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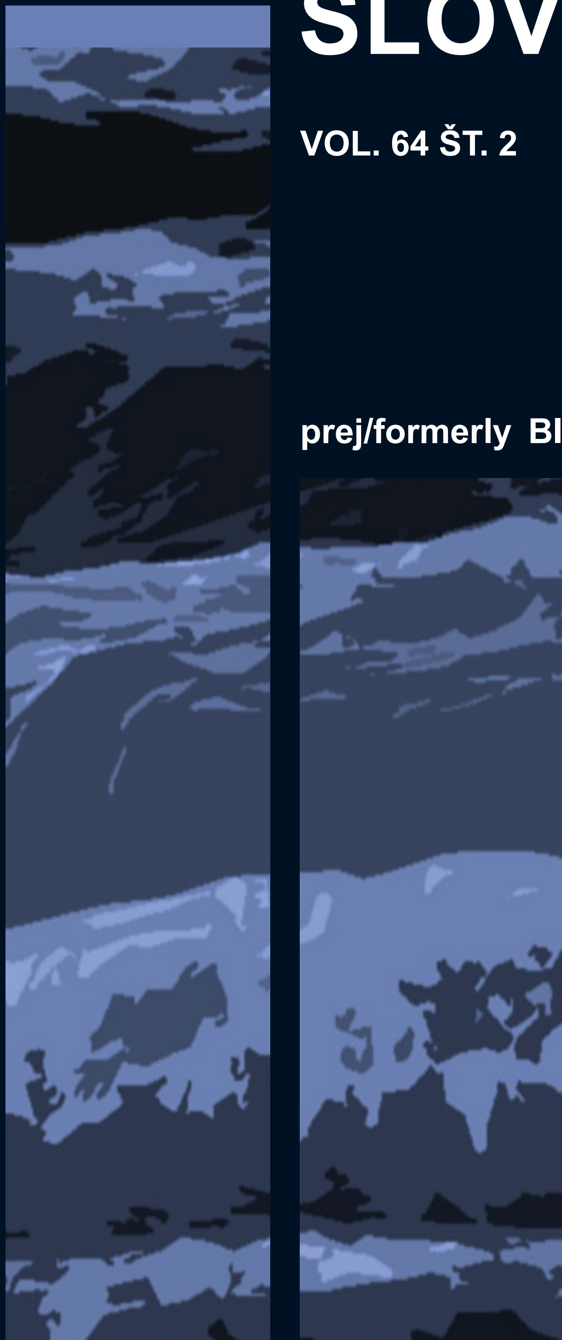

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ACTA BIOLOGICA SLOVENICA
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The issue of Acta Biologica Slovenica honouring Professor Jože Štirn

Številka revije Acta Biologica Slovenica posvečena prof. Jožetu Štirnu

Jadran Faganeli

Marine Biology Station, National Institute of Biology, Fornače 41, 6330 Piran, Slovenia

Correspondence: jadran.faganeli@nib.si

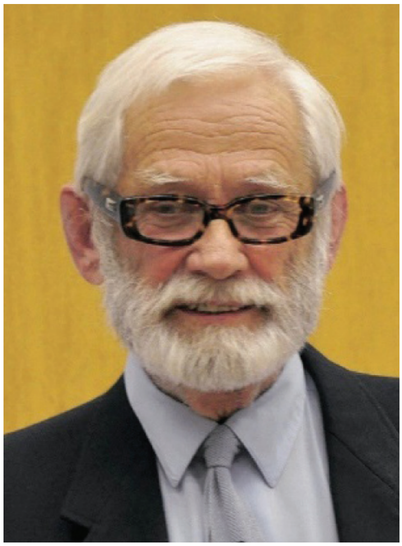

Figure 1: Professor Jože Štirn (1934-2011)
Slika 1: Profesor Jože Štirn (1934-2011)

This issue of Acta Biologica Slovenica is dedicated to the memory of Professor Jože Štirn, a retired senior scientist at the National Institute of Biology and retired Full Professor at the University of Ljubljana, and to honour his contributions to the field of marine phytoplanktology and the broader field of marine ecology. Professor Štirn passed away in August 2021; throughout his career as a researcher, teacher, mentor, colleague, and institute administrator, he greatly influenced the lives of all of those around him. We hope that this volume will serve not only as to honour the memory of Professor Štirn, but also as a way to recognize his major influences and contributions to the fields of coastal marine ecology and related marine pollution.

Professor Štirn received his BSc from the University of Ljubljana in 1965, and his Ph.D. from the University of Ljubljana in 1968, both in Biology. Before being awarded his BS, he founded the Centre for Underwater Research and organised in 1960-61 an echoed biological and diving expedition to Ethiopia and Red Sea. Later, he founded the first Slovene Marine Research Institution in Portorož. In 1968 he moved to the Department of Benthic Invertebrates at the Smithsonian Institution in Salambo (Tunis) and was an institution-member there until 1970. From that time onward he served as a head of the Marine Biology Station in the frame of the Institute of Biology of the University of Ljubljana. In 1980 he moved to IOC UNESCO as a head/member of the educational/research missions in Yemen, Cameroun and Aden. Professor Štirn was acting as a professor at the University of Nice in the period from 1988 to 1990 and later as an emeritus at the International University of Marine Studies in Cagne-sur-mer (France). He moved to the University of Sultan Qaboos in Oman in 1990. In Slovenia, he won awards (1972, 2012) for his research prowess and in 1992 he was elected an associate member of the European Academy of Environmental Affairs in Tubingen.

Professor Štirn was an internationally recognized scientist in the field of biological oceanography and marine ecology. He published approximately 50 peer-reviewed journal articles and book chapters. The majority of these papers were published in international journals and proceedings. Professor Štirn was one of the first to extensively study the pelagic system of the northern Adriatic Sea, including basic physical and chemical oceanographic data, markedly influenced by riverine nutrient input, particularly from the Po River. This work later resulted in his Ph.D. thesis published by the Slovene Academy of Science and Arts. Later, he began a complex research project on the biological, physical and chemical aspects of coastal waters in the northern Adriatic Sea and particularly in the Gulf of Trieste, including riverine inflows, affected by wastewater pollution. He was one of the Mediterranean leaders investigating the consequences of anthropogenic eutrophication and chemical pollution of coastal waters, particularly by metals and pesticides. Professor Štirn was interested in just about everything that touched on the impact of marine eutrophication in the Adriatic area. He co-organised and participated in several Trans-Adriatic research expeditions conducted by the Yugoslav RV Andrija Mohorovičić sixty years after the first Austrian-Italian Adriatic expedition. He was known for his innovative experimental approach and design particularly evident in the mesocosmos pollution experiment in the Lagoon of Strunjan. He was not only innovative but also meticulous with regards to his experimental and field endeavour. Due to his relevant scientific publications, UNEP/FAO engaged him to prepare a research project dealing with the ecological consequences of marine pollution in the Mediterranean Sea. Later, he did similar research in Oman studying biological oceanography and fishery biology of the Arabian Sea mostly in the frame of his Tethys project. A new genus of red algae *Stirnia prolifera* was dedicated to him. Professor Štirn was not only a good scientist and researcher, but also a teacher and mentor of young scientists. He organised (1971-74) the International Summer School of Marine Ecology in Strunjan sponsored by IOC UNESCO. He taught Oceanography, Marine Ecology and Fishery Biology at the University of Ljubljana for many years. His later educational endeavours were in universities in France and Oman. Professor Štirn served on numerous national and international committees, working groups, review panels and workshops over the years. He was a member of the editorial board of the Revue internationale d›océanographie médicale for many years.

His passion for science was equal to his enthusiasm for life. He had a well-known gift for storytelling and his ability to find something humorous in every situation. In describing Professor Štirn, he was a man of many talents: a naturalist, oceanographer, limnologist, marine phytoplanktologist and ecologist as well as scuba diver, and he could have been successful in almost any endeavour he undertook. The papers in this special issue were written by his colleagues and former students and cover a wide range of topics, many of which were central to the work he was involved in over his lifetime. The science contained in these papers is a reflection of his scientific legacy especially in the fields of biological oceanography, marine ecology and fishery biology of the northern Adriatic Sea, and in particular related to anthropogenic eutrophication of coastal waters.

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Short-term changes in microbial communities in the water column around the fish farm in the Bay of Piran

Kratkotrajne spremembe mikrobne združbe v vodnem stolpcu okoli ribogojnice v Piranskem zalivu

Valentina Turk*, Tinkara Tinta

Marine Biology Station, National Institute of biology, Fornače 41, 6330 Piran, Slovenia

*Correspondence: valentina.turk@nib.si

Abstract: A multidisciplinary approach was used to study the impact of fish farming on coastal bacterial communities in the inner part of the Bay of Piran (northern Adriatic). Differences in bacterial abundance, production and the occurrence of selected bacterial groups were studied in the water column around the cage and at different distances from the centre of the fish cage towards the open water, i.e., reference marine station. We also examined the effect of fish feeding on the surrounding system in a short-term *in situ* experiment based on the simultaneous collection of seawater samples from different locations around the fish cage before and after feeding of fish. Our study suggests that fish feeding has a moderate short-term effect on water column parameters, including bacterial abundance and production, only at a limited distance from the fish cages. The nitrifying, ammonia-oxidizing bacterial groups, as determined by the fluorescent *in situ* hybridization method, were represented at a higher percentage in the seawater samples in the middle and around the fish cages. β- *Proteobacteria*, γ-*Proteobacteria* and the *Cytophaga-Flavobacterium* group were represented to a higher percentage at sampling sites in the middle of the Bay of Piran and at the reference marine station. The *Vibrio* group was detected at all sampling sites. The accumulation of organically enriched fish food and waste products released into the seawater during the short-term experiment resulted in a significant increase in particulate matter, orthophosphate and ammonium. In response to the increase in inorganic nutrients, we observed a significant increase in bacterial production, while no significant differences were observed in bacterial abundance in such short time.

Keywords: aquaculture, bacterial abundance, bacterial community composition, bacterial production, fluorescent in situ hybridisation, pollution

Izvleček: Vpliv ribogojstva na bakterijske združbe smo proučevali z multidisciplinarnim pristopom v notranjem delu Piranskega zaliva (severni Jadran). Razlike v številčnosti bakterij, bakterijski produkciji in pojavljanju izbranih bakterijskih skupin smo preučevali v vodnem stolpcu okoli ribje kletke in na izbranih lokacijah od središča ribje kletke do odprtih vod. Spremembe pred in po hranjenju rib smo preučili tudi v kratkoročnem poskusu *in situ*, ki je temeljil na istočasnem vzorčenju morske vode na različnih lokacijah okoli ribje kletke. Naša študija kaže, da hranjenje rib kratkoročno zmerno vpliva na parametre vodnega stolpca, vključno s številčnostjo bakterij in produkcijo, in le na omejeni razdalji od ribjih kletk. Nitrifikacijske bakterije, ki oksidirajo amonij, določene s fluorescentno *in situ* hibridizacijsko metodo, so bile zastopane v višjem deležu v vzorcih morske vode v sredini in neposredni okolici ribje kletke. β-*Proteobakterije*, γ-*Proteobakterije* in skupina *Cytophaga-Flavobacterium* so bile zastopane v višjem odstotku na bolj oddaljenih vzorčnih mestih. Potencialno patogena vrsta *Vibrio* je bila prisotna na vseh mestih vzorčenja. Kopičenje organsko obogatene ribje hrane in odpadnih produktov, ki se sproščajo v morsko vodo v kratkem času po hranjenju, je povzročilo znatno povečanje koncentracij partikulatne organske snovi, ortofosfata in amonija. Kot odgovor na povečanje anorganskih hranil smo izmerili bakterijsko aktivnost, medtem ko razlik v biomasi v vodnem stolpcu ni bilo opaziti.

Ključne besede: akvakultura, bakterijska abundance, bakterijska produkcija, bakterijska vrstna sestava, fluorescentna in situ hibridizacija, onesnaženje

Introduction

In the last 20 years, aquaculture has expanded rapidly both globally and in the Mediterranean, with a focus on the production of sea bass *Dicentrachus labrax* and sea bream *Sparus aurata* (FAO 2018). The rapid expansion of aquaculture has raised increasing concerns about negative environmental impacts, given numerous well-documented cases (Bouwman et al. 2013). These are primarily the impacts of waste products from farming – organic matter, nutrients, chemicals and pharmaceuticals – and the transfer of genes, parasites and diseases between wild and farmed species (Hargrave et al. 1993, McGhie et al. 2000, Karakassis 2000, Kim et al. 2004, Armstrong et al. 2005, Bouwman et al. 2013, Cabello et al. 2013, Wang et al. 2020). The numerous documented negative environmental impacts of aquaculture are mainly related to the accumulation of uneaten feed and faeces in seawater and on the seabed beneath fish cages (Hargrave et al. 1997, Karakassis 2000, Pitta et al. 2006, Vezzulli et al. 2008, Reimers et al. 2013). Fish food consists largely of proteins and the main components of this organic enrichment affect biogeochemical processes and often lead to eutrophication, oxygen deficiency and hypoxia on the seabed (Hargrave et al. 1993, Karakassis 2000). The effects of high organic matter loading on sediment below aquaculture farms have been extensively studied, showing changes in the structure of benthic communities that affect the entire food web and lead to an overall decline in species diversity (Hargrave et al. 1997, Karakassis et al. 1998, Christensen et al. 2000, Grego et al. 2009, Mirto et al. 2012, Luna et al. 2013, Reimers et al. 2013).

Marine microbes drive biogeochemical cycles in coastal areas (Kirchman 1994). They regulate carbon and energy transfer in the food web, the maintenance of water quality, and the health of marine ecosystems (Azam and Malfatti 2007). Marine microbes respond rapidly to environmental perturbations (Galand et al. 2016, Ape et al. 2019) and play an important role in the degradation and remineralization of organic matter. Several studies have shown that organic wastes from fish farms stimulate microbiological metabolism, most of which can be attributed to changes in enzymatic activity (Caruso et al. 2003, Vezzulli et al. 2004), nitrification, denitrification or sulphate reduction (McCaig et al. 1999, Christensen et al. 2000, Asami et al. 2005, Kondo et al. 2012, Dowle et al. 2015) ammonium concentrations, nitrification rates, and ammonia oxidizer most-probable-number counts were determined in samples of sediment collected from beneath a fish cage and on a transect at 20 and 40 m from the cage. The data suggest that nitrogen cycling was significantly disrupted directly beneath the fish cage, with inhibition of nitrification and denitrification. Although visual examination indicated some slight changes in sediment appearance at 20 m, all other measurements were similar to those obtained at 40 m, where the sediment was considered pristine. The community structures of proteobacterial β-subgroup ammonia-oxidizing bacteria at the sampling sites were compared by PCR amplification of 16S ribosomal DNA (rDNA. Increases in bacterial abundance (Mirto et al. 2000, Vezzulli et al. 2002, Bissett et al. 2007), virus-like particles (Garren et al. 2008, Luna et al. 2013), and bacterial production (Navarro et al. 2008, Garren et al. 2008), as well as changes in bacterial community composition (Bissett et al. 2006, Garren et al. 2008, Castine et al. 2009, Kawahara et al. 2009, Quero et al. 2015, Galand et al. 2016, Martins et al. 2018, Kolda et al. 2020) have been detected in the sediments bellow fish cages.

Increased deposition of organic matter on the seabed below fish cages (Kovac et al. 2003, Lojen et al. 2005) has been confirmed for our study area and negatively affects the sediment chemical properties and the abundance and structure of meiofauna (Grego et al. 2009, 2020). The impact of aquaculture on microbes have been studied mainly in sediments, much less in the water column. In our study, we focused on the short-term effects of aquaculture activities on the bacterial community in the upper layer of the water column around the cage and at different locations from the centre of the fish cage to the open water. We mainly investigated the changes in nutrient concentrations, abundance and bacterial production before and after feeding the fish in a short-term *in situ* experiment based on the simultaneous collection of seawater samples from different sites around the fish cage.

Materials and methods

Study site and experimental setup

The study was conducted in October 2005 in the marine fish farm in the inner part of the shallow semi – enclosed Bay of Piran (Gulf of Trieste, northern Adriatic Sea) (Fig. 1). The bay is about 7 km long and 5 km wide and is characterised by limited hydrodynamics. The fish farm consisted of 20 floating cages with a total area of 2019 m^2, where sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus auratus*) were kept. The depth below the fish cages was about 13 m. The total annual production in 2005 was 100 tonnes. The fish were fed three times a day. The daily dietary intake was 12 kg, and consisted

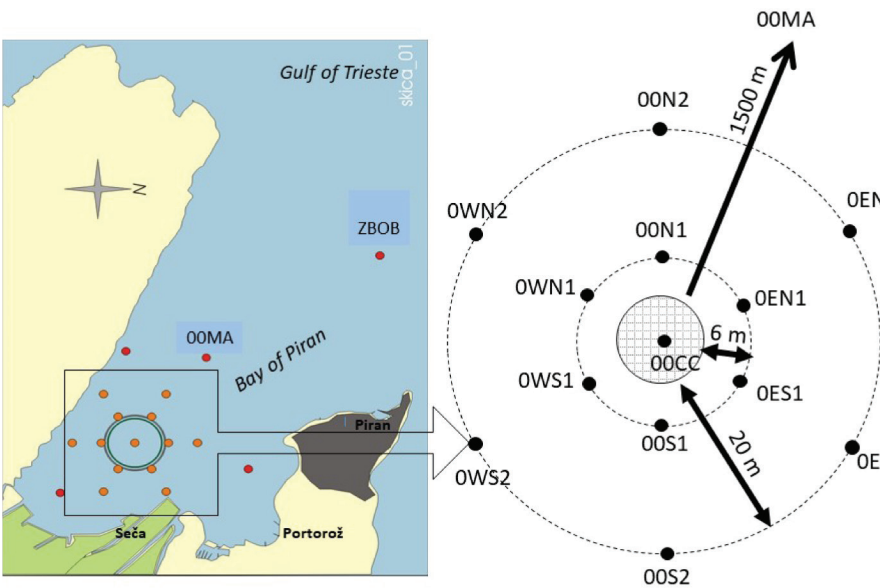

Figure 1: Sampling sites in the middle and around the fish cage at 6 and 20 m intervals, in the inner part of the Bay of Piran, and the reference station in the Gulf of Trieste (ZBOB).

Slika 1: Mesta vzorčenja morske vode v središču in okolici ribje kletke na razdalji 6 m in 20 m, v notranjem delu Piranskega zaliva, ter zunanji referenčni postaji v Tržaškem zalivu (ZBOB).

mainly of proteins (44 %) and lipids (22 %) and a smaller proportion of oligosaccharides (16 %), fibrins (2 %), ash (8 %) and vitamins, according to manufacturer.

To evaluate the impact of aquaculture on the pelagic bacterial community, two different studies were conducted. In the first study, seawater samples were collected at stations along the transect located 6 m, 20 m, 1500 m, and 5400 m from the centre of the fish cage (Fig. 1), at 5 m depth with Niskin samplers. At the same time, basic physical (temperature, salinity) and chemical parameters (total particulate matter, orthophosphate, total phosphorous and nitrogen, nitrate, ammonium) were analysed to determine the level of organic pollution.

For the second study, a system of anchored sampling bottles was constructed at different locations: in the centre of the cage (00CC), sampling points in a circle around the fish cage at a distance of 6 m (00N1, 0EN1, 0ES1, 00S1, 0WS1, 0WN1), and 20 m (00N2, 0EN2, 0ES2, 00S2, 0WS2, 0WN2) (Fig. 1). Each bottle (5 L) was attached to an underwater metal frame at 5 m depth. At the time of sampling, all bottles were opened simultaneously (by small boats and by divers). Seawater samples were collected at each sampling site before (bf) and 3 h after (af) fish feeding and the results for each parameter were expressed as the ratio $K= C_{bf}/C_{af}$ in percentage.

Oceanographic parameters and nutrient analyses

Basic oceanographic parameters (temperature, salinity) were measured using a CTD fine –scale probe (Microstructure Profiler MSS90, Sea & Sun Technology GmbH), and 24-hour current measurements were made (not presented in this study). Total particulate matter (TPM) was measured gravimetrically, and inorganic nutrients (nitrate, ammonium, phosphate) were measured colorimetrically (Grasshoff et al. 1983) using a UV/VIS spectrometer, Perkin Elmer, Lambda 14.

Bacterial abundance and production

Seawater samples for total bacterial counts were fixed with formaldehyde (2% final concentration) and analyzed by the method of Porter and Feig (1980). Samples were stained with the fluorochrome 4’,6’-diamino-2-phenylindole (DAPI, 1 µg mL^{-1} final concentration) and counted under an epifluorescence microscope (Olympus BX51).

Bacterial carbon production (BCP) was measured by incorporation of ^{3}H-leucine into newly synthesized proteins in bacterial cells (Simon and Azam 1989) using the centrifugation protocol of Smith and Azam (1992). Ultima Gold (PerkinElmer) was added to each sample and radioactivity was measured using a liquid scintillation counter (Canberra Packard TriCarb Liquid Scintillation Analyzer, model 2500 TR).

Table 1: List of oligonucleotide probes with sequence and target bacterial group.
Tabela 1: Seznam oligonukleotidnih sond s sekvenco in tarčno skupino bakterij.

Probe	Sequence	Target organisms
Nso 1225	5’- CGC CAT TGT ATT ACG TGT GA – 3’	nitrifying ammonia-oxidizing bacteria
NEU	5’- CCC CTC TGC TGC ACT CTA – 3’	nitrifying ammonia-oxidizing bacteria
EUBI,II,III	5’- GCT GCC TCC CGT AGG AGT – 3’	all bacteria
BET 42a	5’ – GCC TTC CCA CTT CGT TT – 3’	β-proteobacteria
GAM 42a	5’ – GCC TTC CCA CAT CGT TT – 3’	γ-proteobacteria
CF 319a	5’ – TGG TCC GTG TCT CAG TAC – 3’	*Cytophaga-Flavobacterium* group of CFB phylum
GV	5’ – AGG CCA CAA CCT CCA AGT AG – 3’	*Vibrio* group

Fluorescent in situ hybridization (FISH)

The abundance of selected phylogenetic groups was determined by the fluorescence *in situ* hybridization method using Cy3-labelled oligonucleotide probes (Thermo Electron Corporation). The sequences and target organisms of all 16S rRNA-targeted oligonucleotide probes are listed in Tab. 1.

Group specific probes were EUBI, II, III for all bacteria, BET 42a for β-*Proteobacteria*, GAM42 for γ-*Proteobacteria*, CF 319a *Cytophaga-Flavobacterium* cluster of *Cytophaga-Flavobacteria-Bacteroides* CFB phylum and GV for *Vibrio* group. The nitrifying, and ammonia-oxidizing bacteria groups were determined using the Nso 1225 probe specific for the ammonia-oxidizers and the NEU probe complementary to a signature region of most halophilic and halotolerant ammonia-oxidizers.

From each sampling station, 10 ml of sample was fixed overnight with 37% formaldehyde. 5 ml subsamples were filtered onto 0.2 µm polycarbonate filters (47 mm, Poretics) and rinsed with 3 x PBS, 1x PBS solution, and distilled water and stored at -20 ºC. For each probe, a filter piece was placed on a glass slide covered with Parafilm and overlaid with 20 µl hybridization probe prepared by mixing 2 µl probe and 18 µl hybridization solution. The hybridization solution contained 360 µl NaCl (5 M), 40 µl Tris-HCl (1M, pH 7.2-8), 2 µl 10% SDS, the optimal concentration of formamide, 400 µl blocking solution (Roche), and 500 µl distilled water. The filters were incubated in the hybridization oven at 42 ºC for 3h. After hybridization, filters were washed in wash solution (1000 µl Tris/HCl (1M), 700 µl NaCl (5 M), 500 µl EDTA, 25 µl 20% SDS and 47.8 ml distilled water) for 20 min at 48 ºC. Washed and dried filter pieces were stained with 1 µg mL^{-1} 4',6'- diamino-2-phenylindole (DAPI). Cells were counted by epifluorescence microscopy (Olympus BX51). The abundance of each bacterial group detected was expressed as the percentage of each group in the total bacterial count.

Statistical analysis

A three-way ANOVA was conducted to test the main interactive effects of bacteriological and chemical parameters (orthophosphate, total phosphate, ammonium, bacterial abundance and carbon production), feeding activity (before and after feeding), and sampling sites (distance – 0 m, 6 m, 20 m; and directions – south, north, east, west).

Table 2: The results (means and standard deviations) of total particulate matter (TPM), nitrate, ammonium, orthophosphate, total phosphorus (Tot P) and nitrogen (Tot N), bacterial biomass and bacterial carbon production rate (BCP) in seawater samples from different sites as a function of distance from fish cages.

Tabela 2: Rezultati (srednja vrednost in standardni odmik) celokupne partikulatne snovi (TPM), nitrata, amonija, ortofosfata, celokupnega fosforja (Tot P) in nitrogen (Tot N), bakterijske biomase in bakterijske produkcije (BCP) v vzorcih morske vode na različnih merilnih mestih glede na oddaljenost od ribiških kletk.

		Distance from the fish cage (m)				
Parameter	**Cage**	**6**	**20**	**200**	**1500**	**5400**
TPM (mg L^{-1})	2.40	2.12 ± 0.56	2.55 ± 1.79	2.09 ± 0.35	1.67	1.04
Nitrat (µM L^{-1})	0.95	0.33 ± 0.07	0.34 ± 0.12	0.38 ± 0.18	0.51	0.20
Ammonium (µM L^{-1})	0.68	0.59 ± 0.12	0.57 ± 0.04	0.82 ± 0.28	0.76	0.52
Tot N (µM L^{-1})	31.13	33.80 ± 4.86	31.72 ± 3.92	42.46 ± 9.20	45.68	37.01
Orthophosphate (µM L^{-1})	0.12	0.10 ± 0.01	0.11 ± 0.04	0.11 ± 0.01	0.15	0.11
Tot P (µM L^{-1})	0.32	0.29 ± 0.02	0.30 ± 0.04	0.29 ± 0.06	0.33	0.31
Bacterial biomass (µg C L^{-1})	43.95	53.21 ± 12.3	44.15 ± 5.62	37.38 ± 10.3	27.28	21.37
BCP (µg C $L^{-1}day^{-1}$)	10.31	9.27 ± 2.72	10.89 ± 2.26	1.65 ± 1.08	1.34	3.9

Results

Effects of fish farm activity on nutrient and microbial variables along the pollution gradient

Results of chemical and microbiological analyses of the upper layer of the seawater column did not differ significantly among sampling sites, although, higher concentrations of total particulate matter, nitrate, and bacterial carbon production were measured near the fish cage (Tab. 2).

Total particulate matter values ranged from 2.12 to 2.55 mg L^{-1} at sites near the fish cage and were twice the values measured at the site 5400 m from the fish farm. The highest nitrate concentration was at the cage site and was three times lower at all other sites (Tab. 2). Concentrations of ammonium, orthophosphate, total phosphorus (Tot P), and nitrogen (Tot N) in the upper layer of the water column did not differ among stations, or concentrations were slightly higher at the station in the middle of the Bay of Piran.

Bacterial carbon production differed significantly ($F=7.932$, $p < 0.001$) between sites and was on average up to 5-fold higher near the fish cage compared to values measured at the sampling site 1500 m away, and 3-fold higher compared to bacterial production measured at the reference site (Tab. 2, Fig. 2).

Similar trends were observed for bacterial abundance, but the difference was not statistically significant. The average bacterial abundance in the vicinity of the fish cage (0, 6, and 20 m) ranged from 2.4 to 3.1 x 10^9 cells L^{-1} and decreased with increasing distance from the cage. Bacterial abundance was more than twice as high at the fish farm as at the sampling sites at 1500 m and 5400 m (Tab. 2, Fig. 2).

Molecular analyses of targeted ribosomal RNA using the FISH method and various oligonucleotide probes revealed differences in the composition of bacterial groups in seawater along the transect (Fig. 3). Based on the literature data, we selected specific probes for the bacterial groups expected in an environment polluted by aquacultures.

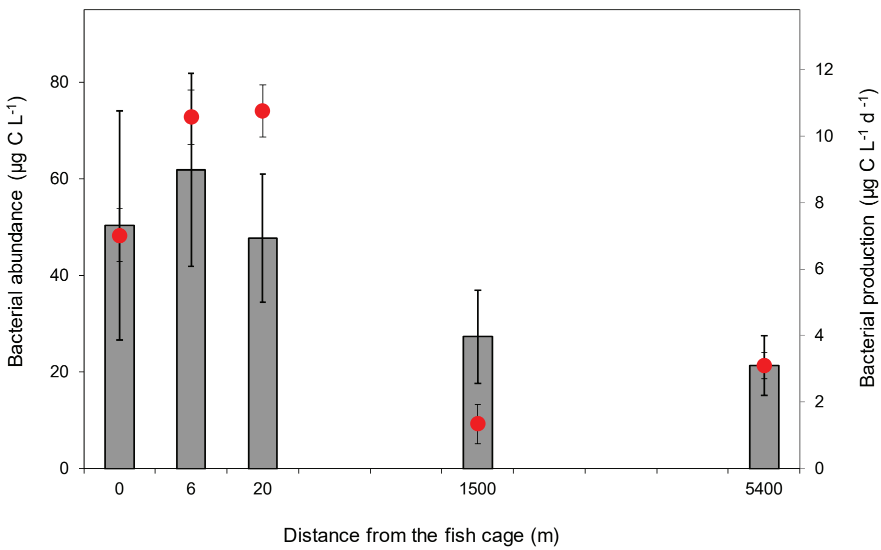

Figure 2: Bacterial biomass (■) and bacterial production (●) at sampling sites at different distances from the fish cages in the Bay of Piran.

Slika 2: Bakterijska biomasa (■) in bakterijska produkcija (●) na merilnih mestih različnih razdalj od ribjih kletk v Piranskem zalivu.

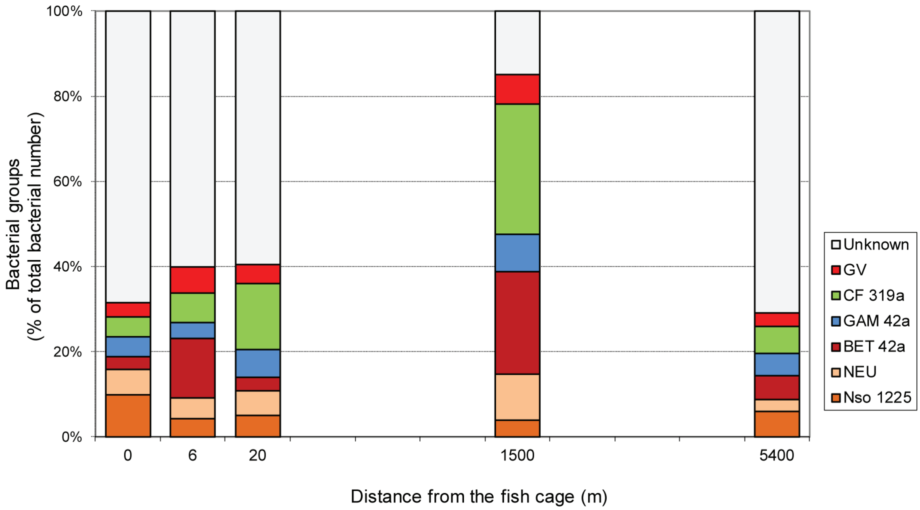

Figure 3: Percentage of bacterial groups determined by the fluorescent *in situ* hybridization method (FISH) at sampling sites at different distances from fish cages in the Bay of Piran.

Slika 3: Procent posamezne skupine bakterij določene z metodo Fluorescentne *in situ* hibridizacije (FISH) na merilnih mestih, ki so različno oddaljeni od ribjih kletk v Piranskem zalivu.

The various groups were represented differently. In the middle and immediate vicinity of the fish cage, all identified bacterial groups accounted for 30-40% of the total number of bacterial cells in the seawater samples, and the composition of the remaining portion was not determined. Within the identified groups, the nitrifying and ammonia-oxidizing bacterial groups Nso 1225 and NEU were represented with a higher percentage, 10 and 6 %, respectively. At other sampling sites, the β-*Proteobacteria*, γ-*Proteobacteria* and the *Cytophaga-Flavobacterium* group of the CFB strain (*Cytophaga-Flavobacteria-Bacteroides*) predominated (Fig. 3). At the sampling site 1500 m from the fish cage, the identified bacterial groups of all selected oligonucleotide probes accounted for 85 % of the total bacterial count. Compared to the other stations, there was a higher percentage of all bacterial groups, especially the β-*Proteobacteria*, and *Cytophaga-Flavobacterium* groups, except for the ammonia-oxidizers of the β-*Proteobacteria* subgroup (the Nso 1225 probe), which was represented with the highest percentage at station 00CC. At the reference marine site, all bacterial groups were evenly distributed, however accounted for only 30 % of the total bacterial count and most of the community composition was unknown, possibly due to untargeted bacterial groups of the α-*Proteobacteria* that may predominate in coastal, more oligotrophic waters. The *Vibrio* group (up to 6% of the total bacteria count) was detected at all sampling sites.

Effects of fish feeding on nutrient levels, bacterial abundance and production in the water column in a short period of time

The results of chemical and bacteriological analyses of seawater, sampled simultaneously at all sampling sites before and 3 hours after feeding, showed changes in the concentrations of several parameters. The results of the relative difference (%) of total particulate matter, orthophosphate and ammonium concentrations after fish feeding in relation to before fish feeding at the sites around the fish cage (in the middle, 6 m and 20 m away) are shown in Fig. 4.

With few exceptions, all values increased 3 hours after feeding compared with results before fish feeding. Overall, concentrations of total particulate matter, increased from 2.12 ± 0.5

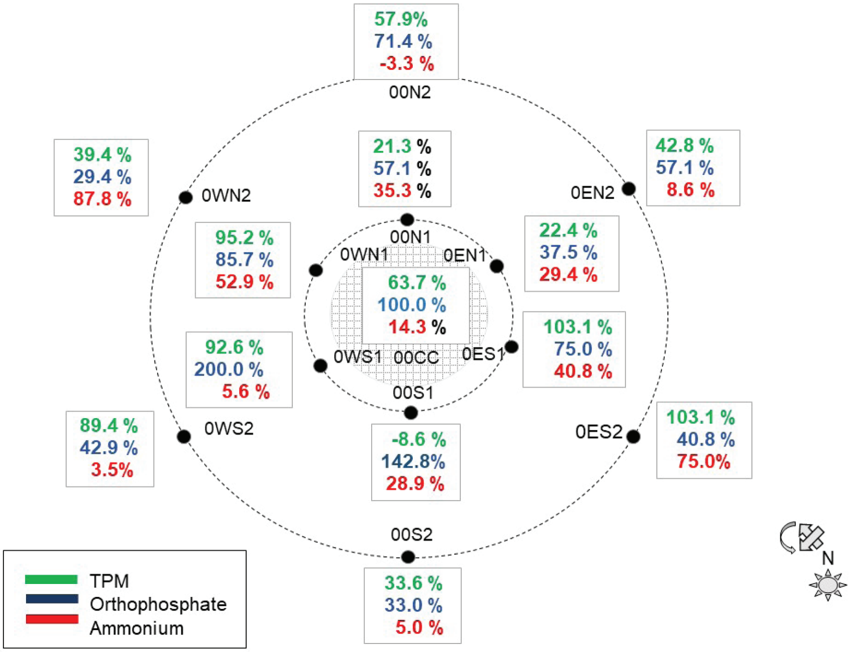

Figure 4: Results of relative difference (%) of total particulate matter (TPM) (green), orthophosphate (blue) and ammonium (red) concentrations after fish feeding in relation to before fish feeding at the sites around the fish cage (in the middle, 6 m and 20 m away).

Slika 4: Rezultati relativne razlike (%) v koncentracijah totalne partikulatne snovi (TPM), ortofosfata in amonija po hranjenju rib glede na vrednosti pred hranjenjem v okolici ribje kletke (v centru, 6 m in 20 m stran).

mg L^{-1} to 5.65 ± 1.1 mg L^{-1}. A similar trend was observed in the concentrations of orthophosphate, which doubled from 0.07 ± 0.01 µmol L^{-1} to 0.14 ± 0.04 µmol L^{-1}, ammonium increased from 0.59 ± 0.1 µmol L^{-1} to 0.77 ± 0.1 µmol L^{-1}, and nitrate from 0.33 ± 0.07 µmol L^{-1} to 0.77 ± 0.2 µmol L^{-1}. A three-way ANOVA showed a significant increase in concentrations of orthophosphate (F=25.09, $p < 0.0001$), total phosphorus (F=37.08, $p < 0.001$), ammonium (F=8.61, $p < 0.009$) after the feeding. But we found no significant difference between distance and direction of sampling sites.

During the experiment, bacterial carbon production increased at almost all stations around the cage at radius of 6 m and 20 m. The average rate of bacterial carbon production was significantly higher 3 hours after feeding (F=18.81, $p < 0.001$) within the 20 m radius and an overall increase from 9.66 ± 2.8 µg C L^{-1} d^{-1} to 14.54 ± 2.9 µg C L^{-1} d^{-1} was recorded. The highest increase was observed towards the south and southwest, up to 163% at the station 00S2 and 145% at station 0WS1 and in the middle of the cage up to 125% (at the station 00CC) (Fig. 5 A).

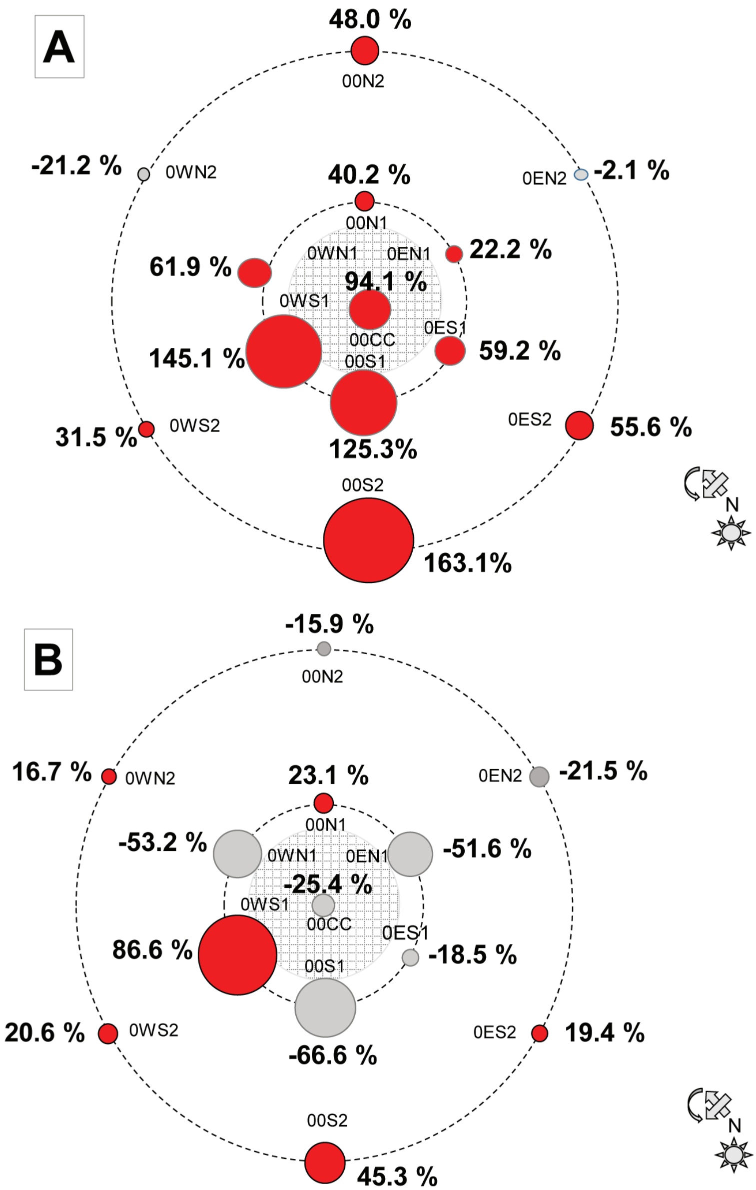

Figure 5: Relative difference (%) in bacterial carbon production **(A)** and bacterial biomass **(B)** measured before and after fish feeding at the sites around the fish cage (in the middle, 6 m and 20 m away). The size of the circle shows the increase (red color) or decrease (grey color).

Slika 5: Relativna razlika (%) v izmerjeni bakterijski produkciji ogljika **(A)** in bakterijski biomasi **(B)** pred in tri ure po hranjenju rib v okolici ribje kletke (v centru, 6 m in 20 m stran). Velikost kroga ponazarja % povečanja (rdeč krog) ali znižanja (siv krog).

Bacterial biomass, recalculated from bacterial counts using the 19.8 fg C cell^{-1} conversion factor (Lee and Fuhrman 1987), changed between sites, but no significant differences in concentrations were observed after feeding. Mean values decreased from 48.8 µg C L^{-1} ± 9.8 to 46.2 ± 19.7 µg C L^{-1}, showing an increase after feeding only at some sites in the southwest direction. No changes or even a slight decrease was observed at all other sites (Fig. 5 B). The production/biomass (P/B) ratio changed from 0.20 ± 0.06 to 0.38 ± 0.2 after feeding and was on average up to 4.5 times higher near the fish cages than at other sampling sites, reflecting higher daily biomass production.

Discussion

Fish farming changes the load of organic and inorganic nutrients according to the increase of organic wastes (uneaten food, faeces, soluble excreta), which potentially negatively affects the marine environment, especially shallow areas, gulfs and semi-enclosed bays. Our study suggests that fish farming has moderate impact on water column parameters, including bacterial abundance and production, but only at a limited distance from fish cages (< 20 m), while at other distances no effects were detectable using our approach. Direct effects of nutrient pollution from fish farms on water quality and secondary effects on production and bacterial communities have also been reported in previous studies in the region (Turk and Malej 2003) and in other coastal areas (Price et al. 2015, Šestanović et al. 2016). In this study, we only analysed the effects on the pelagic microbial community, while the effects of increased organic matter on the benthic microbial community were not analysed in detail. Uneaten food and faeces lead to the accumulation of a large amount of organic matter on the seabed below the cages in our study area (Lojen et al. 2005) since the sedimentation rates in the vicinity of the fish cages were significantly higher (eight times higher) than background values for Piran Bay (Kovac et al. 2003). Accumulation of organic and inorganic nutrients showed significant changes in redox potential and concentrations of chlorophyll *a* and phaeopigments in the sediment (Štrukelj 2008), as well as structure and abundance of meiofauna (Grego et al. 2009, 2020). The negative influence of fish farm on the phytoplankton and bacterioplankton in immediate proximity was confirmed with in situ experiment with incubation in dialysis bags (Štrukelj 2008).

In addition, we investigated the effects of feeding of the sea bass and sea bream on bacterial abundance and production in a short-term *in situ* experiment based on the simultaneous sampling of seawater from different sites around the fish cage before and after fish feeding (Fig. 5). Feeding fish with organically enriched fish food resulted in significant increases in total particulate matter, dissolved organic and inorganic nutrients in the seawater of the cage and surrounding area. Increased concentrations of ammonium and orthophosphate could have triggered intense bacterial metabolism, which might be reflected in the recorded increase of the bacterial production. The observed higher P/B ratio reflects higher daily biomass production near the fish cages as compared to other sampling sites, although no significant differences in bacterial abundance were observed over the 3 h period. Previous results from the P-limited northern Adriatic showed that dissolved phosphorus concentrations in fish farms were elevated in the water column compared to reference stations (Matijević et al. 2009), resulting in changes in abundance and production of phytoplankton and bacterioplankton (Šestanović et al. 2016). In our study area, correlations between the bacterial community and orthophosphate concentration have been documented (Sjöstedt et al. 2013, Malfatti et al. 2014, Tinta et al. 2015), as well as rapid turnover and uptake of orthophosphate (Turk et al. 1992). Similar effects of organic material input from fish farms on bacterial activity, especially enzymatic activity, have been described in different aquaculture areas (Vezzulli et al. 2002, Caruso et al. 2003).

The effects of dissolved wastes (inorganic and organic nutrients) depend on the rate at which these nutrients are diluted before being assimilated by the pelagic ecosystem. It is assumed that in cases where the flushing time (i.e., the time required to exchange the local water volume with new coastal water) is shorter than the typical microbial generation time and the risks of an increase in nutrient concentrations caused by the fish farming do not result in a measurable increase in local microbial biomass (Black 2001). Smaller pelagic organisms such as bacteria near fish farms have access to highly available organic nutrients and have short generation times (Kirchman 2016). Because the generation time of heterotrophic bacteria in the Bay of Piran ranges from 5 to 82 hours (Turk et al. 2020), we did not detect differences in bacterial abundance in our 3-hour experiment. Changes in the concentrations of inorganic and organic nutrients mainly affect bacterial production. During the field experiment currents near the fish cage showed north-westerly to south-westerly direction with a velocity of 7 cm s^{-1} (Malačič and Forte 2003), which could influence the higher concentrations of inorganic nutrients and bacterial production at sampling sites located in this direction.

The method of fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes is widely used for cultivation-independent identification, quantification, and visualization of microbes in different environments (Amann et al. 1990). Nitrifying bacteria are particularly difficult to culture, because they have a long generation time and poor counting efficiency. Analysis with oligonucleotide hybridization probes specific for different ammonia-oxidizing bacteria (probes Nso 1225 and NEU) showed that at least two different species of ammonia-oxidizing bacteria were present with a higher percentage in the seawater samples in the middle and around the fish cage. The importance of nitrifying bacteria in the context of fish culture has been studied previously (McCaig et al. 1999, Bissett et al. 2006). McCaig et al. (1999) demonstrated the importance of sediment contamination in fish farms on nitrification rates and the composition of ammonium-oxidizing bacteria. Using 16S rDNA clone library analysis, Bissett et al. (2006) demonstrated the presence of ammonium-oxidation *Nitrospira* associated clones and differences in the microbial community as a function of sediment pollution level. The groups of β-*Proteobacteria*, γ-*Proteobacteria* and *Cytophaga – Flavobacterium -Bacteriodes* reached a higher percentage at more distant sampling sites. The importance and seasonal changes in these bacterial communities has been demonstrated in recent studies but only on benthic communities using next -generation sequencing methods (Garren et al. 2008, Fodelianakis et al. 2014, Quero et al. 2015, Martins et al. 2018, Duarte et al. 2019, Ape et al. 2019, Kolda et al. 2020, Roquigny et al. 2021). Species belonging to the genus *Vibrio* are heterotrophic bacteria that increases rapidly when nutrients are available (Thompson et al. 2006). Some *Vibrio* species are known pathogens of fish (Austin and Austin 2016), and the presence of *Vibrio* near the fish cages could be an important reservoir for fish pathogens that could spread over large geographic areas.

Conclusions

Given the rapid development of aquaculture, concerns remain about the environmental impact of intensive aquaculture on water and sediment quality, particularly in shallow and semi-enclosed bays. Our study suggests that aquaculture has moderate effects on water column parameters, and can cause changes in bacterial abundance, production and community composition, but only at a limited distance from fish cages. However, we did not follow change in the sediment where in fact these effects might be more pronounced. We also confirmed that concentrations of organic and inorganic nutrients change in a relatively short time after fish feeding, which affect the bacterial production. Changes in bacterial activity and community composition can lead to changes in biogeochemical cycling and ecological status of the environment. Sedimentation of organic matter has a strong impact on sediment quality and benthic community, but changes in the concentration of organic and inorganic nutrients in the water column, as well as microbial abundance, production, and community composition in a relatively short time are not negligible. Preliminary fluorescence *in situ* hybridization results indicated changes in abundance of certain bacterial groups in response to pollution, as well as the presence of bacteria pathogenic to humans and fish. In the future, seasonal changes and long-term consequences should be investigated in the water column as well as sediment, to better understand changes in microbial community structure, and biogeochemical processes.

Povzetek

Dokumentirani številni negativni vplivi marikulture na okolje so povezani predvsem s kopičenjem ne-zaužite hrane in ekskrecijskih produktov v morskih vodi in na/v sedimentu. Rezultati naših meritev prostorskih razlik števila in hitrosti rasti populacije heterotrofnih planktonskih bakterij, kot tudi analize vsebnosti hranilnih snovi, so pokazali povišane vrednosti merjenih parametrov v bližini ribogojnice, ki pa se z oddaljenostjo od vira onesnaženja zmanjšujejo. Rezultati analize sestave bakterijskih združb z metodo fluorescentne *in situ* hibridizacije in izbranih bakterijskih oligonukleotidnih sond so pokazali prisotnost amonij-oksidirajočih bakterij, β-*Proteobacteria*, γ-*Proteobacteria* and *Cytophaga – Flavobacterium* kot tudi potencionalno patogenega seva *Vibrio*. Kopičenje organsko obogatene ribje hrane in odpadnih produktov, ki se sproščajo v morsko vodo znotraj ribje kletke in okolici v relativno kratkem času po hranjenju, je povzročilo znatno povečanje koncentracij partikulatne organske snovi, ortofosfata in amonija. Kot odgovor na povečanje anorganskih hranil smo izmerili znatno povečano bakterijsko produkcijo, medtem ko razlik v številu bakterij ni bilo opaziti.

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Status of the invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) in Slovenia

Status invazivne tujerodne modre rakovice *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) v Sloveniji

Lovrenc Lipej[a*], Manja Rogelja[b]

[a] Marine Biology Station, National Institute of biology, Fornače 41, 6330 Piran, Slovenia
[b] Aquarium Piran, Academic, Electronics and Maritime High School, Bolniška 11, 6330, Piran, Slovenia

*Correspondence: lovrenc.lipej@nib.si

Abstract: Authors present data regarding the presence of the invasive blue crab *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) based on records gathered from local fishermen. This alien species was firstly recorded in Slovenian coastal waters in March 2019 and it was subsequently found in six other cases in 2019, 2020 and 2021. Crabs were collected or sighted in shallow coastal areas along the Slovenian coast. In addition, authors discussed about the status of the blue crab in marine and coastal habitats of Slovenia.

Keywords: Adriatic Sea, *Callinectes sapidus* Rathbun, 1896, invasive species, presence, Slovenia

Izvleček: Avtorja poročata o prisotnosti modre rakovice *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) na podlagi podatkov, pridobljenih s strani lokalnih ribičev. Prvič smo to tujerodno vrsto v slovenskem obrežnem morju potrdili marca 2019, potem pa je bila zabeležena še v šestih primerih v letih 2019, 2020 in 2021. Rakovice so bile v vseh primerih ulovljene ali opažene v plitvem obalnem morju vzdolž slovenske obale. Avtorja nadalje razpravljata o statusu modre rakovice v obmorskih in morskih življenjskih okoljih v Sloveniji.

Ključne besede: *Callinectes sapidus* Rathbun, 1896, invazivne vrste, Jadransko morje, prisotnost, Slovenija

Introduction

The blue crab *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae), is considered an economically important species throughout its native area of distribution (Nehring 2011) which comprises the western Atlantic coast from Nova Scotia to Argentina (Manfrin et al. 2016). Its population supports intensive fishery, which currently supplies over one-third of all US commercial blue crab landings (Miller et al. 2011). It is characterized by early maturity, rapid growth and high reproductive potential, strong swimming capacity and aggressive behaviour and is able to efficiently exploit available resources in euryhaline and eurytherm environments (Piras et al. 2019). Nowadays, the blue crab is rapidly spreading its distribution worldwide (Mancinelli et al. 2021). During the last decades an expansion of the blue crab has been witnessed in almost all Mediterranean countries (Galil 2011, Cerri et al. 2020). The blue crab is considered as one amongst the most invasive crab species (Streftaris and Zenetos 2006). Up to date it was reported in different habitat types, mainly in coastal lagoons, estuaries and coastal sea. The first record of this species in Europe originated from harbour of Rochefort in France in 1900 (Bouvier 1901). The first evidenced and documented record in the Mediterranean Sea was reported by Giordani Soika (1951) in the Lagoon of Marano and Grado in 1949, however, there are some findings showing that it may arrived already in the 1937 in the Aegean Sea (Nehring 2011). Since then the crustacean started emerging in different areas of the Mediterranean Sea (Cerri et al. 2020). The invasion of the blue crab is aided by warming sea temperatures (Nehring 2011). During the last two decades the records of blue crab specimens have been reported from different localities along the Adriatic coast in its southern (Onofri et al. 2008; Beqiraj and Kashta 2010, Dulčić et al. 2010, 2011, Cilenti et al. 2015), middle (Florio et al. 2008; Castriota et al. 2012) and northern parts (Scaravelli and Mordenti 2007, Manfrin et al. 2016). However, the species is considered established only in southern Croatia, northern Albania and southern Italy (Castriota et al. 2012).

Some authors mentioned different vectors as reponsible of Mediterranean introduction of the blue crab, such as dispersal of larvae by ballast water (Nehring 2011, Garcia et al. 2018) or by natural spreading due to its swimming ability (Galil et al. 2002). As it is a highly valued seafood, the possibility of an intentional introduction for aquaculture reasons could not be neglegted, as well (Giordani-Soika 1951, Nehring 2011).

The aim of this contribution is to present all available data regarding the presence of the invasive alien crab in marine and coastal areas of Slovenia and to discuss its current status in the area.

Material and methods

Blue crab specimens were obtained by local fishermen who caught them in fishing nets or photographed them *in situ*. Blue crabs were captured accidentally from March 2019 to October 2021 in the Slovenian coastal waters. The collected crab specimens were measured by Vernier calliper to the nearest millimetre and weighed with digital balance. The biometric parameters which were measured were the carapace length (CL), carapace width (CW), abdomen length (AL), abdomen width (AW), propodus length (PL), dactylus length (DL), cheliped length (ChL), cheliped width (ChW) and cheliped height (ChH), and rostrum width (RW). The gender was easily assessed through the colour pattern and abdomen characters of specimens. According to the classification of Harding (2003), where blue crabs are divided in three size categories, e.g. small crabs if CW is lower than 80 mm, medium crabs, when CW range from 80 – 120 mm and large crabs, when CW is bigger than 120 mm.

Results and discussion

Specimens were collected at seven different localities (Tab. 1, Figs. 1, 2), which were all characterized by their shallowness and muddy bottom. The earliest record of the blue crab originates from Seča (Fig. 2a), in front of the Jernej's canal on 15th March 2019 (Lipej and Rogelja 2021). Altogether 12 specimens were recorded, among which were 10 females and two males. Six specimens (5 females and one male; Fig 3) were collected, while another six specimens were sighted and photographed on 28 July 2020 (Table 1). All specimens were found in the period from March to October. The carapace width ranged between 159.5 mm to 182 mm, while their weight ranged between 173 g and 402 g (Tab. 2). Therefore, all individuals recorded in Slovenia were classified as large sized specimens.

Unlike in male blue crab, sexual maturity in females is established at the terminal moult, during which the coloration of the abdomen darkens and its shape changes from triangular to semi-circular presumably to provide a larger surface area for brooding eggs (Van Engel 1958,

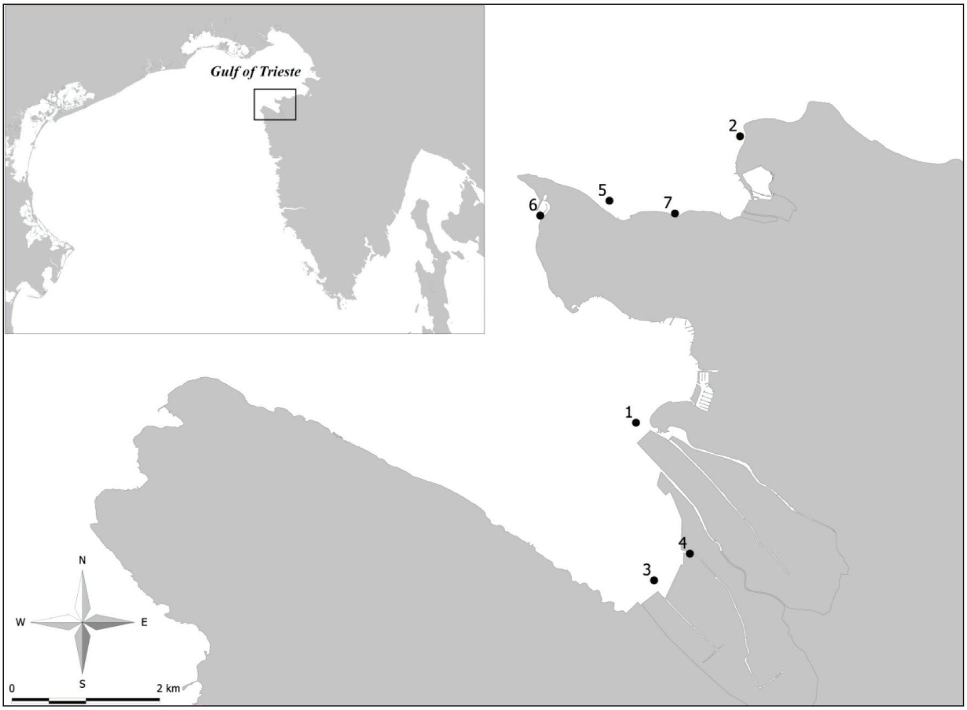

Figure 1: Map of the Slovenian coastal sea and adjacent waters of the Gulf of Trieste. Black points denote the records of the blue crab in the area. Legend: 1. Jernej's channel, Seča, 2. Strunjan, 3. In front of the Sečovlje Salina, 4. Chanell in the Sečovlje salina, 5. Fiesa, 6. Piran and 7. Pacug.

Slika 1: Zemljevid slovenskega obalnega morja in okoliških voda. Črne pike označujejo lokalitete, kjer so bile potrjene modre rakovice. Legenda: 1. Jernejev kanal, Seča, 2. Strunjan, 3. Pred Sečoveljskimi solinami, 4. Kanal v Sečoveljskih solinah, 5. Fiesa, 6. Piran in 7. Pacug.

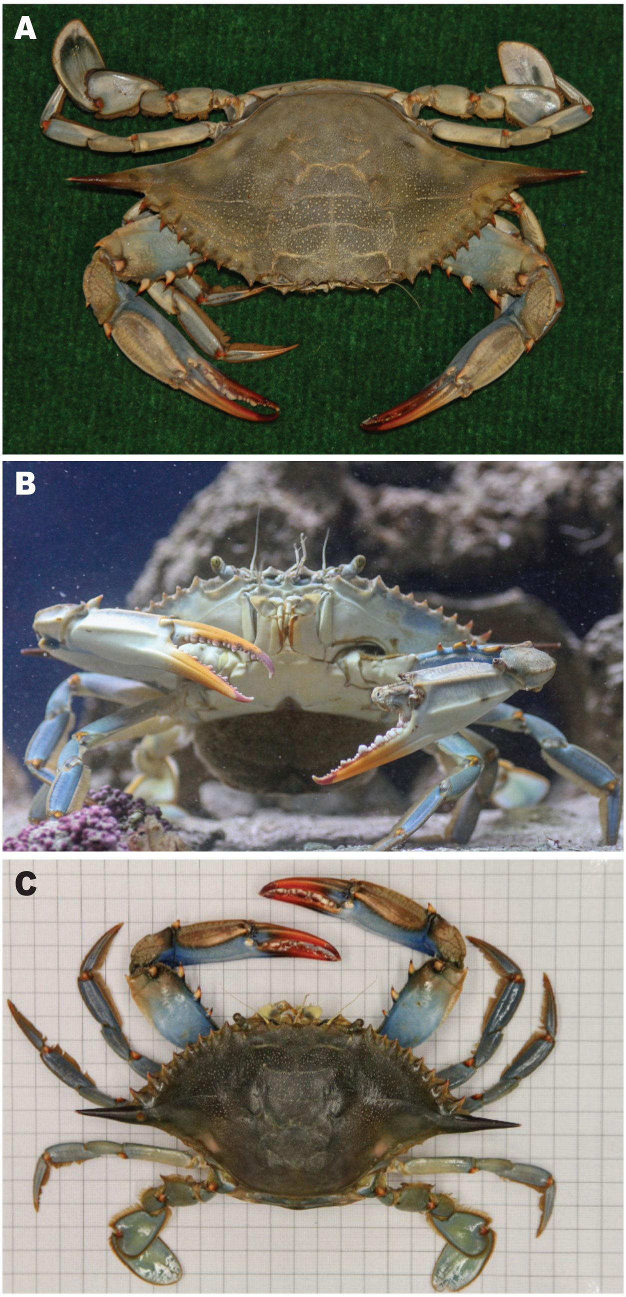

Figure 2: Blue crabs, recorded in the marine waters of Slovenia. (A) - female blue crab, captured on 15 March 2019, (B) - An ovigerous female, recorded on 9 April 2020, (C) - female, captured recorded on 16 October 2019.

Slika 2: Modre rakovice, ulovljene v slovenskih morskih vodah. (A) - samica, ujeta 15 marca 2019, (B) - ovigerna samica, ujeta 9. aprila 2020, (C) - samica, ujeta 16. oktobra 2019.

Figure 3: A male specimen of the blue crab, captured at 14 October 2021 in Pacug.
Slika 3: Samec modre rakovice, ujet 14 oktobra 2021 v Pacugu.

Jivoff et al. 2007). Based on these characteristics all five collected females are considered as sexually mature. Females have the only opportunity to mate immediately after the pubertal moult. They store the spermatophores containing sperm and can therefore produce multiple broods over several years, reaching 1 – 3 broods in temperate waters and up to 7 in tropical waters per year (Hines et al. 2003). The female collected on 9th April 2020 (Fig. 2b) and kept in the tank developed an egg mass beneath its aprons that was firstly noticed on 9th July 2020. The eggs were already brown and got darker in the period of four days, showing that the egg development was reaching its final stage. The egg mass was not noticed before as the female was less active and buried in the sand during the day. On 13th July the female was preventively translocated to a quarantine tank where it was kept until the release of larvae, so no accidental escape of larvae to the sea could be possible. On 17th July the female was returned to the aquarium in the public area.

The majority of specimens were caught in shallow waters on sedimentary bottom or in estuaries and channels. As an opportunistic species the blue crab is able to tolerate wide oscillations of temperature and salinity, typical conditions of brackish waters and adjacent eurytherm and euryhaline habitats (Piras et al. 2019). Such environment is characterized by low number of native species and seems to offer many non occupied ecological niches (Nehring 2006, 2011). Brackish waters are also in general known as environments where alien species are very successful in term of colonisation (Paavola et al. 2005).

The first record of *C. sapidus* in the northernmost part of the Adriatic Sea originated before seventy years (Giordani Soika 1951), however, only recently new findings were discovered in the area (Manfrin et al. 2016). Such records were reported only during the past five years. It is reasonable to speculate that the blue crab arrived in Slovenian waters from the Italian part of the Gulf of Trieste where it was recently discovered in Marano and Grado Lagoon (Manfrin et al. 2016). The second possibility is that the species came from the south, *i.e.* from Istria, where it is considered as frequent from Savudrija to Pula (Sladonja, personal communication), although there are no published records in that regard. However, Gljuščić (2019) in his Master thesis mentioned a case of the blue crab finding in the vicinity of Šćuza lagoon, near Pomer bay in southern Istria. The third possibility is that the blue crabs recruited to the Gulf of Trieste and Slovenian waters as settled larvae from ship's ballast waters (Manfrin et al. 2016).

Table 1: Occurrence of the blue crab in Slovenian coastal waters.
Tabela 1: Pojavljanje modre rakovice v slovenskih obalnih vodah.

			GPS position		Depth	
N	**Date**	**Locality**	**N**	**E**	**(m)**	**Ind.**
1	15.03.2019	Jernej's canal	45.50055556	13.58416667	6	1♀
2	16.10.2019	Strunjan	45.535630	13.601313	5	1♀
3	09.04.2020	Portorož	45.48138889	13.58777778	3	1♀
4	28.07.2020	Sečovlje salina	45.48472222	13.59388889	1	5♀,1♂
5	13.09.2021	Fiesa	45.52750000	13.57888889	9	1♀
6	03.10.2021	Piran	45.52555556	13.56694444	4	1♀
7	14.10.2021	Pacug	45.526111	13.590278	3	1♂

Taking into account recently published data of Benabdi et al. (2019) and Shaiek et al. (2021) the occurrence of *C. sapidus* is so far confirmed in 18 out of the 23 countries surrounding the Mediterranean Sea. Since the species was recently confirmed also in Slovenian waters (Lipej and Rogelja 2021), Slovenia represents the 19th country. On the basis of regular monitoring of non-indigenous species in marine waters of Slovenia up to date at least 57 non-indigenous species were reported in the Slovenian part of the Adriatic Sea (Orlando Bonaca et al. 2020, Mavrič et al. 2021). Among them the blue crab is the only alien crab. Since in the close proximity other alien crab species were recorded, such as *Callinectes danae* (Mizzan 1993), *Dyspanopeus sayi* (Mizzan 1995) and *Rhitropanopeus harrisii* (Mizzan and Zanella 1996) from the northern Adriatic lagoons, *Hemigrapsus sanguineus* from waters off Rovinj (Schubart 2003) and *Eriocheir sinensis* (Bettoso and Comisso, 2015) from the Lagoon of Grado and Marano, some of them could be recorded in Slovenia in nearby future.

According to some authors (Nehring 2011) the blue crab may benefit from global warming. It has been hypothesized that as global temperatures rise, blue crabs will probably experience a northward range expansion (Johnson 2015), which facilitate their colonisation and dispersion in new environments (Nehring 2011).

Although the blue crab was recorded in three successive years in Slovenian coastal waters it is still not possible to consider the species as an established one. In fact, all findings were recorded in the warmer period of the year. Due to the low winter temperatures, the species is probably not able to survive such harsh conditions occurring in winter. However, there are some evidences from the estuary of the Neretva river where "muddy" individuals, which may emerged after overwintering buried in the sediments, were observed (Mancinelli et al. 2016).

In its native area, the Chesapeake Bay, the average winter temperatures on the sea bottom reach 3.4 °C. Rome et al. (2005) showed that significant mortality may not occur unless water temperatures drop significantly below 3° C for an extended period of time. Since the average winter temperature in the Slovenian sea is higher, with the average in the coldest month of February 7.3° C and minimal 6.3° C (ARSO, 2019), the blue crab could be able to over-winter in the studied area.

The arrival of this invasive alien crab in the waters off Slovenia was expected since it was previously recorded in the immediate vicinity at Italian sites (see Manfrin et al. 2016) and could be attributed to higher water temperatures. Although only few records have been reported to us so far, the local occurrence and the further dispersion of this species deserve to be cautiously monitored,

Table 2: Biometrical parameters, measured on the collected specimens of blue crab, recorded in the coastal waters of Slovenia. All measurements are in millimetres.
Tabela 2: Biometrični parametri, izmerjeni na zbranih primerkih modre rakovice, ulovljenih v slovenskih obalnih vodah. Vse meritve so izražene v milimetrih.

Date	**15. 03. 2019**	**16. 10. 2019**	**9. 04. 2020**	**13. 09. 2020**	**3. 10. 2021**	**14. 10. 2021**
Sex	♀	♀	♀	♀	♀	♂
Weight (g)	173	234	188	287	189	402
Carapace width	182	186	174	170.5	159.5	172
Carapace length	68.5	73.5	69	74	65	78.5
Rostrum width	36	38	36.1	45	39	46.5
Abdomen length	51	50	49	47	47	55
Abdomen width	55	55	54	55.5	52	54.5
Right chelipod length	74	82.5	75.5	83.9	72	112.1
Left chelipod length	76	80	77.5	80	63	109
Right propodus length	33	36	30	35.5	31.5	37.5
Left propodus length	31	33.5	32	33	26.5	44.2
Right chelipod height	24.4	26.5	20	30.3	15	34.5
Left chelipod height	20.5	22.5	23	23	18.5	30
Right dactylus length	39.5	41	38	43	34.8	34
Left dactylus length	39	40	-	42	33.1	55.2
Right chelipod width	17.5	19	15	20.8	18	25
Left chelipod width	16	17	16.5	18	13	23

especially in order to gain data on the functional role that this taxon could play within benthic food webs in Mediterranean coastal habitats. According to Mancinelli et al. (2017) the blue crab can prey on a wide spectrum of native crab and fish or compete with them, which could inflict potentially high ecological impacts on the native biota. Since in its native area of distribution the blue crabs plays a major role in energy transfer within the food web of estuaries and lagoons, it may also be responsible for certain impacts in the invaded environments.

Povzetek

Modra rakovica *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae) je med najbolj invazivnimi vrstami rakovic, ki so jo doslej potrdili v 19 od 23 sredozemskih držav. Prvič smo to tujerodno vrsto v slovenskem obrežnem morju potrdili marca 2019, potem pa je bila zabeležena še v šestih primerih v letih 2019, 2020 in 2021. Skupaj je bilo popisanih 12 primerkov, od katerih je bilo 10 samic in dva samca. Vse najdbe pa izvirajo iz toplejšega dela leta. Rakovice so bile v vseh primerih ulovljene ali opažene v evrihalinih in evritermnih življenjskih okoljih v plitvem obalnem morju vzdolž slovenske obale. Gre za oportunistično vrsto, ki je zmožna tolerirati velika nihanja temperature in slanosti, ki

so značilna za brakične vode. Premer karapaksa rakovic je bil med 159.5 mm in 182 mm, telesna masa pa je bila med 173 g and 402 g. Čeprav je bila modra rakovica doslej najdena le na manjšem številu lokalitet, je njeno pojavljanje in razširjanje smiselno spremljati, še posebej z vidika pridobivanja podatkov o funkcionalni vlogi, ki jo ima ta vrsta v pridnenem prehranjevalnem spletu sredozemskih obrežnih habitatov.

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ABS ACTA BIOLOGICA SLOVENICA
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Umetni podvodni grebeni in umetnost možnega

Artificial reefs and the art of the possible

Tom Turk

Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, Večna pot 111, 1000 Ljubljana, Slovenija

Korespondenca: tom.turk@bf.uni-lj.si

Izvleček: V številnih evropskih državah so umetni podvodni grebeni (UPG) dobro sprejet način za povečevanje lokalne biodiverzitete. Ideja o umestitvi UPG na dno slovenskega morja je stara 25 let. Ta ideja še vedno ni realizirana, kljub jasnim dokazom, da imajo UPG pozitiven vpliv na lokalno morsko okolje in biocenozo, kar nam sporočajo izkušnje z italijanske strani Tržaškega zaliva. Zaradi različnih vzrokov tako na uresničitev postavitve prvega UPG v slovenskem morju še vedno čakamo. Prepričani smo, da bi bile take potopljene strukture pomemben substrat za naselitev (repopulacijo) morskih habitatov z bentoškimi vrstami, ki zaradi občasnih hipoksij in anoksij na morskem dnu množično poginjajo.

Ključne besede: bentos, biocenoza, hipoksija, repopulacija, umetni podvodni greben

Abstract: In many European counties artificial underwater reefs (AUR) are well established means to increase local biodiversity. In Slovenian part of Adriatic Sea the idea of establishing an artificial reef system was first brought to light 25 years ago. So far, despite strong evidence on the benefits to the local environment and biocenosis brought by AUR in the Italian part of Gulf of Trieste, due to various reasons we are still waiting for the first AUR in Slovenian part of Northern Adriatic Sea. We believe that such a structure would be an important center for the repopulation of local habitats after mass mortality due to the hypoxic or anoxic conditions that occasionally occurs at the benthic communities.

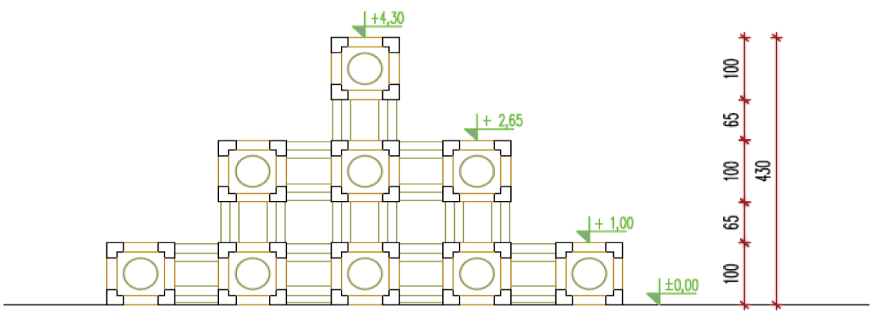
Keywords: artificial underwater reef, benthos, biocenosis, hipoxy, repopulation

Uvod

Ker je ta prispevek namenjen spominu na pokojnega prof. dr. Jožeta Štirna, naj ga začnem kar z njegovo mislijo, ki jo je izrekel v intervjuju za *Sobotno prilogo* pred 12 leti: »*Nenavadno je, da se morskim grebenom upira veliko biologov, češ da to ne bi prineslo nič za biodiverziteto. To je seveda nesmisel. Slovenski obali manjka ravno globoka skalna obala, ki omogoča najbolj pestra naselja pridnenih organizmov in rib. Vsak ribič ve, da se ribe zbirajo in zadržujejo ob grebenih ali pod večjimi in zasidranimi plavajočimi predmeti. V Jemnu sem spremljal projekt FAO, pri katerem so v morje metali lesene plavajoče kolute in čez dobro leto so imeli okoli njih toliko tunov, da si lahko samo gledal. Še na Kon Tikiju so vedeli, da ribe iščejo senco in zatočišče pod splavom. Nasprotovati podvodnim grebenom je nonsens. Dokaznega gradiva je na vseh koncih sveta dovolj,* *da bi že morali verjeti. V biologiji ne zadostuje, da poznaš eno samo vrsto. Moraš gledati celoto. Da ne govorim o ekologiji. Pri današnjih ekologih je tudi veliko enosmernega razmišljanja.«*

Ugotovitve v tem besedilu so del projektne študije o umestitvi enega samega pilotnega umetnega podvodnega grebena UPG v slovensko morje kakor tudi zapisanega v enem od dokumentov v postopku pridobitve vodnega soglasja za postavitev UPG, ki ga je od izvajalca projekta zahteval MOP (Turk 2020). Načrt za postavitev UPG je izdelal zavod Populus v skladu z idejo Zavoda za trajnost morske krajine /Institute for Sustainable Seascape po načrtih krajinske arhitekte dr. Darje Marinček Prosenc (Marinček Prosenc, Mladenović, Ljubej, 2020). Prvotno je bilo načrtovanih več UPG na različnih lokacijah, nazadnje je ostala le ena sama v neposredni bližini desne kanalizacijske cevi približno 1 navtično miljo od rta Madone (Piranska punta) na globini 23 m. Prav tako se je prvotni tloris grebena z 13.6 x 13.6 m zmanjšal na 7.6 x 7.6 m, višina grebena pa iz 7.6 m na 4.3 m. Načrtovani greben bi sestavljali kvadratni votli betonski bloki povezani z mozniki. Greben naj bi utrjevala bazaltna armaturna mreža, betonski elementi grebena pa bi bili zgrajeni iz morju prijaznega naravnega betona s pH vrednostjo 9. Tako načrtovan UPG bi nudil dovolj skrivališč za ribe in rake in dovolj površin za naselitev pritrjenih bentoških organizmov. Razmiki med votlimi kockami in mozniki so dovolj veliki, da bi omogočali zadostno strujanje morske vode med posameznimi elementi UPG, s tem pa tudi dotok hranil in larvalnih stadijev bentoških živali (Sl. 1).

V številnih evropskih morjih so različne oblike umetnih podvodnih grebenov že dolgo dobro sprejet in uveljavljen način za povečevanje biodiverzitete, varovanje obale, povečanje ribjega fonda ali preprosta turistična atrakcija, ki privablja potapljače (Jensen in sod. 2000). Ideja o postavitvi umetnih podvodnih grebenov v Tržaškem zalivu ni nova, porodila se je že pred desetletji tako pri slovenskih kakor italijanskih biologih. Medtem ko so Italijani načrte tudi uresničili, prvi slovenski UPG še vedno že dolga leta čaka na umestitev na morsko dno in se še vedno bolj ali manj neuspešno prebija skozi administrativne ovire. Prvi načrt modela umetnega podvodnega grebena v Piranu je predstavil že biolog Ugo Fonda, davnega leta 1996. Fonda je izrazil potrebo po postavitvi umetnih podvodnih grebenov kot enega od načinov, s katerim bi lahko delno omejili negativne vplive delovanja človeka na morske ekosisteme, ki naj bi bili pomemben vzrok za izginjanje nekaterih vrst kot so: morska mačka (*Scyliorhinus canicula*), rdeča bodika (*Scorpaena scrofa*), romb (*Bothus maximus*), zobatec (*Dentex dentex*) in veliki morski pajek (*Maja squinado*) iz slovenskega dela Jadranskega morja. Vzroki, ki so porodili idejo za postavitev polja UPG, so bili tudi geomorfološke in fizikalne narave, npr. plitvina morja, majhna razgibanost dna, večja izpostavljenost nalaganju sedimenta, velika temperaturna nihanja ter občasne hipoksije - prav te predstavljajo v Tržaškem zalivu ogrožajoče življenjske razmere za mnoge vrste (Fonda 1996). Poleg Fonde sta v novejšem času problematiko postavitve UPG v slovenskem delu Jadranskega morja obravnavali tudi dve dip-



Slika 1: Prikaz načrtovane strukture grebena z osnovno stranico 7.6 m in skupno višino 4.3 m.
Figure 1: Graphical presentation of the planned artificial reef with a base length of 7.6 m and total height of 4.3 m.

lomski deli iz dveh različnih fakultet Univerze na Primorskem (Cernich 2015; Žabar 2016).

V italijanskih vodah Tržaškega zaliva so konec sedemdesetih let začeli graditi pilotne umetne podvodne grebene v študijske namene (Orel in sod. 2006). V Tržaškem zalivu (vsi v italijanskem delu) je danes enajst umetnih podvodnih grebenov, ki so bili ciljno postavljeni v morsko okolje. Eden izmed teh leži v zavarovanem območju Miramar (Odorico in sod. 2008). Skupina umetnih podvodnih grebenov v bližini peščene plitvine pri Križu (»Dosso di Santa Croce«) pa je bila zavarovana po postavitvi kompleksa na morsko dno. Italijanske izkušnje postavljanja umetnih podvodnih grebenov v Tržaškem zalivu so pokazale, da številne izmed teh struktur privabljajo različne organizme, medtem ko nekatere predstavljajo tudi pomembna območja za razmnoževanje lignjev (*Loligo* sp.), sip (*Sepia* sp.) in predstavnikov polžev zaškrgarjev (Opistobranchia) (Odorico in sod. 2008). Podrobne raziskave glede naseljevanja in vpliva, ki ga imajo umetni podvodni grebeni v Tržaškem zalivu, so opravili v sklopu projektov INTERREG II (2001) in INTERREG III (2006), kjer so se osredotočili na skupino umetnih morskih struktur na peščeni plitvini pri Križu. Rezultati preliminarnih raziskav, ki so bile izvedene v obdobju med drugim in tretjim letom starosti umetnih struktur, so pokazale, da je prišlo že po tako kratkem času od postavitve umetnih grebenov do povečane biodiverzitete na območju umetnih podvodnih grebenov (Specchi in sod. 2001; Miletić in sod. 2001). Prišlo je tudi do povišanja abundance larvalnih stadijev rib, ki so značilne za morske grebene, kar nakazuje, da lahko umetni podvodni grebeni omogočijo nastanek nove ribje združbe (Miletić in sod. 2001). V Tržaškem zalivu je prišlo tudi do poskusa postavitve UPG s ciljem izboljšanja kvalitete morske vode (Stachowitsch 1998). Taki UPG delujejo na principu izrabljanja naravnih sposobnosti čiščenja vode filtratorskih organizmov, katerih larvalni stadiji so že naravno prisotni v planktonu. Rezultati raziskave so pokazali, da lahko gosto naseljena obrast prečrpa več 1000 litrov vode na dan. S postavitvijo majhnih struktur, ki so se hitro obrasle s filtratorskimi organizmi, so nastale majhne »biološke precejevalne postaje«, te pa so kasneje prispevale tudi k (hitrejšemu) obnavljanju izvirne bentoške skupnosti (Stachowitsch 1998).

Material in metode

Ekološko stanje in prevladujoči tip biocenoze na širšem območju postavitve UPG

Celotno ožje območje načrtovane postavitve pilotnega UPG lahko prištejemo v t.i. biocenozo muljastega detritnega dna oz. po EUNIS klasifikaciji v MC6 oz. EUNIS A5.381, po RAC/SPA klasifikaciji pa v IV.2.1.(*Pregled habitatnih tipov in njihova opredelitev na nivoju kategorizacije EUNIS in RAC/SPA,* glej Lipej in sod. 2018). Bolj podrobno je to facies z vodilno vrsto *Ophiothrix quinquemaculata.* Za to biocenozo so značilne globoke plasti mulja, ki jih pokriva organski detrit, slabo oziroma praktično odsotno strujanje morske vode in, predvsem v toplejših obdobjih leta, močna evtrofikacija, zelo slaba vidljivost ter možnost nastanka hipoksije v pridnenem sloju vode (približno do 2 m nad morskim dnom). Občasne hipoksije ali celo anoksije so glavna ekološka grožnja temu tipu biocenoze. Pomanjkanje kisika je posledica slabega mešanja morske vode (stratifikacija) ter že omenjenega velikega števila planktona in organskega detrita. Pojav je izrazit predvsem v poznem poletju, ko zaradi povečane produkcije in odmiranja planktonskih organizmov, posledično pa zaradi povečanega števila mikroorganizmov, ki razgrajujejo biomaso, zmanjka kisika. To lahko povzroči obsežne pomore pritrjenih bentoških organizmov, ki živijo na dnu ali so zakopani v mulju. Taki organizmi se pomanjkanju kisika ne morejo izogniti, zato množično poginjajo in zaradi mikrobne razgradnje dodatno prispevajo k porabi kisika v pridnenih slojih vode. Ker se ustvarijo anoksične razmere to favorizira razvoj anaerobnih mikroorganizmov, ki na morskem dnu z anaerobnim dihanjem (t.i. sulfatno respiracijo) tvorijo žveplovodik (H_2S), ta pa se nato kemijsko pretvori v sivo prevleko železovega sulfita. Hipoksije in anoksije so bile v Tržaškem zalivu zelo obsežen pojav predvsem v 80 letih 20. st. (najhujša leta 1983), so se pa sporadično, sicer v manjšem obsegu, pojavljale tudi v naslednjih letih vse do danes.

Značilnosti biocenoze muljastega detritnega dna

Za muljasto detritno dno je značilna ORM biocenoza (združba kačjerepov iz rodu *Ophiotrix*, spužev iz rodu *Reniera* in kozolnjakov/plaščarjev iz rodu *Microcosmus*). Spužve in kozolnjaki se na površini mulja naselijo na odmrle ostanke/lupine morskih organizmov, predvsem večjih školjk in morskih ježkov, vmes pa so prepleteni sicer gibljivi kačjerepi. Spužve in kozolnjaki so filtratorji, kačjerepi pa prav tako pobirajo hrano iz bogate »hranilne juhe«, ki jih obdaja. Na ta način na enolični muljasti površini mehkega dna nastajajo nekakšni otočki biogeno učvrščenega sekundarnega dna, ki v takem okolju predstavljajo edino površino za naseljevanje drugih pritrjenih organizmov, prav tako pa tudi edino trdnejše okolje, kamor se lahko skrijejo in zatečejo sicer gibljivi bentoški organizmi, npr. razne ribe in raki. Številni drugi organizmi, ki živijo v tej biocenozi, so t.i rovači. To so organizmi, ki so bolj ali manj zakopani v mulj ali rijejo po in v njem. Sem spadajo številne školjke (npr. velika pokrovača (*Pecten jacobeus*), še bolj raznovrstni mnogoščetinci, pa tudi redko videne živali, kot so npr. pršivci (Sipunculida). Med značilne živali take združbe spadata tudi velik plenilski rak morska bogomolka (*Squilla mantis*) in redka listasta morska zvezda (*Anseropoda placenta*). Od rib so v taki biocenozi najpogostejše ribolovno pomembne vrste, kot so bokoplute (npr. morski listi, rod *Solea*) ter nekateri bradači (*Mullus*) in riboni (*Pagellus*) pa tudi številne manjše vrste rib (Tab. 1).

Tabela 1: Nekaj značilnih organizmov muljastega detritnega dna, pogostost in način življenja.
Table 1: Some typical organisms inhabiting muddy bottom, their basic biology and frequency.

Slovensko ime vrste	Latinsko ime vrste	Način življenja	Opomba
Bodičasti kačjerep	*Ophiothrix quinquemaculata*	prosto gibljiva vrsta na površini dna	vodilna vrsta biocenoze
Kačjerep	*Amphiura chiajei*	prosto gibljiva vrsta na površini dna ali delno zakopana	značilna vrsta
Brazdasti ježek	*Shizaster canaliferus*	zakopana vrsta, rovač	značilna vrsta
Morska kumara	*Cucumaria planci*	na površini dna, v pokončnem položaju	pogosta vrsta
Zeleni morski ježek	*Psammechinus microtuberculatus*	na površini dna	pogosta vrsta
Listnata morska zvezda	*Anseropoda placenta*	zakopana vrsta	redka vrsta
Nagubani kozolnjak	*Microcosmus* sp.	pritrjena vrsta	značilna vrsta
Velika pokrovača	*Pecten jacobeus*	na površini dna, delno zakopana	pogosta vrsta
Spužve več vrst	*Reniera* spp.	pritrjene vrste	značilne vrste
Morska bogomolka	*Squilla mantis*	v rovih, plenilec	pogosta vrsta
Vetrnični samotarec	*Pagurus prideaux*	gibljiv na površini dna	pogosta vrsta, skupaj v simbiozi z morsko vetrnico *Adamsia palliata*
Pršivci	*Sipunculus* spp.	zakopane vrste	pogoste vrste
Mnogoščetinci	Polychaeta	zakopane vrste	pogoste vrste
Bokoplute, npr. morski listi	*Solea* spp.	na površini dna ali delno zakopane	pogoste vrste

Opis neposrednega območja za postavitev predvidenega UPG in ocena sedanjega stanja

Lokacija predvidenega UPG leži na globini med 22.5 in 23 m v neposredni bližini desne kanalizacijske cevi, približno 1 navtično miljo od Piranske punte. Enkratni ogled lokacije smo opravili v začetku meseca julija 2020 s pomočjo avtonomne potapljaške opreme, tipično biocenozo dna pa smo slikovno dokumentirali s pomočjo v vodotesno ohišje nameščenega fotoaparata Olympus TG6 s pripadajočo podvodno bliskavico. Ocenjeno območje ogleda je bilo približno 50 m x 50 m. V času potopa je bila vidljivost zelo slaba, na površini okrog 3 m, na dnu pa le slab meter, kar je v tem akvatoriju v poletnih mesecih dokaj pogosto stanje. Temperatura morja je bila (izmerjena s potapljaškimi instrumenti) na površini 25^{o}C, pri dnu pa dve stopinji manj. Termokline ni bilo. Stanje morskega okolja lahko opišemo z 11 deskriptorji (D1-D11) ali ključnimi značilnostmi, ki na integralen način opisujejo stanje morja oziroma morskega okolja, tako da obravnavajo oziroma upoštevajo ključne pritiske in vplive nanj. Mi smo jih uporabili pet, kakor sledi: (i) biotska raznolikost (D1), (ii) tujerodne vrste (D2), (iii) ribji stalež oz. komercialne vrste rib in lupinarjev (D3), (iv) evtrofikacija ali onesnaženje s hranili (D5) in (v) morski odpadki (D10).

Cilji postavitve pilotnega UPG

S postavitvijo pilotnega UPG bi lahko dosegli naslednje cilje:

1. Ustvariti večjo trdno površino za naseljevanje bentoških organizmov, s čimer lahko povečamo biodiverziteto na območju postavitve.
2. Omogočiti možnost umika in nadomestni habitat gibljivim organizmom v primeru nastanka hipoksije ali anoksije na morskem dnu (višina predvidenega grebena je 4,3 m).*
3. Zagotoviti repopulacijsko jedro za ponovno naseljevanje organizmov na morsko dno, če pride do pogina organizmov zaradi hipoksije ali anoksije.*
4. Ustvariti površino za preučevanje sukcesije naseljevanja bentoških organizmov na večjo trdno podlago na sicer mehkem muljastem morskem dnu.
5. Zagotoviti dodaten habitat in primerna skrivališča za nekatere vrste bentoških rib in rakov.

* Zavedati se moramo, da postavitev UPG v primeru hipoksije ali anoksije ne predstavlja trenutne rešitve za pritrjene vrste ali tiste vrste, ki so slabo gibljive in živijo v mulju, ker se taki organizmi ne morejo umakniti na greben.

Rezultati in razprava

Biotska raznolikost (D1). Na lokaciji smo zasledili združbo, ki jo večinoma predstavljajo pritrjeni organizmi filtratorji, kot so spužve in plaščarji. Na dnu so prevladovali organizmi, ki so za združbo muljastega detritnega dna dokaj značilni in pogosti. (Tab. 2). V vodnem stolpcu pa smo opazili nenavadno veliko prisotnost kolonijskih plaščarjev *Salpa maxima.* To je občasen in dokaj redek pojav, ki kaže na veliko prisotnost hranil, rastlinskega ter živalskega mikroplanktona v morju.

Na lokaciji nismo opazili tujerodnih vrst (D2). Tujerodnih vrst na dnu nismo opazili. V vodnem stolpcu so bile prisotne rebrače, verjetno tujerodne vrste *Mnemiopsis leidyi.* Na lokaciji pri ogledu nismo opazili komercialnih vrst rib ali lupinarjev. Prav tako nismo zasledili večjih rakov, razen nekaj rovov, v katerih domujejo morske bogomolke (*Squilla mantis*) (deskriptor (D3 ribji stalež oz. komercialne vrste rib in lupinarjev). Glede na kriterij D5 (evtrofikacija ali onesnaženje s hranili) smo ugotovili, da je bila na lokaciji v času potopa izredno slaba vidljivost, morska voda zeleno obarvana, kar kaže na veliko število rastlinskega planktona. V vodnem stolpcu pa je bilo veliko število želatinoznega živalskega planktona (kolonijski plaščarji - salpe in rebrače). To potrjuje, da je bilo v morju polno organskih hranil, stanje morja pa evtrofno. Glede kriterija D10 (morski odpadki) lahko zapišemo, da na lokaciji planiranega UPG nismo našli odpadkov antropogenega izvora, kar pa ne izključuje prisotnosti mikro- in nanoplastike tako v okolju kakor v organizmih.

Tabela 2: Prevladujoči opaženi organizmi na muljastem dnu in njihova ogroženost.
Table 2: Predominant species inhabiting muddy bottom and their vulnerability.

Slovensko ime vrste	Latinsko ime vrste	Število	Ogroženost
Spužve več vrst	*Reniera* spp., *Suberites* sp.	več kot 20	niso ogrožene
Bodičasti kačjerep	*Ophiothrix quinquemaculata*	več kot 50	ni ogrožen
Vetrnični samotarec	*Pagurus prideaux*	3	ni ogrožen
Nagubani kozolnjak	*Microcosmus* sp.	5	ni ogrožen
Zeleni morski ježek	*Psammechinus microtuberculatus*	okrog 10	ni ogrožen

V slovenskem morju obstaja kar nekaj habitatnih tipov, nekateri od teh so izpostavljeni hudim okoljskim pritiskom tako naravnega kakor antropogenega izvora (Lipej in sod. 2006). Eno najbolj ranljivih okolij je nedvomno muljasto-detritno dno s sekundarno utrjenimi biogenimi formacijami (Lipej in sod. 2016). Ta tip dna kaže veliko biodiverziteto, vendar je vsaj občasno izpostavljen prekomerni evtrofikaciji in posledično pomanjkanju kisika. Dodatni večji otoki trdnega dna (tudi v obliki UPG) bi lahko prispevali k ohranitvi in večji naseljenosti vrst na takem dnu. Predvidevamo dolgoročnejši pozitivni vpliv UPG na lokalno biocenozo zaradi možnosti, da se bodo na dvignjeno trdno podlago lahko naselili/naseljevali pritrjeni filtratorski organizmi kot so kozolnjaki/plaščarji, mahovnjaki in spužve ter tudi drugi pritrjeni ali na trdno podlago vezani organizmi. Ti lahko z reprodukcijo sčasoma nadomestijo odmrle organizme na morskem dnu in tako predstavljajo repopulacijsko jedro za obnovo prvotne biocenoze muljastega detritnega dna, zlasti k obnavljanju sekundarnih otočkov trdnega dna na muljasti podlagi. Širših vplivov na ekosistem in že vzpostavljeno naravno ravnovesje ne bi smelo biti, saj predstavlja postavitev pilotnega UPG (konstrukcije tlorisa 7,6 m x 7,6 m in višine 4.3 m) zelo omejen poseg na zanemarljiv del morskega dna (56 m^2 od skupnih 406 kvadratnih kilometrov teritorialnega morja). Še toliko več, če načrtovani poseg primerjamo z vplivi drugih že izvedenih in predvidenih posegov v morje, kot so poglabljanje morskega dna, izgradnja pomolov, sidrišč, postavitev marikultur in drugo oblikovanje - urbanizacija morske obale. Ocenjujemo, da je glede na ugotovljeno biološko-ekološko stanje, postavitev pilotnega UPG smiselna, ker lahko pripomore k povečanju biodiverzitete na omenjenem območju in hkrati ob pomanjkanju kisika v pridnenem sloju vode omogoči nadomestni začasni habitat za nekatere gibljive bentoške organizme. Hkrati lahko zagotovi repopulacijsko jedro za ponovno naselitev nekaterih pomembnih členov biocenoze muljastega detritnega dna po morebitnem poginu tovrstne biocenoze zaradi pomanjkanja kisika na morskem dnu. Na podlagi izkušenj z do sedaj nelegalno in legalno postavljenih struktur v Piranskem zalivu (kip madone, betonske cevi, sidrišča za školjčišča, privezi in boje) in znanstvenih raziskav že nameščenih UPG v Tržaškem zalivu bodo UPG pripomogli k povečanju biodiverzitete. Omogočili bi lahko tudi samoobnovitvene procese v bentoških združbah, ki so jih prizadele občasne hipoksije ali anoksije na morskem dnu. Zato je postavitev UPG nedvomno ukrep ohranjanja ali bolje renaturacije določenega življenjskega okolja. Zaradi majhne velikosti objekta širših vplivov ni pričakovati, bi pa na podlagi spremljanja obrasti UPG ter vpliva na okolje v bližini takega pilotnega objekta lahko prišli do trdnejših spoznanj o smiselnosti vključevanje tovrstnih struktur v strategije prostorskega načrtovanja za doseganje okoljskih ciljev Direktive 56/2008/ES.

Kot avtor tega prispevka se popolnoma zavedam, da je nujno na lokaciji postavitve UPG opraviti precej bolj poglobljen monitoring obstoječega stanja, kot sem ga opravil sam v zelo kratkem času. Pretehtati moramo vse mogoče vplive takega posega na morsko okolje, je pa res, da v Tržaškem zalivu to ne bi bil prvi UPG in da izkušnje italijanskih kolegov kažejo na večino pozitivne posledice in odsotnost negativnih vplivov na biocenoze v bližini UPG. Načeloma se tudi strinjam s pristopom, da je treba habitate in morske ekosisteme predvsem ohranjati in če se le da, čim manj posegati vanje. Toda zaradi specifičnih hidrografskih razmer na dnu Tržaškega zaliva bi lahko UPG, če drugega ne, pomagali pri obnavljanju biocenoze muljasto-detritnega dna, ki je občasno podvržena pomanjkanju kisika in posledično poginom velikega števila pritrjenih ali slabo gibljivih organizmov na morskem dnu. Poskus potopitve prvega UPG v slovensko morje se zaradi birokratsko-administrativnih ovir in tudi zgoraj navedenih pomislekov vleče že več kakor pet let, osnovna ideja Uga Fonde pa je stara že četrt stoletja. Umetnost umeščanja umetnih podvodnih grebenov v slovensko morje torej še naprej ostaja v domeni umetnosti možnega. Vse skupaj pa potrjuje izhodiščno misel velikega slovenskega morskega biologa dr. Jožeta Štirna, kateremu spominu je ta prispevek tudi namenjen.

Summary

Attempts to built artificial underwater reef(s) (AUR) in Slovenian coastal waters are longlived, albeit unsuccesfull due to various, mainly administrative reasons. The idea is quite old, born in the end of former century by biologist Ugo Fonda, who planned a network of AUR in order to enrich biodiversity and protect some of the vanishing species. While Italian collegues realized at least a modest number of AUR in the Italian part of Gulf of Trieste, up to now there is none in the Slovenian part. Since Italian AUR proved to be a succesfull way to enrich and sustain biodiversity, new attempt to put at least one pilot AUR in the Gulf of Piran started few years ago. In this paper we describe the projects and the results of the brief monitoring at the location of future AUR. We also disccuss the benefits as well as possible drawback such a project might have to local benthis community particularly in regards of sporadic hypoxic or anoxic conditions in such environment.

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The impact of sewage discharge on nutrients and community production in a lagoon environment (Lagoon of Strunjan, Gulf of Trieste, northern Adriatic Sea) – a revisited experiment

Vpliv vnosa komunalnih odplak na hranila in produkcijo v lagunarnem okolju (Strunjanska laguna) – ponovni ogled poskusa

Jadran Faganeli

Marine Biology Station, National Institute of Biology, Fornače 41, 6330 Piran, Slovenia

Correspondence: jadran.faganeli@nib.si

Abstract: A specially constructed enclosure in the lagoon environment (Lagoon of Strunjan, Gulf of Trieste, northern Adriatic Sea) received sewage daily while another was kept clean and used as a reference. Nutrients and community production changes were monitored approximately bimonthly over a year. Nutrients introduced by the sewage discharges and diluted by tides were immobilized by enhanced community production, in particular benthic macroalgae. The dead organic matter afterwards settled and decomposed producing anoxic conditions and high levels of dissolved and suspended organic nutrients in the water and total nitrogen in the sediments. The daily mean gross community production showed no quantitative differences between the two enclosures during the study. Differences arose in the temporal succession of the studied events. An intensive nutrient recycling emerged from this study.

Keywords: carbon, lagoons, nutrients, northern Adriatic, oxygen, production

Izvleček: V posebej zgrajeni bazen v Strunjanski laguni smo dnevno uvajali komunalne odplake mesta Piran, medtem ko je drugi služil za primerjavo. V odbobju enega leta smo s približno dvomesečno frekvenco spremljali gibanja koncentracij hranil in produkcije. Vnešena hranila, redčena s plimovanjem, so povečala produkcijo predvsem bentoških makroalg. Odmrla organska snov se je nato posedla in razgrajevala ter povzročila nastanek anoksije in visokih koncentracij raztopljene in suspendirane organske snovi in celotnega dušika v sedimentu. Srednja dnevna bruto produkcija v celoletnem obdobju ni pokazala velikh razlik med bazenoma, medtem ko so bile lete opazne v časovnem poteku študiranih procesov. Iz poskusa je razvidno intenzivno kroženje vnešenih hranil.

Ključne besede: hranila, kisik, lagune, ogljik, produkcija, severni Jadran

Introduction

Pollution problems are particularly evident in coastal, estuarine and lagoon environments although the long-term effects extending over wider marine areas and oceans now deserve great attention (Clark 2001). However, most studies have dealt with the pollution of stressed coastal and estuarine environments (Kennish 1997, Prepas and Charette 2005). The application of mesoscale seawater enclosures (mesocosmos) allows for the testing of the responses of the isolated communities to various pollutants and provides a possibility for experimental manipulation and control. The requirements, advantages and disadvantages of this approach, an intermediate between microcosmos and field observations, were extensively reviewed in the past by Grice and Reeve (1982).

Due to their shallowness and penetration of sunlight to the bottom, lagoons, areas of great economic importance and influenced by pollution, are characterised by intense benthic primary production and respiration and are places of intense accumulation and recycling of organic carbon and nutrients (Nixon et al. 1976; Nowicki and Nixon 1985, McGlathery et al. 2001, Cloern 2001). Sediments with benthic communities are the most sensitive compartments of the lagoon system affected by eutrophication and oxygenation (Sfriso et al. 1992, Boynton et al. 1996). Due to the large storage capacity of organic matter and nutrients, their sediments have an important regulatory function. They influence the oxygen budget of bottom waters and releasing nutrients to the overlying water and affect the benthic and pelagic primary production (Jorgensen 1996). Due to the high rate of microbial processes, lagoon sediments are anoxic except for a thin surface layer and around the infauna burrows containing oxygen. The depth of the oxic-anoxic interface changes seasonally, mostly dependent on organic matter accumulation and oxygen levels in the overlying water. At present, our knowledge of the carbon and nutrient fluxes in these environments, which is the key factor to understand the functioning of lagoon and coastal systems, is still limited (Boynton et al. 1996).

In the present experiment, designed by the late professor J. *Štirn*, conducted in the period 1975-78 in the Lagoon of Strunjan (Gulf of Trieste, northern Adriatic Sea), the ecological (Malej et al. 1979) and pollution (Salihoglu et al. 1980, Stegnar et al. 1980) consequences of domestic sewage on the lagoon environment were studied. In this article, the results of an approximately year-long study on nutrients and community production changes are presented and discussed in the new perspective.

Materials and methods

Study area and design of the controlled pollution experiment

The Lagoon of Strunjan (<1 km^2) is located in the southeastern part of the Gulf of Trieste (Slovenia), the northernmost part of the Adriatic Sea (Fig. 1). The lagoon is very shallow (0.6 m, on average) and at lower low tide some portions of the bottom are exposed to the air. The lagoon is characterised by semi-diurnal tidal fluxes (65 cm mean). Freshwater inflows are scarce and limited mostly to the northwestern part. Salinity varies between 33 and 38. Water temperature ranges from maximum values (28 °C) in summer to minimum values in winter (4 °C).

Sediment from the Lagoon of Strunjan is composed mainly of calcite (31%) and quartz (29%) and consists of >90% of silt-clay sized material (Ogorelec et al. 1991). A well-defined colour stratigraphy determined by the relative abundance of Fe-oxides and sulphides is present. The top centimetre was brown, followed by a black layer which coincides with reduced redox conditions in sediment.

The controlled lagoon ecosystem consisted of two stony enclosures each of 63 m^2 with an average depth of 0.6 m. The enclosures were connected to the main lagoon to allow for tidal oscillations. Each enclosure contained 38 m^3 of seawater at the mean tidal level. One enclosure (PB) was treated daily with 300 l of primary settled sewage (Tab. 1) during the lowest level of seawater. The sewage was transported from the Piran sewerage system monthly and stored in a 5 m^3 plastic tank. The addition of the sewage to the polluted enclosure was done via a single discharge outlet. The other enclosure (CB) was kept clean as a control.

Naturally, the lagoon sediment was mainly inhabited by sea grasses (*Cymodocea nodosa* and *Zoosterella noltii*) with some branches of *Laurentia obtusa* and *Cystoseira barbata*, while the central part of the PB was occupied by some islands of *Ulva rigida* and *Enteromorpha compressa*. In the CB, *UIva riqida* reached its normal spring peak and coexisted with *Laurentia obtusa, Cystoseira barbata* and *Giqartina acicularis*, whereas the PB became literally filled up with *Ulva*. At the end of

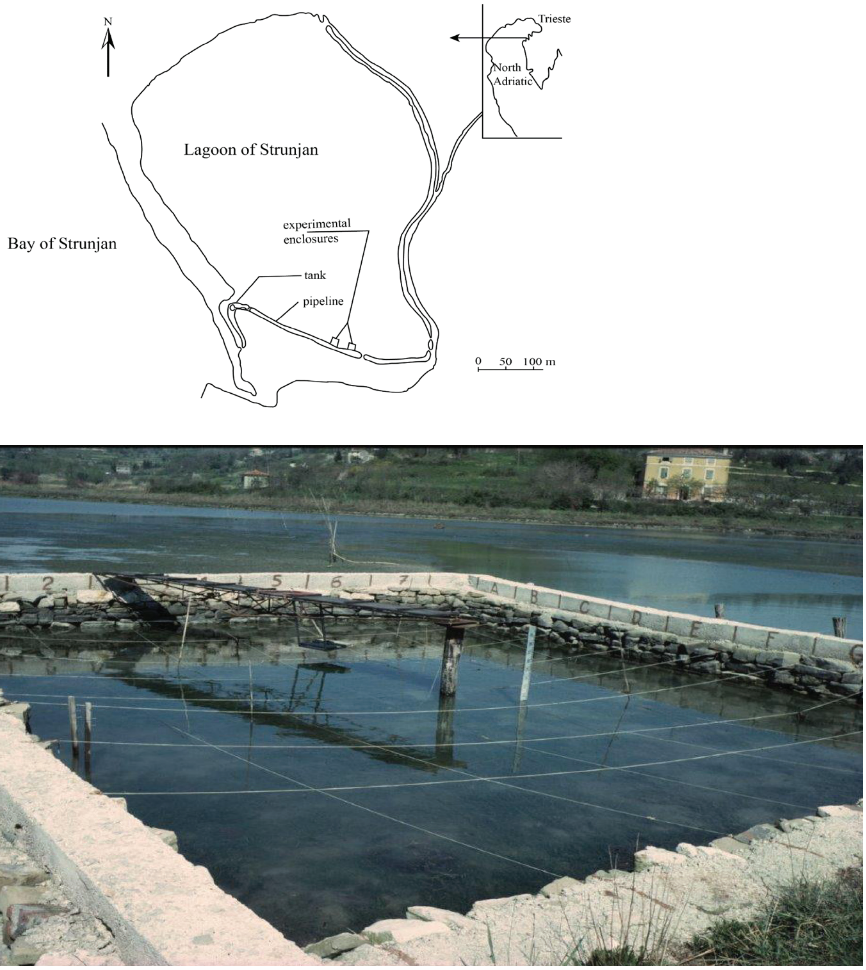

Figure 1: Location and design of the controlled ecosystem pollution experiment at Strunjan, Gulf of Trieste, northern Adriatic Sea

Slika 1: Lokacija in shema poskusa v Strunjanski laguni v Tržaškem zalivu, Severni Jadran.

spring all the vegetation decayed and bare sediment covered by Cyanophycea and Bacteria was left behined. In the CB, after the decay of *Ulva*, sea grasses and some macroalgae (*Cladophora battersi, Laurentia obtusa, Cladophora echinus, Cystoseira barbata, Polysiphonia tenerrirna*) developed according their normal seasonal dynamics (Malej et al. 1979). Natural lagoon macrofauna typically associated with sea grass modified under the stress of experimental pollution, and a few supertolerant organisms, increased in biomass and abundance: *Neantes succinea, Scolelepis fuliginosa and Capitella capitata,* a shrimp *Upogebia litoralis* and some Amphipodes which were the most abundant (Malej et al. 1979). The main groups of meiofauna in both experimental enclosures were Nematoda, Harpacticoidea, Polychaeta, Olygochaeta and Ostracoda, while Kinorhyncha, Turbellaria, Cumacea and some others were only accidental, these being found in the PB only at the beginning of the experiment (Vrišer 1982). The main phytoplankton genera in both enclosures

Table 1: Average daily discharged nutrients to the polluted enclosure by primary settled sewage from the town of Piran (in grams per 300 L of sewage) (N = 7).

Tabela 1: Povprečni dnevni vnos hranil v onesnaženi bazen s primarno čiščeno piransko komunalno odplako (v gramih na 300 L odplake) (N = 7).

Nutrient	Quantity
NO_3^-	0.03
NO_2^-	0.03
NH_4^+	2.50
DON	1.61
PN	2.82
PO_4^{3-}	3.75
DOP	0.09
PP	3.40
SiO_4^{4-}	4.26
POC	6.89
Total suspended matter	**25.60**

Abbreviations:
DON, dissolved organic nitrogen, raztopljeni organski dušik; DOP, dissolved organic phosphorus, raztopljeni organski fosfor; PN, particulate nitrogen, suspendirani dušik; PP, particulate phosphorus, suspendirani fosfor; POC, particulate organic carbon, suspendirani organski ogljik.

were *Navicula*, *Nitzschia*, *Amphora*, *Amphiprora* and *Gymnodinium* as well as microflagellates which were more numerous in the PB, while diatoms were more abundant in the CB (Fanuko 1984). Conversely the phytoplankton biomass and abundance were lower in the PB (Fanuko 1984). During the experiment the zooplankton community in the PB showed some regressive modifications since some organisms found in the CB were not detected in the PB: *Sarsia qemmifera*, *Muggiacea kochi*, *Ctenocalanus vanus*, *Clytemnestra sp.*, *Sapphirina sp.*, *Corycaeus sp.*, *Oikoplura lonqicauda*, *Oikopleura fusiformis*. In the first phase of the experiment the biomass and abundance of zooplankton organisms increased, the inhibitory effects of pollution later prevailed (Malej 1979).

Sampling

Seven diurnal samplings were conducted in both enclosures: September 16-17, 1976, November 15-16, 1976, February 28 – March 1, 1977, April 19-20, 1977, June 22-23, 1977, August 3-4, 1977, and October 4-5, 1977. Seawater samples were taken every 5-6 hours just below the surface and at a depth of 0.5 m using a Van Dorn sampler in a horizontal position. Undisturbed sediment cores were taken by pushing a plexiglass tube (6 cm i.d., 20 cm length) into the sediment. The top 2 cm was used for analyses.

Analyses

Dissolved O_2 in seawater was analysed via the Winkler method (Grasshoff 1976) using an automated titration system (Mettler Toledo, DL 21). The reproducibility of the method was 5 %. H_2S was determined spectrophotometrically after trapping with Zn acetate (Grasshoff 1976). The reproducibility of the method was 10 %. Dissolved inorganic carbon (DIC) was determined using a Van Slyke gas apparatus (Strickland and Parsons 1968). The reproducibility of the method was between 1.5–3 %. Nutrients analyses in unfiltered seawater and filtered sewage samples, through preignited glass fibre filters Whatman GF/C, were performed photometrically for ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-). phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}), using standard methods (Strickland and Parsons 1968; Grasshoff 1976). Total dissolved nitrogen (TDN) and phosphorus (TDP) in the samples filtered through preignited glass fibre filters Whatman GF/C were analysed by irradiation for 3.5-4 hours using short wavelength UV radiation (1200 W, Hanovia, USA) in the presence of a few drops of 30 % H_2O_2 (Armstrong et al. 1966). Dissolved organic nitrogen (DON) and phosphorus (DOP) were calculated as the difference between TDN and dissolved inorganic nitrogen and between TDP and PO_4^{3-}, respectively. The precision of nutrient and DON and DOP analyses was 3%.

Analyses of organic C (C_{org}) and total N (N_{tot}) in freeze-dried particulate matter (particulate organic carbon - POC and particulate nitrogen - PN), collected on preignited Whatman GF/C glass fibre filters, and freeze-dried and homogenized sediment samples were performed using a Coleman C, H (Konrad et al. 1970) and N (Keeney and Bremner 1967) elemental analysers at combustion temperatures of 650 and 900 °C, respectively. Total P in freeze-dried particulate matter (PP), collected on preignited Whatman GF/C glass fibre filters, and sediments was determined by digestion of the sample with a mixture of perchloric and nitric acid followed by colorimetric detection of the phosphate produced (Strickland and Parsons 1968). The precision for C_{org},, N_{tot} and $P_{tot.}$ was about 3%.

Gross production was calculated from diurnal cycles of dissolved O_2 and DIC, examining the differences between extremes and estimating system parameters directly from ΔO_2 and ΔDIC (Odum 1956).

Production

Substantial diurnal O_2 and DIC changes were observed in both enclosures during the series of measurements. Inverse correlation appeared between diurnal O_2 and DIC changes in the experimental enclosures daily (Fig. 2). Differences in O_2 concentrations between the two enclosures started in spring 1977 and higher O_2 concentrations were observed in PB. In June 1977 (Fig. 2) in PB anoxic conditions prevailed during the night and H_2S was observed (up to 160 µM). Later (in autumn) the O_2 concentrations became uniform in both basins. The production (Tab. 2) measured from O_2 concentrations showed the highest values in CB in June and August 1977, while this occurred in PB in February 1977. Similar situation was found for the metabolism dynamics based on DIC measurements, considering that DIC concentrations in the northern Adriatic are largely attributed to the production and decomposition of

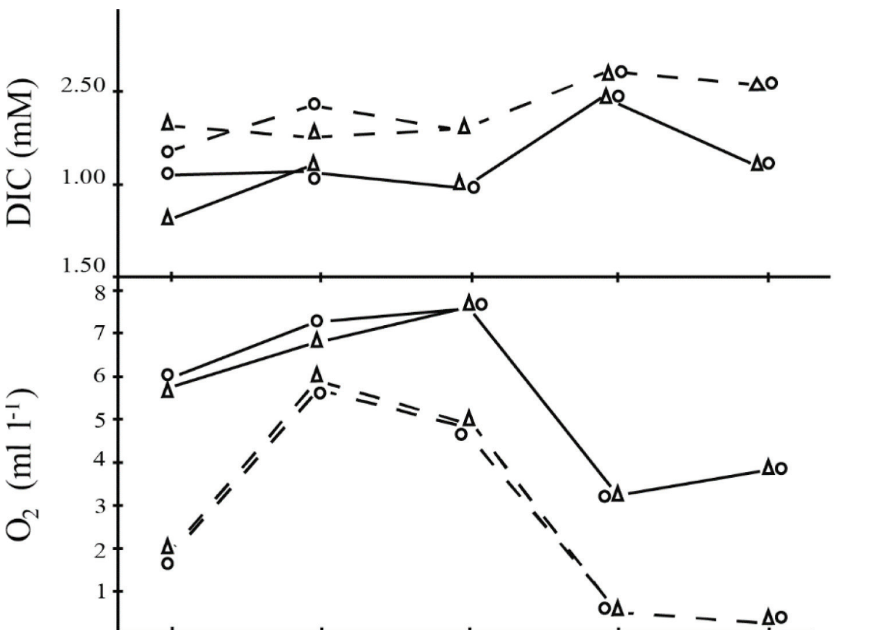

Figure 2: Diurnal variations of dissolved inorganic carbon (DIC) and oxygen (O_2) in clean enclosure (solid line) and polluted enclosure (dotted line) in surface (○) and bottom (Δ) layer in June 1977.

Slika 2: Dnevne variacije raztopljenega anorganskega ogljika (DIC) in kisika (O_2) v čistem bazenu (polna črta) in onesnaženem bazenu (črtkano) na površini **(○)** in pri dnu **(Δ)** junija 1977.

Table 2: Daytime community gross production in clean (CB) and polluted (PB) enclosure in the Lagoon of Strunjan (g m^{-2} day^{-1})
Tabela 2: Dnevna bruto produkcija v čistem (CB) in onesnaženem (PB) bazenu v Strunjanski laguni (g m^{-2} dan^{-1})

		O_2 production		DIC production	
Date	**Temperature (°C)**	**CB**	**PB**	**CB**	**PB**
9/28-29/1976	19.0-22.5	2.19	1.73	2.20	1.80
11/16-17/1976	12.3-18.7	2.00	2.18	3.02	2.04
2/28-3/1/1977	4.2-11.3	3.59	6.22	2.00	4.57
4/19-20/1977	10.9-16.6	2.28	5.46	2.02	3.27
6/22-23/1977	22.3-25.0	6.47	3.11	4.12	1.71
8/3-4/1977	22.0-26.7	5.44	3.62	-	-

Abbreviations: DIC, dissolved inorganic carbon; - , no data.
Okrajšavi: DIC, raztopljeni anorganski ogljik; -, ni podatka.

organic matter and less to carbonate dissolution and precipitation (Ogrinc et al. 2003). The highest values in CB and PB were found in February and June 1977, respectively. The production in PB in spring (March-April 1977) exceeded that of CB. Photosynthetic quotients (PQ, $\Delta O_2/\Delta DIC$) were mostly less than 1.0, except during high production in February 1977 in CB and in June 1977 in PB, probably as the consequence of the precipitation of carbonates (Cermelj et al. 2001) and the nonalgal incorporation of CO_2 (Johnson et al. 1981). Annual daily mean gross production from O_2 concentration variations was 3.7 g O_2 m^2 day^{-1} in both enclosures, those from DIC concentrations was 2.7 g C m^2 day^{-1}.

Nutrients

Nitrogen

Diurnal fluctuations of NH_4^+ concentrations were the most pronounced of all inorganic nitrogen compounds. The lowest NH_4^+ concentrations were observed in general during the photoperiodin both enclosures, while the highest concentrations were observed in PB just after the sewage input (Fig. 3). Great differences of NO_3^- and NO_2^- concentrations between the two enclosures were not observed during the diel cycles. The levels of inorganic nitrogen were higher during the winter (February 1977). In general, somewhat higher inorganic nitrogen, DON and PN concentrations were observed in PB (Fig. 4). The dissolved organic forms of nitrogen were lower than inorganic during the experiment except during the period of anoxia in June 1977 (Fig.4). The $N_{tot.}$ content in sediments averaged approximately 0.15 % in both enclosures, except in spring and summer 1977, when much higher levels (0.4-0.5 %) were found in PB (Fig. 4).

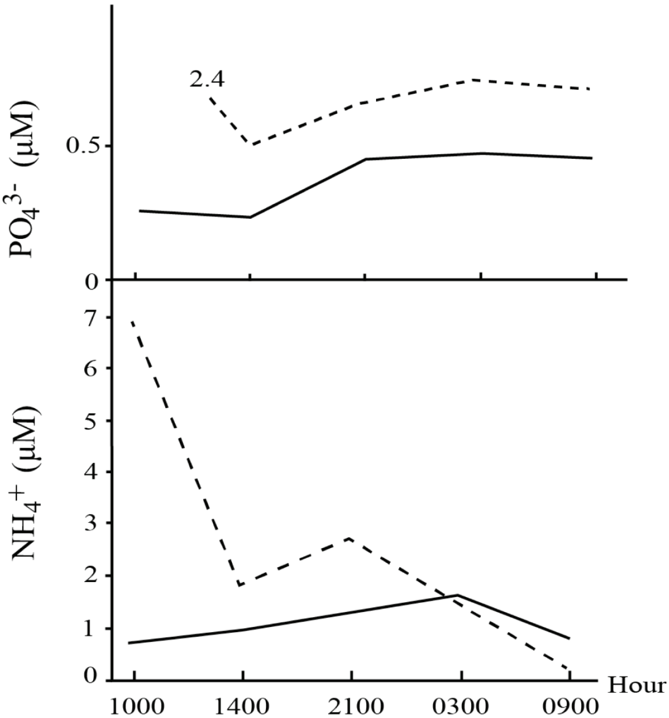

Figure 3: Diurnal variations of PO_4^{3-} and NH_4^+ in clean enclosure (solid line) and polluted enclosure (dotted line) in June 1977.

Slika 3: Dnevne variacije PO_4^{3-} and NH_4^+ v čistem bazenu (polna črta) in onesnaženem bazenu (črtkano) junija 1977.

Phosphorus

Diurnal fluctuations of PO_4^{3-} concentrations again showed lowest values during the photoperiod (Fig. 3). Somewhat higher PO_4^{3-} was detected in PB, especially just after sewage input, but the PO_4^{3-} concentrations dropped relatively fast to a level approximately equal to that in CB (Fig. 3). The lowest PO_4^{3-} concentrations in both enclosures during the experiment were observed in autumn (November 1977). The levels of DOP and PP were up to 100-times higher than that of PO_4^{3-} with the highest concentrations in PB during anoxia in June 1977 (Fig. 5). The DOP and PP concentrations in PB as well as the $P_{tot.}$ content in sediments of PB were in general slightly higher than in CB (Fig. 5). The $P_{tot.}$ contents in sediments of both enclosures nearly doubled (0.10-0.15 %) in spring 1977.

Silica

Diurnal fluctuations of SiO_4^{4-} concentrations (not presented) were similar to those of inorganic nitrogen and phosphorus, usually with lower concentrations during the photoperiod. The lowest concentrations in both enclosures were observed in February 1977 and the differences between the two enclosures were negligible (Fig. 6).

Organic carbon

Seasonal variations of POC concentrations showed the highest values in PB during anoxia (June 1977) and starting from 1977 higher levels were observed in PB (Fig. 6). Conversely, seasonal fluctuations of the $C_{org.}$ content in sediments in both enclosures were similar averaging approximately 2 % except in April 1977 when higher levels (3-4 %) were found (Fig. 6).

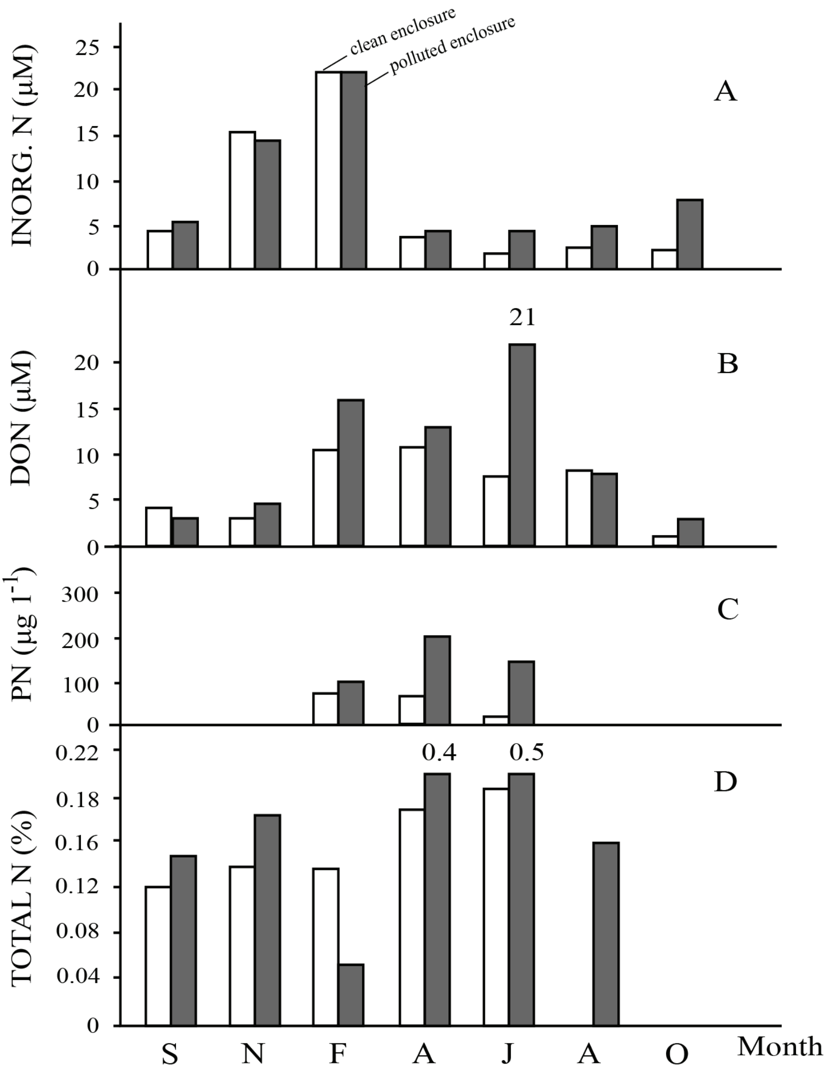

Figure 4: Variations of daily mean total inorganic nitrogen (NO_2^-+NO_3^-+NH_4^+) (A), dissolved organic nitrogen DON (B) and particulate nitrogen PN (C) concentrations in seawater, and total nitrogen content (D) in sediment in the experimental enclosures during the period September 1976 - October 1977.

Slika 4: Variacije povprečnih dnevnih koncentracij celotnega anorganskega dušika (NO_2^-+NO_3^-+NH_4^+) (A), raztopljenega organskega dušika DON (B) in suspendiranega dušika PN (C) v vodi ter koncentracij celotnega dušika v sedimentu (D) v poskusnih bazenih med septembrom 1976 in oktobrom 1977.

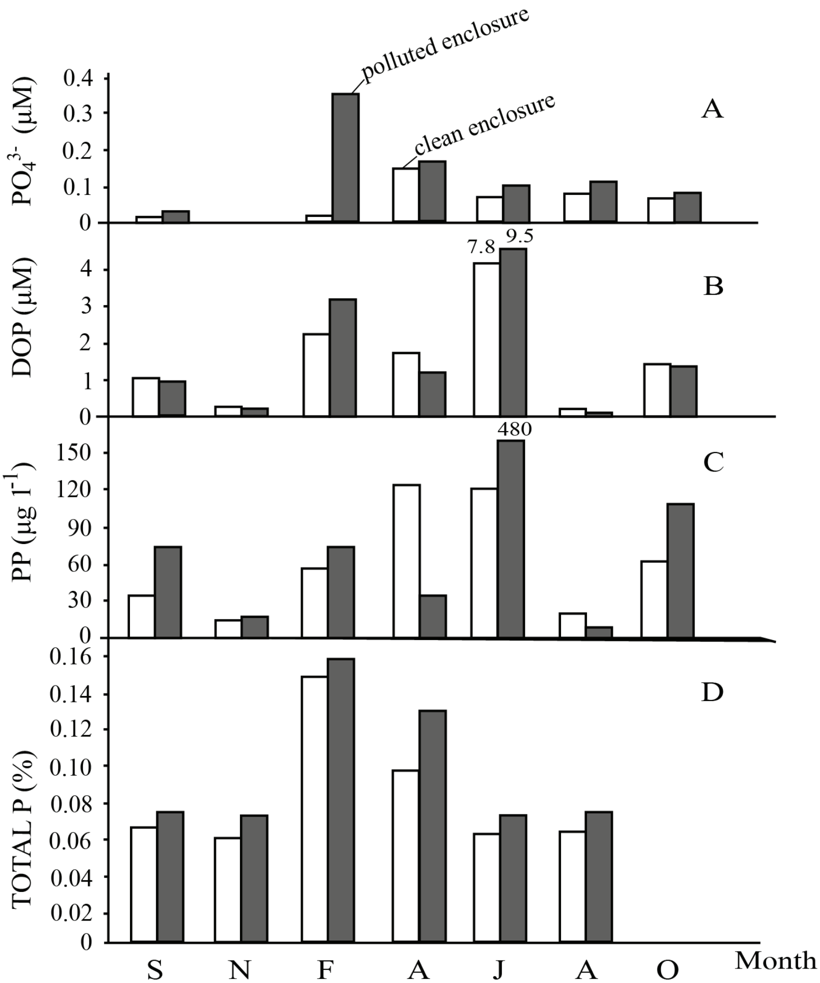

Figure 5: Variations of daily mean phosphate (A), dissolved organic phosphorus DOP (B) and particulate phosphorus PP (C) concentrations in seawater, and total phosphorus content in sediment (D) in the experimental enclosures during the period September 1976 – October 1977.

Slika 5: Variacije povprečnih dnevnih koncentracij fosfata (A), raztopljenega organskega fosforja DOP (B) in suspendiranega fosforja PP (C) v vodi ter koncentracij celotnega fosforja v sedimentu (D) v poskusnih bazenih med septembrom 1976 in oktobrom 1977.

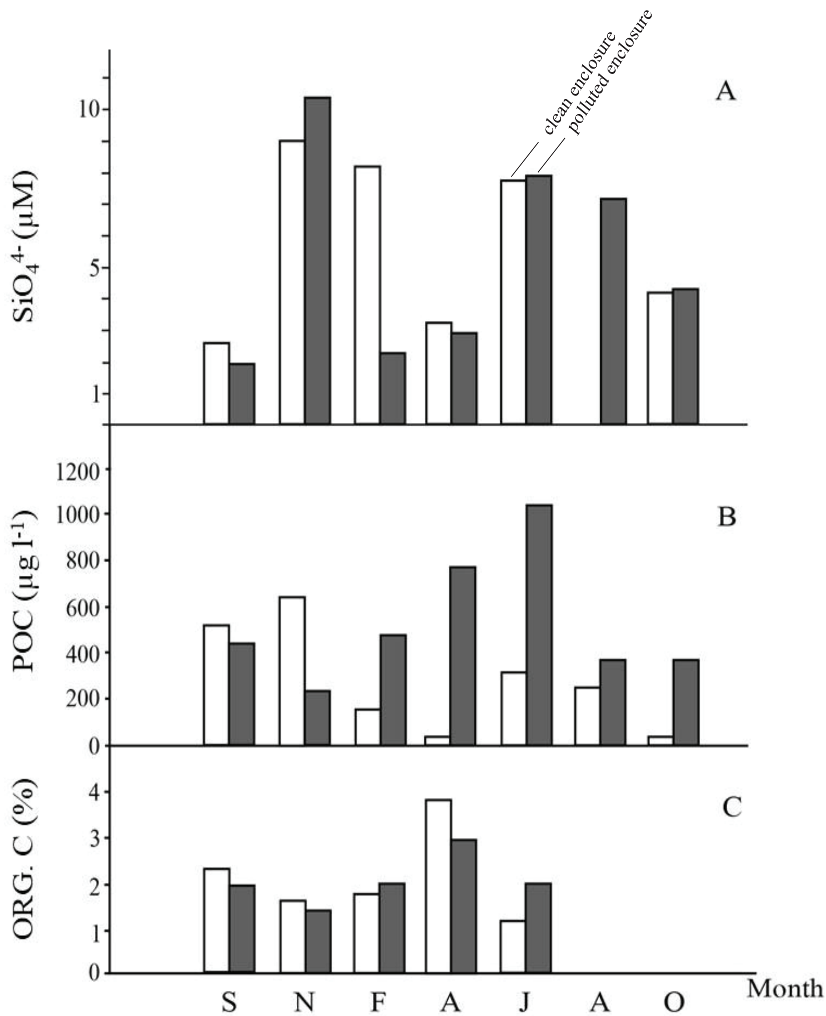

Figure 6: Variations of daily mean silicate (A) and particulate organic carbon POC concentrations (B) in seawater, and organic carbon content in sediment (C) in the experimental enclosures during the period September 1976 - October 1977.

Slika 6: Variacije povprečnih dnevnih koncentracij silikata (A) in suspendiranega organskega ogljika POC (B) v vodi ter koncentracij organskega ogljika v sedimentu (C) v poskusnih bazenih med septembrom 1976 in oktobrom 1977.

Discussion

From the results it is evident that the community in PB assimilated high nutrient input from the sewage discharges quite quickly. In addition, the dilution present due to the tidal exchange of water, particularly in spring and late summer, between the enclosures and the surrounding lagoon led to uniform nutrient concentrations after some hours in the two basins. Nutrients introduced in PB were also adsorbed onto suspended and sedimented particles, such as the adsorption of NH_4^+ on aluminosilicates (Faganeli et al. 1991), and PO_4^{3-} on Fe-oxides and the precipitation of authigenic P minerals (Ogrinc and Faganeli 2006) which was reflected in higher $P_{tot.}$ content in the PB sediments compared to CB. Rather low SiO_4^{4-} concentrations (2-3 μM) detected in September 1976 and April 1977 in both enclosures were likely due to the assimilation of Si by benthic diatoms (Welker et al. 2002) while high levels found in other periods may indicate dissolution of biogenic Si accumulating in sediments over time (Faganeli and Ogrinc 2009, Škrinjar et al. 2012). A significant decrease of NH_4^+ concentrations in PB after the sewage input, which prevails over NO_3^- in both enclosures, suggested NH_4^+ as an active inorganic nitrogen nutrient in assimilation processes. The high NH_4^+ concentrations could be also attributed to decomposition and mineralization of DON and bacterial reduction of NO_3^- during night anoxia in PB in June 1977 (Canfield et al. 2005). Levels of N, P and Si nutrients in CB, around those described for the Gulf of Trieste in the same period (Faganeli and Tušnik 1983, Faganeli 1983), supported rather high biomass in the lagoon. This indicated an efficient recycling of biogenic elements within the lagoon community (Mee 1978). High inorganic N/P ratios (>15, molar) observed in both enclosures suggest that an excess of nitrogen was present and that the whole lagoon ecosystem should be phosphorus limited in accordance with phosphorus limitation of the Sečovlje saltern (Škrinjar et al. 2012), Grado and Marano Lagoon (De Vittor et al. 2012, Petranich et al. 2018) and of pelagic primary production in the waters of the Gulf of Trieste (Faganeli and Tušnik 1983) postulated by high inorganic N/P ratios. Nutrient regeneration and fluxes at the sediment-water interface, significantly impacted by the infauna bioturbation activity (Cermelj et al. 1997; Thouzeau et al. 2007) and redox conditions (Faganeli and Ogrinc 2009; Rigaud et al. 2013), is likely the primary natural source of nutrients available for assimilation processes (De Vittor et al. 2012, Petranich et al. 2018, Testa et al. 2021) in CB, especially in the warmer period of the year, since the natural nutrient input by freshwater inflows into the lagoon is limited. High benthic effluxes of NH_4^+ and in lesser extent of PO_4^{3-} were measured during the summer period in the shallow Grado and Marano Lagoon (De Vittor et al. 2012) as well in the Gulf of Trieste (Bertuzzi et al. 1997). The lowest inorganic N/P ratios observed in April 1977 in both enclosures, and in June 1977 in CB, in correlation with high community production, were due to decreasing inorganic nitrogen content in sea water probably as a result of enhanced assimilation by primary producers influencing the limitation conditions. The highest concentrations of DON, DOP, POC, PN, and PP in seawater and $N_{tot..}$ in sediment observed in June 1977 in PB was mostly the consequence of macrophyte decomposition after an intense growth illustrated by the high community production measured in April 1977. The high oxygen consumption of decomposing organic matter caused the night anoxia and the proliferation of sulphate reducing bacteria producing H_2S and pyrite in sediments (Hines et al. 1997).

The gross production estimations based on O_2 and DIC measurements indicated the stimulation effect of nutrients added by the sewage discharges in PB in spring 1977. No attempt was made to discriminate between the production of phytoplankton and benthic macroalgae, but phytoplankton biomass measurements in PB clearly indicated the reduction of phytoplankton biomass in parallel with the increased biomass of benthic macroalgae (Fanuko 1984). In the production experiments in an open lagoon in Florida (USA) it was found that in shallow water (<1 m deep) the benthic macrophytes and microalgae dominated the primary production of the lagoon community (Mee 1978). This was likely also the case of the Lagoon of Strunjan. The estimated yearly production in both enclosures were similar, despite the higher production in PB during spring 1977. Decomposition of dead macroalgae and the anoxic conditions stopped the vigorous primary production in PB. The production in CB reached the highest values only in summer. A comparison with the Marano and Grado Lagoon (De Vittor et al. 2012) where the waters are deeper shows higher (gross) production values in our study area.

The lagoon environment at Strunjan accumulated nutrients introduced via sewage discharges, in particular through benthic macroalgae assimilation and subsequent deposition and intense decomposition of dead plant material on the bottom. The tentative mas balance, assuming the Redfield ratio (Redfield et al. 1963) for N and P, and 50 % of those of C for Si assimilation (Brezezinski et al. 2003) and estimated from the carbon net production (assumed as ½ of the gross production), showed that <2, 0.2 and 10% of inorganic N, P and Si introduced by sewage, respectively, were assimilated in PB. The great majority of introduced chemical species were, therefore, exported by tides in the surrounding lagoon and deposited in PB especially PN and PP, which are composed of both organic (especially N) and inorganic (especially P) fractions, reflected in the low $C_{org.}/P_{tot.}$ ratios (4-16, molar) in surface sediments. The low $C_{org.}/P_{tot.}$ ratios could also be attributerd to formation of phosphate minerals, e.g. apatite (Ogrinc and Faganeli 2006). The high $C_{org.}/N_{tot.}$ ratios (>14, molar) in surface sediments indicated the prevalent degradation of N over C in the sedimentary organic matter in both enclosures (Ogrinc et al. 2005). Lower values (<14) found in November 1976 and June 1977 were more the direct consequence of the presence of microalgae, with a typical $C_{org.}/N_{tot.}$ ratio <10 (Faganeli et al. 2009), and *Ulva*, with typical $C_{org.}/N_{tot.}$ ratio of 11 (Faganeli et al. 1986), respectively.

Conclusions

The present study indicates that nutrients introduced by sewage into a partially closed lagoon environment and diluted by tides were immobilized by enhanced production, especially by benthic macroalgae. Afterwards, the dead organic matter settled and decomposed producing anoxic conditions in late spring. Decomposition led to high levels of dissolved and suspended organic nutrients in the water and $N_{tot..}$ in sediments bearing in mind that the nutrients introduced with sewage were also to some extent adsorbed on suspended and sedimented particles. The daily mean gross production estimated on an annual basis showed no quantitative differences between the two enclosures. Differences arose in the temporal succession of the studied events. This lagoon environment provides an example of quite intensive nutrient recycling.

Povzetek

Med poskusom v Strunjanski laguni v obdobju 1976-77 smo dnevno uvajali 300l primarno čiščenih komunalnih odplak mesta Piran v posebej zgrajeni bazen. Drugi bazen je služil za primerjavo. V odbobju enega leta smo s približno dvomesečno frekvenco spremljali nihanja koncentracij hranil N, P in Si ter bruto produkcije na osnovi dnevnih variacij koncentracije O_2 in DIC. Na osnovi visokega anorganskega razmerja N/P sklepamo na limitativnost P. Vnešena hranila, redčena s plimovanjem, so povečala produkcijo predvsem bentoških makroalg, ki so odmrle pozno pomladi. Odmrla organska snov se je nato posedla in razgrajevala ter povzročila nastanek anoksije ponoči in visokih koncentracij raztopljene in suspendirane organske snovi in celotnega dušika v sedimentu. Srednja dnevna bruto produkcija v celoletnem obdobju ni pokazala velikh razlik med bazenoma, medtem ko so bile le-te opazne v časovnem poteku študiranih procesov. Iz poskusa je razvidno dokaj intenzivno kroženje vnešenih hranil.

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Pol stoletja morskih ved na Morski biološki postaji Piran Nacionalnega inštituta za biologijo (1969 – 2019)

Half a century of marine science at the Marine Biology Station Piran of the National Institute of Biology (1969 – 2019)

Patricija Mozetič

Morska biološka postaja, Nacionalni inštitut za biologijo, Piran, Fornače 41, 6330 Piran

Korespondenca: patricija.mozetic@nib.si

Morska biološka postaja Piran, ena od raziskovalnih oddelkov Nacionalnega inštituta za biologijo (v nadaljevanju MBP), je leta 2019 je praznovala častitljivih 50 let (Slika 1). V pol stoletja dolgem delovanju, se je MBP od skromnih začetkov razvila v pomembno znanstveno–raziskovalno ustanovo, ki se lahko z znanjem in rezultati dela ter sodobno raziskovalno infrastrukturo mirno postavi ob bok sredozemskim in drugim morskim raziskovalnim postajam širom sveta.

Vse se je začelo l. 1969, ko je bila, takrat kot del Inštituta za biologijo Univerze v Ljubljani, ustanovljena MBP, in so ji bili dodeljeni prostori v vili z lepim vrtom na Sončni poti v Portorožu. Zasluge za ustanovitev MBP je treba pripisati tedanjemu direktorju Inštituta za biologijo, prof. Francu Sušniku in prof. Jožetu Štirnu, ki je že pred formalno ustanovitvijo MBP uvidel potrebo po tovrstni raziskovalni ustanovi v Sloveniji. Začetki so bili skromni, z maloštevilno skupino 11 zaposlenih, od tega z enim doktorjem znanosti in šestimi biologi, osnovno laboratorijsko in vzorčevalno opremo in lesenim čolnom, ki nam je več desetletij zvesto služil, danes pa še vedno razveseljuje novega lastnika.

Pod vodstvom prof. Štirna in kasneje prof. Miroslava Zeia je v 70. in 80. letih postaja rasla, vpeljala znanstvene pristope raziskav, ki so naslavljale ekološke značilnosti pelaških in bentoških združb, populacijsko ekologijo malih pelaških rib in evtrofikacije severnega Jadrana, ter postala aktivna tudi v medvladnih organizacijah oz. njihovih programih (FAO, UNESCO, UNEP/MAP), ki so med drugim omogočili nakup prepotrebnih instrumentov in laboratorijskega materiala. Veliko kasneje, po osamosvojitvi Slovenije, je MBP in postala središčno mesto UNEP-ovega programa MED POL (program monitoringa onesnaženja Sredozemskega morja), akcijski naslov Nacionalnega odbora Medvladne oceanografske komisije pri UNESCO -u, Slovenija pa članica Sredozemske komisije za raziskovanje morja. Zasluge, da je mlada država postala članica medvladnih »morskih« organizacij oz. programov, gredo v veliki meri prav MBP.

V 70. in 80. letih so se sodelavci MBP večkrat udeležili jadranskih ekspedicij z vojaško raziskovalno ladjo »Andrija Mohorovičić, ki je raziskovalne mornarje popeljala po vsem Jadranu - od Tržaškega zaliva pa vse do Jonskega morja. To je bilo tudi obdobje izjemno plodne izobraževalne dejavnosti v okviru mednarodne podiplomske poletne šole s tujimi študenti in predavatelji, ki jo je organiziral prof. Štirn, zaradi česar je Medvladna oceanografska komisija pri UNESCU podelila MBP status regionalnega izobraževalnega centra za temeljno in aplikativno ekologijo.

Laboratoriji na Sončni poti so kmalu postali premajhni in neprimerni za raziskovalno delo, zato se je postaja l. 1980 ob pomoči piranske občine, takratne Raziskovalne skupnosti Slovenije in delno tudi lastnega vložka preselila v preurejene prostore opuščene tovarne mila Salvetti na Fornačah v Piranu, kjer je na temeljih nekdanje tovarne še danes.

V drugi polovici 80. let je vodenje postaje prevzela prof. Alenka Malej in to delo zavzeto opravljala 23 let. To so bili razburkani časi z veliko prelomnicami, ki so pomembno zaznamovale razvoj postaje, zato jih velja nekaj izpostaviti:

- V letih 1990-1993 je potekal do tedaj največji mednarodni projekt v okviru delovne skupine Alpe-Jadran, ki je povezal raziskovalne institucije ob severnem Jadranu iz Rovinja, Pirana, Trsta in Benetk, pa tudi veliko bolj oddaljeno Univerzo na Dunaju, z namenom proučevanja izvora, sestave in razvoja enega večjih ekoloških problemov tistega časa - želatinastih agregatov, med ljudmi pa se je za ta nevšečni pojav prijel izraz sluzenje morja. Projekt je omogočil do takrat prva in kasneje neponovljiva, časovno usklajena mesečna vzorčenja omenjenih inštitutov, ki so podala sinoptično sliko razmer v severnem Jadranu za razumevanje tega pojava.
- Leta 1994 je MBP prvič postala del mednarodnega konzorcija za evropski projekt iz 4. Okvirnega programa, ki je bil sploh prvi tovrstni projekt Inštituta za biologijo. Sledili so mu številni drugi mednarodni projekti, ki so omogočili raziskovalno delo, mreženje, nakup velike opreme za operativno oceanografijo in posodobitev visokega šolstva. Omeniti velja tudi več kot 30 bilateralnih znanstvenoraziskovalnih sodelovanj z raziskovalci iz 12 držav.
- Pomemben mejnik pri razvoju operativne oceanografije je pomenila ustanovitev Infrastrukturnega centra MBP leta 1995, ki je sprva vključeval obe plovili in obalno oceanografsko postajo ali bojo, zasidrano dve milji stran od piranske punte, kasneje pa še visokofrekvenčni radar in večjo laboratorijsko opremo. Najnovejša pridobitev centra je vrstični elektronski mikroskop, ki je bil leta 2020 kupljen iz sredstev projekta LifeWatch-Slovenija. Skozi relativno kratko zgodovino operativne oceanografije so se zvrstile tri boje; zadnja in tudi najbolj izpopolnjena boja Vida je tudi ena izmed bolj poznanih delov oz. ponudnikov storitev MBP med laično javnostjo. Meteorološki in oceanografski podatki, ki se na boji nepretrgoma beležijo od leta 2002 dalje, so uporabni v znanstvene namene, potrebni za prognostične modele Agencije RS za okolje, preko spleta pa prosto dostopni vsem zainteresiranim - od kopalcev, ribičev do jadralcev.
- Sploh prvo popolnoma novo raziskovalno plovilo je MBP dobila šele leta 2000, torej po dobrih 30 letih obstoja. 12-metrska raziskovalna ladja Sagita s pripadajočo merilno in vzorčevalno opremo in delovno površino predstavlja plavajoč laboratorij, na kateri je možno opravljati tudi zahtevnejša laboratorijska dela in je v svoji 20-letni zgodovini odplula vse do Mljetskih jezer na Hrvaškem.
- Zares veliko prelomnico pa je zaznamoval začetek gradnje nove stavbe MBP leta 2001, za kar je, poleg tedanje vodje, dr. Alenke Malej, najbolj zaslužna tedanja direktorica NIB prof. Tamara Lah Turnšek, ki je s svojim prizadevanjem in vztrajnostjo prepričala pristojne na ministrstvu o nujnosti novih in večjih prostorov na Fornačah. V času gradnje, ki je potekala v dveh fazah, smo od leta 2001 do 2004 oz. 2006 živeli dobesedno na gradbišču, a kljub vsemu ni izpadlo niti eno vzorčenje in opravljene so bile vse naloge, ki so bile predvidene v različnih projektih.

Danes je MBP multidisciplinarna skupina z 42 zaposlenimi, od tega je 23 raziskovalcev z doktoratom in 6 mladih raziskovalcev. Naša najpomembnejša raziskovalna področja so biologija in ekologija morja in obalnega pasu, biogeokemično kroženje snovi, fizikalna oceanografija, podnebne spremembe, onesnaževanje morja, varstvo narave in okolja ter morska biotehnologija. Zanimivo pa je, da so raziskave biodiverzitete, tako kot pred 50. leti, osrednji steber naše dejavnosti tudi danes in so vključene v mnoge temeljne in aplikativne projekte, a se dopolnjujejo in povezujejo z modernimi metodami in drugimi biološkimi področji oz. naravoslovnimi vedami. Glavi okvir temeljnih raziskav predstavlja raziskovalni program Agencije za raziskovalno dejavnost RS »Raziskave obalnega morja«, ki skupaj z Instrumentalnim centrom MBP zagotavlja do neke mere stabilen vir zgolj polovice vseh prihodkov skupine. Preostali del pridobimo z izvajanjem javne službe za organe ministrstev, iz drugih domačih in mednarodnih projektov, manjši del pa iz sodelovanja z gospodarstvom.

Prav gotovo pa so širjenje znanja o morju, ozaveščanje javnosti ter promocija znanosti tista področja, po katerih smo še posebej prepoznavni. Sodelavci MBP predavajo na štirih slovenskih univerzah in na Mednarodni podiplomski šoli Jožefa Stefana. Zelo bogata je tudi naša publicistična dejavnost; od pionirskih del prof. Štirna in prof. Zeia pa do danes je izšlo 17 znanstvenih in 14 strokovnih monografij.

Naj predstavim le nekaj številk, ki nas opisujejo in označujejo naše dosežke v prve pol stoletja:

- Na MBP je ustvarjalo 92 raziskovalcev ter strokovnih, tehničnih in administrativnih sodelavcev.
- Skozi zgodovino MBP se je zvrstilo 6 vodij: poleg že omenjenih dr. Štirna, Zeia in Malejeve, so postajo v zadnjem desetletju vodili dr. Vlado Malačič, dr. Andreja Ramšak in avtorica tega zapisa.
- Na MBP je nastalo 45 doktorskih disertacij, kar vključuje tako doktorande kot mentorstva. Med doktorandi je 26 takih, ki so se izobraževali v okviru programa mladih raziskovalcev in stažistov. Še veliko več je bilo mentorstev pri diplomskih in magistrskih nalogah ter študentov in dijakov, ki so tu opravljali študentsko prakso ali delo.
- Pohvalimo se lahko s tremi Fulbrightovimi štipendisti, prejemnico individualne štipendije iz Marie Sklodowska Curie programa ter številnimi štipendijami iz ZDA, Japonske, VB, Švedske, Italije, Francije, mednarodnih mrež odličnosti, stanovskih društev in Ad Future, ki so omogočile izpopolnjevanje v tujini za mesec dni do dveh let.
- Prejeli smo 22 priznanj in nagrad, tako domačih kot tujih, ki so bile podeljene posameznikom ali skupinam raziskovalcev.
- Odkar smo l. 2007 prvič organizirali Dan odprtih vrat MBP, nas je obiskalo več kot 3000 učencev in drugih obiskovalcev.

Naj zaključim z mislijo iz prispevka, ki je izšel v posebni številki revije Proteus ob 25. letnici Morske biološke postaje. Belgijski kralj Leopold I je dejal nekako takole: »*Dežela, ki ima morje, ni nikoli majhna*«. Prepričana sem, da so v prvih 50-ih letih obstoja k zavedanju Slovenije o pomenu morja svoj kamenček v mozaiku prispevali sodelavci Morske biološke postaje Piran z raziskovalnim delom in večanjem morske pismenosti.

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Slika 1: Slavnostna prireditev ob 50-letnici Morske biološke postaje Piran Nacionalnega inštituta za biologijo je bila 10. 10. 2019 v Avditoriju Portorož. Na sliki stojijo od leve proti desni: mag. Robert Turk (Zavod RS za varstvo narave), prof. dr. Tamara Lah Turnšek (nekdanja direktorica NIB), mag. Dejan Židan (tedanji predsednik Državnega zbora), prof. dr. Alenka Malej (nekdanja vodja MBP), izr. prof. dr. Matjaž Kuntner (tedanji direktor NIB), izr. prof. dr. Patricija Mozetič (vodja MBP). (Foto: Marko Alpner)

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Moji spomini na prof. Štirna

My memories of prof. Štirn

Mihael Jožef Toman

Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, Večna pot 111, SI-1000 Ljubljana, Slovenija

Korespondenca: MihaelJozef.Toman@bf.uni-lj.si

Zaslužni profesor dr. Jože Štirn, v 70 letih moj profesor biologije morja, je odplul na svoje zadnje potovanje. Moja zgodba povezana z njim je zelo osebna, pa tudi profesorska in akademska. V moje življenje je vstopil pred natanko 50 leti, leta 1971 v Strunjanu, kjer sem kot gimnazijec in zmagovalec na biološkem tekmovanju dobil teden dni nagradnega bivanja in spoznavanja z življenjem v morju. Za Gorenjca je bil to moj prvi stik z morjem in življenjem v njem. Prof. Štirn mi je deloval kot prijazen morski volk, s čepico na glavi, s čudnimi kovinskimi flašami ob sebi, oblečen v čudno črno obleko in masko na glavi. Prijaznost in neposrednost profesorja sta premagala vse moje strahove. Moji prvi koraki v morski svet so bili njegovo delo. In kasneje tudi v svet celinskih voda. Nikoli nisem pozabil mikroskopov in lup v Strunjanski vili na obali, kjer so danes apartmaji, lesenih lop ob obali, kjer so hranili akvalunge in kjer je upokojeni prof. Valentinčič delal poskuse z morskimi zvezdami in profesor Štirn je bil pogosto z njim. Tam sem spoznal Andreja Avguština, strastnega potapljača, ki je bil povezan z morsko biološko postajo od vsega začetka. Imel je podjetje za proizvodnjo različnih plastičnih izdelkov, med njimi so bile številne posodice za shranjevanje bioloških vzorcev in še danes jih imam nekaj s fiksiranimi morskimi organizmi. Močno sva se spoprijateljila in bil je moj mecen v pravem pomenu besede vse do svoje smrti. Z njegovo finančno podporo sem lahko študiral, takšni časi so zagotovo minili. Profesor Štirn pa je postal moj neformalni mentor, ki me je spremljal v času študija in tudi kasneje, ko sem že sam postal profesor te ljubljanske univerze in mojega Oddelka za biologijo.

Še kot gimnazijec sem se v Strunjanu spoznaval z vzorci morskega dna in zame z neznanimi organizmi. Mišo, kot so ga klicali le prijatelji, je znal v mladih poglobiti ljubezen do biologije, narave, morja in življenja nasploh. Še preden je minilo prvo poletje najinega druženja sva vzljubila eden drugega in več kot le poznanstvo je trajalo do konca. Kako osebno je bilo najino druženje pove tudi dejstvo, da me je vedno klical Metuljček in bil je eden tistih, ki mi je ta vzdevek tudi prilepil. Metulji so bili moja prva biološka ljubezen. Veliko kasneje sem ga sam upal poklicati Mišo.

Redno sva se srečevala na fakulteti, poslušal sem njegova predavanja o morski biologiji, navduševal je s pripovedovanjem zgodb s svojih ekspedicij po svetu. Vedno nam je govoril, da se ne smemo zapirati v ozke meje svojega okolja, pojdite v svet je dejal, raziskujte naravo in ljudi. Zato je bil zame tudi eden prvih glasnikov varstva okolja, ko to še ni bilo modno. Vizionarsko je začutil neumnosti, kot je dejal, ki jih vsak dan znova in vsak dan intenzivneje počnemo ljudje, v želji, da bi naravo nadvladali, jo pokorili in izkoriščali. Naravo in okolje je razumel kot povezano celoto, zato ni nerazumljivo, da se je z enako močno vnemo loteval onesnaževanj morij, kopnega in jezer. Veliko časa je preživel na Bledu in bil posebej zavzet za reševanje Blejskega jezera. Kritično je pisal o sanaciji, ki so jo vodili predvsem inženirci, se izjemno angažiral pri ohranitvi lehnjakotvornih helokrenih izvirov Brje pri Bledu, ki jim je grozila potopitev in druga akumulacija na Savi za akumulacijo Moste. To je tudi bilo najino skupno zavzemanje za delček lepote pod Triglavom, ki sva jo čutila, jaz kot domorodec, on kot občasni prebivalec blejskega okoliša. Veliko sem se takrat naučil od njega, predvsem argumentirane strpnosti v pogovorih z drugače mislečimi. Tudi za nastopanje v javnosti me je navduševal. Neštetokrat je poudaril, da smo raziskovalci in univerzitetni profesorji dolžni, da izražamo svoja mnenja v družbi, da se ne zapiramo v svoje kabinete in raziskujemo samo zato, ker nas nekaj zanima in so naši rezultati v znanstvenih člankih dostopni omejenemu številu bralcev. In prav to je danes še kako pomembno, ko je ekologija postala varstvo okolja in varstvo okolja ekologija in ko je stroka pogosto obrobna, necenjena, v ospredju pa so samooklicani ekologi brez meja. Ja, profesor Štirn je bil tudi pionir ozaveščanja o okoljskih problemih in njegovo sporočilo, da smo akademsko izobraženi naravoslovci prvi glas ljudstva proti pogoltnosti kapitala in onesnaževanja vseh vrst bo vedno aktualno.

In na koncu, dragi Mišo, tvoja izjemna srčnost in dobrota sta za vedno ostali v meni, ko sem kot mlad raziskovalec na Kemijskem inštitutu ostal z mlado družino, nekaj mesečno hčerko tako rekoč na cesti v Ljubljani, si nam ponudil svoje stanovanje in preživeli smo najtežja leta. Dobrota pa je tisto najvrednejše, ki vedno ostane za nekom, ki je odšel. Mišo, hvala ti za vse.

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Dragi Mišo,

V imenu Nacionalnega inštituta za biologijo se ti kot direktorica NIB iskreno zahvaljujem za vso pionirsko znanstveno in pedagoško delo ter organizacijske sposobnosti, ki so pripeljale do ustanovitve in napredovanja Morske biološke postaje, ki je enkraten in neprecenljiv del našega inštituta, pa tudi slovenske znanosti in družbe. V svojem imenu pa orisujem svoj osebni pogled na velikega znanstvenika in človeka, ki je bil moj stric in prijatelj.

Imela sem veliko srečo, da si bil od malih nog moj vzornik v občudovanju narave in taborniških veščinah. Slednje lahko ponazorim z dejstvom, da sem bila tudi sama navdušena tabornica, pripravil pa si me tudi do tega, da sem se skupaj s tvojo hčerko Senjo na skrivaj kopala v ledeno mrzli Soči blizu izvira in to ob 5^h zjutraj, saj si nama razložil, da se tako kalijo pravi taborniki. Naučil si me tudi kaditi srobot, človek nikoli ne ve kdaj, ob kakšnem mrzlem dnevu, takšne veščine pridejo prav. Medtem ko me je tvoj vpliv raziskovanja zaznamoval za celo življenje, me na srečo kajenje srobota ni tako prevzelo, da bi imelo dolgoročne posledice.

Bil si vedno zanimiv sogovornik in pripovedovalec zgodb, komaj sem čakala, da sva poklepetala, ko sva se po daljši odsotnosti videla. Z ženo Baby sta mi kot otroku vedno prinesla kakšno arabsko darilce, kar je bilo imenitno, saj je dišalo po eksotičnih krajih. V zrelih letih si bil navdušen ribogojec in kmetovalec, sadove tega tvojega hobija pa je preizkušala tudi širša družina.

Še posebno mi je bilo všeč, da si svoje bogato znanje uporabil pri iskanju praktičnih rešitev, tako pri reševanju okolijskih vprašanj kot pri marikulturi in pomorstvu. Imel si moč in pogum, da si se vedno strokovno in družbeno izpostavil kadar si precenil, da je tvoje znanje in prepričanje potrebno izpostaviti, ne glede na možne posledice in občasno nestrinjanje okolice. V resnici si bil človek akcije in si ves čas stopal pred časom. Tak si bil, ker ti je bilo mar in ker si bil družbeno odgovoren.

Zagotovo me je kot mnoge mlade zaznamovala tvoja velika znanstvena radovednost, s katero si začaral sogovornike in jih popeljal v svet znanosti, kjer vedno odkrivamo nekaj novega.

Veliko sva razpravljala o različnih vidikih znanosti in družbenega dogajanja nasploh. Zanimalo te je s čim se raziskovalno ukvarjam. Hvala ti za vse pozornosti, zanimive pogovore in pogled na znanstvena dejstva iz drugega zornega kota.

Zelo te bomo pogrešali, a vem, da smo bili vsi, ki smo te poznali od blizu, privilegirani. Tvoj iskrivi intelekt in tvoje zgodbe bodo vedno z nami, saj so prav zgodbe tisto, kar ljudje tako močno potrebujemo.

V imenu Nacionalnega inštituta za biologijo in vseh, ki so te imeli radi, še posebno v imenu naravoljubcev, ki si jih zapeljal v svoj prečudoviti svet.

Prof. dr. Maja Ravnikar
Direktorica Nacionalnega inštituta za biologijo

INSTRUCTIONS FOR AUTHORS

1. Types of Articles

SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). In this category ABS also publishes methodological articles, in so far as they present an original method, which was not previously published elsewhere, or they present a new and original usage of an established method. The originality is judged by the editorial board if necessary after a consultation with the referees. The recommended length of an article including tables, graphs, and illustrations is up to fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

REVIEW ARTICLES will be published in the journal after consultation between the editorial board and the author. Review articles may be longer than fifteen (15) pages.

BRIEF NOTES are original articles from various biological fields (systematics, biochemistry, genetics, physiology, microbiology, ecology, etc.) that do not include a detailed theoretical discussion. Their aim is to acquaint readers with preliminary or partial results of research. They should not be longer than five (5) pages. Brief note articles shall be subject to peer review by one expert in the field.

CONGRESS NEWS acquaints readers with the content and conclusions of important congresses and seminars at home and abroad.

ASSOCIATION NEWS reports on the work of Slovene biology associations.

2. Originality of Articles

Manuscripts submitted for publication in *Acta Biologica Slovenica* should not contain previously published material and should not be under consideration for publication elsewhere.

3. Language

Articles and notes should be submitted in English, or as an exception in Slovene if the topic is very local. As a rule, congress and association news will appear in Slovene.

4. Titles of Articles

Title must be short, informative, and understandable. It must be written in English and in Slovene language. The title should be followed by the name and full address of the authors (and if possible, fax number and/or e-mail address). The affiliation and address of each author should be clearly marked as well as who is the corresponding author.

5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is up to 250 words, and for brief note articles, 100 words. Article must have an abstract in both English and Slovene.

6. Keywords

There should be no more than ten (10) keywords; they must reflect the field of research covered in the article. Authors must add keywords in English to articles written in Slovene.

7. Running title

This is a shorter version of the title that should contain no more than 60 characters with spaces.

8. Introduction
The introduction must refer only to topics presented in the article or brief note.

9. Illustrations and Tables
Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided in electronic form. Tables should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2; Tab. 1 and Sl. 1). A full name is used in the legend title (e.g. Figure 1, Table 2 etc.), written bold, followed by a short title of the figure or table, also in bold. Subpanels of a figure have to be unambiguously indicated with capital letters (A, B, …). Explanations associated with subpanels are given alphabetically, each starting with bold capital letter, a hyphen and followed by the text (A - text...).

10. The quality of graphic material
All the figures have to be submitted in the electronic form. The ABS publishes figures either in pure black and white or in halftones. Authors are kindly asked to prepare their figures in the correct form to avoid unnecessary delays in preparation for print, especially due to problems with insufficient contrast and resolution. Clarity and resolution of the information presented in graphical form is the responsibility of the author. Editors reserve the right to reject unclear and poorly readable pictures and graphical depictions. The resolution should be 300 d.p.i. minimum for halftones and 600 d.p.i. for pure black and white. The smallest numbers and lettering on the figure should not be smaller than 8 points (2 mm height). The thickness of lines should not be smaller than 0.5 points. The permitted font families are Times, Times New Roman, Helvetica and Arial, whereby all figures in the same article should have the same font type. The figures should be prepared in TIFF, EPS or PDF format, whereby TIFF (ending *.tif) is the preferred type. When saving figures in TIFF format we recommend the use of LZW or ZIP compression in order to reduce the file sizes. The photographs can be submitted in JPEG format (ending *.jpg) with low compression ratio. Editors reserve the right to reject the photos of poor quality. Before submitting a figure in EPS format make sure first, that all the characters are rendered correctly (e.g. by opening the file first in the programs Ghostview or GSview – depending on the operation system or in Adobe Photoshop). With PDF format make sure that lossless compression (LZW or ZIP) was used in the creation of the *.pdf file (JPEG, the default setting, is not suitable). Figures created in Microsoft Word, Excel, PowerPoint etc. will not be accepted without the conversion into one of the before mentioned formats. The same goes for graphics from other graphical programs (CorelDraw, Adobe Illustrator, etc.). The figures should be prepared in final size, published in the magazine. The dimensions are 12.5 cm maximum width and 19 cm maximum height (width and height of the text on a page).

11. Conclusions
Articles shall end with a summary of the main findings which may be written in point form.

12. Summary
Articles written in Slovene must contain a more extensive English summary. The reverse also applies.

13. Literature
References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (Trinajstić and Franjić 1994); if a work by three or more authors is cited, (Pullin et al. 1995); and if the reference appears in several works, (Honsig-Erlenburg et al. 1992, Ward 1994a, Allan 1995, Pullin et al. 1995). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c,

etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5–6).The bibliography shall be arranged in alphabetical order beginning with the surname of the first author, comma, the initials of the name(s) and continued in the same way with the rest of the authors, separated by commas. The names are followed by the year of publication, the title of the article, the full name of the journal (periodical), the volume, the number in parenthesis (optional), and the pages. Example:

Mielke, M.S., Almeida, A.A.F., Gomes, F.P., Aguilar, M.A.G., Mangabeira, P.A.O., 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of Genipa americana seedlings to soil flooding. Experimental Botany, 50(1), 221–231.

Books, chapters from books, reports, and congress anthologies use the following forms:

Allan, J.D., 1995. Stream Ecology. Structure and Function of Running Waters, 1st ed. Chapman & Hall, London, 388 pp.

Pullin, A.S., McLean, I.F.G., Webb, M.R., 1995. Ecology and Conservation of *Lycaena dispar*: British and European Perspectives. In: Pullin A. S. (ed.): Ecology and Conservation of Butterflies, 1st ed. Chapman & Hall, London, pp. 150-164.

Toman, M.J., 1992. Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1-7.

14. Format and Form of Articles

The manuscripts should be sent exclusively in electronic form. The format should be Microsoft Word (*.doc) or Rich text format (*.rtf) using Times New Roman 12 font with double spacing, align left only and margins of 3 cm on all sides on A4 pages. Paragraphs should be separated by an empty line. The title and chapters should be written bold in font size 14, also Times New Roman. Possible sub-chapter titles should be written in italic. All scientific names must be properly italicized. Used nomenclature source should be cited in the Methods section. The text and graphic material should be sent to the editor-in-chief as an e-mail attachment. For the purpose of review the main *.doc or *.rtf file should contain figures and tables included (each on its own page). However, when submitting the manuscript the figures also have to be sent as separate attached files in the form described under paragraph 10. All the pages (including tables and figures) have to be numbered. All articles must be proofread for professional and language errors before submission.

A manuscript element checklist (For a manuscript in Slovene language the same checklist is appropriately applied with a mirroring sequence of Slovene and English parts):
English title – (Times New Roman 14, bold)
Slovene title – (Times New Roman 14, bold)

Names of authors with clearly indicated addresses, affiliations and the name of the corresponding author – (Times New Roman 12)
Author(s) address(es) / institutional addresses – (Times New Roman 12)
Fax and/or e-mail of the corresponding author – (Times New Roman 12)
Keywords in English – (Times New Roman 12)
Keywords in Slovene – (Times New Roman 12)
Running title – (Times New Roman 12)
Abstract in English (Times New Roman 12, title – Times New Roman 14 bold)
Abstract in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)

Introduction – (Times New Roman 12, title – Times New Roman 14 bold)
Material and methods – (Times New Roman 12, title – Times New Roman 14 bold)
Results – (Times New Roman 12, title – Times New Roman 14 bold)
Discussion – (Times New Roman 12, title – Times New Roman 14 bold)
Summary in Slovene – (Times New Roman 12, title – Times New Roman 14 bold)
Figure legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, figure designation and figure title – Times New Roman 12 bold)
Table legends; each in English and in Slovene – (Times New Roman 12, title – Times New Roman 14 bold, table designation and table title – Times New Roman 12 bold)
Acknowledgements – (Times New Roman 12, title – Times New Roman 14 bold)
Literature – (Times New Roman 12, title – Times New Roman 14 bold)
Figures, one per page; figure designation indicated top left – (Times New Roman 12 bold)
Tables, one per page; table designation indicated top left – (Times New Roman 12 bold)
Page numbering – bottom right – (Times New Roman 12)

15. Peer Review

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. With articles written in Slovene and dealing with a very local topic, both reviewers will be Slovene. In the compulsory accompanying letter to the editor the authors must nominate one foreign and one Slovene reviewer. However, the final choice of referees is at the discretion of the Editorial Board. The referees will remain anonymous to the author. The possible outcomes of the review are: 1. Fully acceptable in its present form, 2. Basically acceptable, but requires minor revision, 3. Basically acceptable, but requires important revision, 4. May be acceptable, but only after major revision, 5. Unacceptable in anything like its present form. In the case of marks 3 and 4 the reviewers that have requested revisions have to accept the suitability of the corrections made. In case of rejection the corresponding author will receive a written negative decision of the editor-in-chief. The original material will be erased from the ABS archives and can be returned to the submitting author on special request. After publication the corresponding author will receive the *.pdf version of the paper.

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ZNANSTVENI ČLANKI – SCIENTIFIC ARTICLES:

KRATKA NOTICA / BRIEF NOTE:

IN MEMORIAM: