121 t in 2015 (http://www.fao.org/fishery/countrysector/naso_montenegro/en). Although it shows a mild growth over the past few years, it is still at very low level, in comparison with Croatia and other Mediterranean countries with relatively small production.

Similar to presented Croatian overview of legislative issues regarding mariculture, all the relevant issues in Montenegro are regulated by the National Laws. The Law on Marine Fishery and Mariculture [50] lays down the conditions for farming of fish and other marine organisms in locations planned in line with the Spatial Plan of Special Purpose Costal Zone [51], the Law on Environmental Impact Assessment [52], Law on Environmental Protection [53] and in line with the other laws that apply on the basis of the Laws above mentioned.

For each new fish farm it is obligatory to make assessment on the environmental impact, and based on the zero state of the site to estimate the minimum distance between locations for mariculture. To ensure proper and final site selection, additional studies with analysis of the sediment (granulometry, organic matter) benthic fauna, water quality (salinity, dissolved oxygen, temperature, chlorophyll-a, suspended solids, nutrients), oceanographic conditions, sanitary control and monitoring of biotoxins were recommended [54].

Programme of water quality monitoring and biomonitoring on the farms and around the farms is regulated by the Law on Marine Fishery and Mariculture [50]. The aim is to give farm owners timely information about the appearance of pollution and natural phenomena, if any, which may have a negative impact on the mariculture zone and on mariculture products. According to the Law the monitoring should be carried out on the basis of the Water Quality Monitoring and Biomonitoring Program in the fishing sea, adopted by the Ministry at the proposal of the competent institution (Institute of Marine Biology in Kotor). Monitoring is conducted by the Institute or an accredited laboratory [50].

Monitoring of mussel farms conducted in Boka Kotorska Bay included mostly analysis in the water column such as nutrient composition, microbiological parameters, and qualitative and quantitative data on phytoplankton [55]. For fish farm monitoring there are no specified parameters to be analysed.

To expand mariculture in the draft of the Special Purpose Spatial Plan for the Coastal zone of Montenegro, as potential new locations, nine new sites have been included in the open sea area (http://www.fao.org/fishery/countrysector/naso_montenegro/en). Fisheries Strategy of Montenegro 2015–2020 with an Action Plan [56] recognized aquaculture as one of the development activities of fisheries sector. Strategy predicts development of sustainable aquaculture through modernization of existing capacities in order to increase the production as well as to strengthen the efficiency of this sector, while respecting high environmental, animal health and welfare standards (http://www.fao.org/fishery/countrysector/naso_montenegro/en).

Considering the brief overview on Montenegrin state of mariculture activities and its perspective it is obvious that number of fish farm sites will increase in the future as well as the need for the protection of the marine environment regarding ecological impact of fish farming. According to the given information there are no specified parameters to be monitored at fish farms in Montenegro, so presented Croatian monitoring design of physicochemical parameters in water column, and particularly in sediments, can be applicable in future monitoring practice on the Montenegrin fish farm facilities.

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