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THE INFLUENCE OF FISH CAGE AQUACULTURE ON BACTERIOPLANKTON IN THE BAY OF PIRAN (GULF OF TRIESTE, ADRIATIC SEA)

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ABSTRACT

The impact of fish cages on bacterioplankton was examined in enclosures containing seawater from cage and non-cage sites and natural plankton from the Bay of Piran (northern Adriatic). Nutrient enriched seawater stimulates bacterial production and abundance. This observation was further investigated in situ, *where for 3 months submerged bio-filter with* Schizobrachiella sanguinea *dominating fouling community was enclosed in mesocosm. Based on the results of bacterial production and abundance, we assumed that filter feeding could remove significant amount of bacteria attached on suspended particles and thus depleting bacterioplankton in the water column.*

Key words: aquaculture, environmental impact, bacterioplankton, cyanobacteria, Adriatic Sea

INFLUENZA DELLA PISCICOLTURA IN GABBIE SUL BATTERIOPLANCTON NELLA BAIA DI PIRANO (GOLFO DI TRIESTE, MARE ADRIATICO)

SINTESI

L'impatto di un allevamento di pesci in gabbie sul batterioplancton è stato esaminato in contenitori da laboratorio contenenti acqua marina proveniente dall'allevamento, da un sito prossimo all'allevamento e da una stazione di controllo con plancton naturale della baia di Pirano (Adriatico settentrionale). L'acqua proveniente dall'allevamento di pesci, arricchita in nutrienti, stimola la produzione e l'abbondanza batterica. Tale osservazione è stata successivamente investigata in situ *con l'immersione per tre mesi di bio-filtri con una comunità di fouling con predominante* Schizobrachiella sanguinea *inclusa nel mesocosmo. In base ai risultati di produzione ed abbondanza batterica, gli autori concludono che l'alimentazione di filtri può rimuovere quantità significanti di batteri legati al particellato sospeso e quindi diminuire il batterioplancton nella colonna d'acqua.*

Parole chiave: acquacoltura, impatto ambientale, batterioplancton, cianobatteri, mare Adriatico

INTRODUCTION

Bacteria play a central role in all major nutrient cycles in the marine environment (Azam, 1998). They are also most important organisms involved in different systems designed to treat domestic wastewaters. During the last few years, a series of papers was published addressing the impact of fish farming on water column chemistry and the effect on plankton distribution (Pitta *et al.*, 1999; Alongi *et al.*, 2002). However, the role of bacteria in the environment impacted by the caged fish culture has received little attention. Bacterioplankton community and abundance in pelagic system can be regulated by bottom-up and top down regulating forces. The fish farming activity may have direct and indirect effects on the components of the microbial food web either by changing nutrient status of the environment or by altering prey and predator community composition.

The present study is part of the EU funded project BIOFAQs (Bio-filtration and Aquaculture: an Evaluation of Hard Substrate Deployment Performance Within Mariculture Developments) (Angel, 2001; Black *et al.*, 2001). The main objective was to assess the effectiveness of deployment of artificial substrates (bio-filters) in the water column in reducing the environmental impacts of cage fish culture. By providing surface area for sessile biota and microbial colonization, bio-filters would facilitate uptake of organic and inorganic matter released by farmed fish. Within this project the microbial dynamics was followed in the enrichment experiment, using seawater from fish cage as nutrient source for enclosed plankton population, and seawater collected at unimpacted area. Additionally, microbial abundance and production was measured in mesocosm with enclosed bio-filters together with surrounding water during 2 diel cycles.

MATERIAL AND METHODS

Enrichment experiment was undertaken in July 2001 and lasted for 5 days (from 30th July to 5th August). Seawater was collected with Niskin sampler in the middle of the fish cage (treatment C), at the station about 200 m from the cages (station CL, 5 m depth) (treatment B), and at the stations in the middle of the Bay of Piran (45°30.20; 13°34.20) (control – treatment A) (Plate I: Fig. 1). Seawater was filtered through 0.22 μm pore size filters (Millipore), poured into the transparent polyethylene bottles (Nalgen, 8 litres), and one litre of concentrated seawater with entire plankton community was added. Experimental plankton communities were collected above the thermocline layer (14 m depth) in the middle of the Bay of Piran. Enclosures were incubated *in situ* at a depth of 2 m, to provide natural temperature and light conditions.

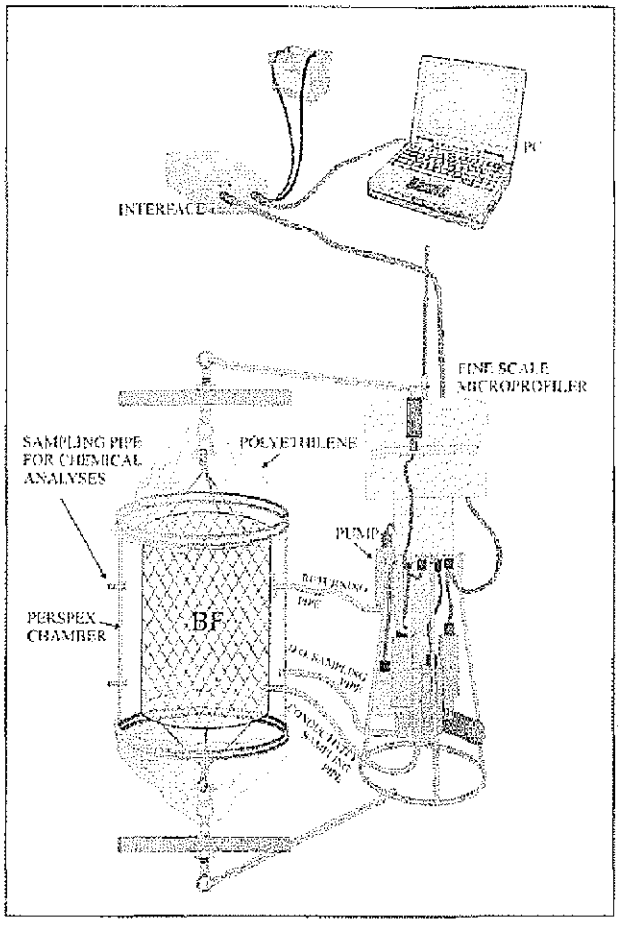

Fig. 1: Scheme of the mesocosm** in situ **enclosure experiment.
Sl. 1: Shema mezokozemskega eksperimenta v naravnem okolju.

In situ enclosure (mesocosm) (Fig. 1) experiments were performed on the 3rd and 5th months after bio-filters immersion into natural environment (Plate I: Fig. 1) at the station near fish cages (station SL), and at the station 200 m from the cages (station CL) (Plate II: Fig. 14). The mesocosm represented an *in situ* enclosure of selected bio-filter in natural environment. Each selected bio-filter was enclosed within a clear acrylic plastic octagon box, with a volume of about 110 litres (Fig. 1). A fine scale profiler with sensors (Sea Bird and Sea Tech) was connected to the chamber to measure, each hour, temperature, conductivity, dissolved oxygen and fluorescence. Divers collected samples for chemical and biological parameters from the chamber five times over 24 hours, from 26 to 27 September and 21 to 22 November at the station CL and at the station SL from 27 to 28 September 2001 and from 23 to 24 November 2001. At the same time intervals water column characteristics were performed using fine-scale profiler (CTD - University of Australia). The seawater for chemical and bio-

logical parameters was collected at three different depths (5 m, 8 m, 11 m) using a membrane pump (flow rate 20 l per minute).

Heterotrophic bacteria were counted according to the Porter and Feig protocol (Porter & Feig, 1980) and the biovolume of bacteria was converted into carbon biomass using 20 fg C cell^{-1} as the conversion factor (Lee & Fuhrman, 1987). Cyanobacteria were counted in green excitation using an epifluorescence microscope (Takahashi *et al.*, 1985). Bacterial production (BP) was measured by ^{3}H-leucine incorporations according to procedures of Smith & Azam (1992). Each time, 1.7 ml of seawater was incubated with L-[4,5-^{3}H] leucine (20 nM final, Amersham) for 1 hour at *in situ* temperature. All samples were done in triplicate. Bacterial production was calculated as in Simon & Azam (1989). At the same time, ^{3}H-thymidine incorporation method was used in parallel samples (Fuhrman & Azam, 1982). Triplicates of each sample were incubated for one hour at *in situ* temperature with 250μCi ^{3}H-thymidine l^{-1} (sp. act. 80 Ci mmol^{-1}, Amersham). Moles of thymidine incorporated were converted to cells produced by the conversion factor 2 x 10^{18} cells mole^{-1}.

RESULTS AND DISCUSSION

An enrichment experiment was set up to examine the impact of fish farming on nutrients and microplankton distribution. During the five days of incubation, natural bacterioplankton community showed significant enhancement on production rates and biomass accumulation in the enclosures with the water from the fish cage (treatment C) and nearby station (treatment B), compared to the seawater from the non-impacted area (treatment A). Abundance of bacteria increased from 0.5 to 4.3 x 10^{6} cells l^{-1} in the treatment B, from 0.6 to 3.8 x 10^{6} cells l^{-1} in the treatment C three days after the inoculation (Fig. 2). The highest bacterial production was measured on the second day with the value of 2.02 μg C l^{-1} h^{-1} in the treatment B, compared to the production of 1.01 μg C l^{-1} h^{-1} in the treatment C (Fig. 2). Cyanobacteria were more abundant in the treatment with seawater from fish farm (treatment C) and increased from 3.3 to 7.8 x 10^{7} cells l^{-1} in treatment B, and from 3.1 up to 11.0 x 10^{7} cells l^{-1} in treatment C within four days of incubation, and only up to 2.8 x 10^{7} cells l^{-1} in the control bottle. Natural population of heterotrophic bacteria quickly responded to the nutrient enriched seawater, preceding autotrophic organisms, which was also reported in previous studies (Pitta *et al.*, 1996; Malej *et al.*, 2003).

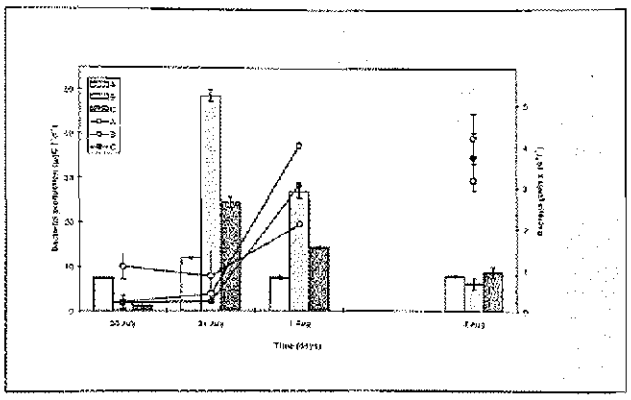

Fig. 2: The bacterial abundance (—) and production (□) in different nutrient treatments during the enrichment experiment lasting from 30th July to 5th August 2001.
Sl. 2: Gostota bakterij (—) in produkcija (□) v različnih hranilnih razmerah v obogatitvenem poizkusu v času od 30. julija do 5. avgusta 2001.

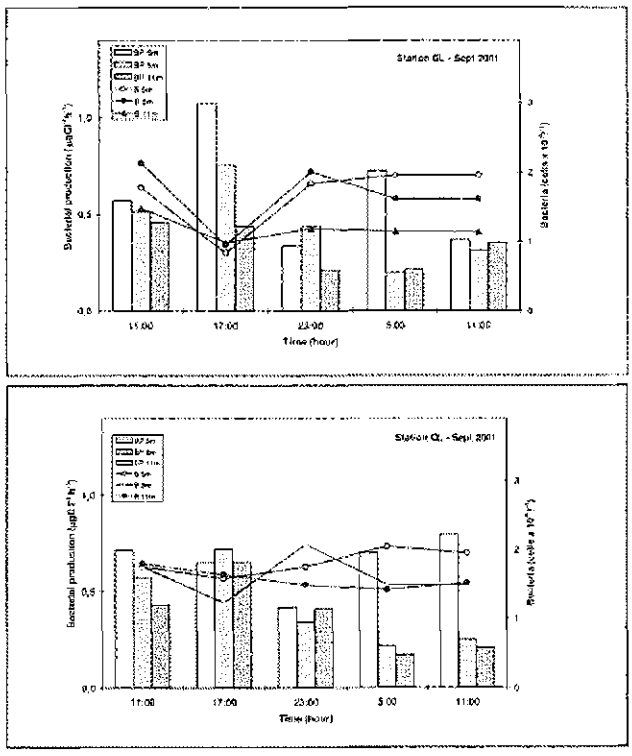

Fig. 3: Bacterial abundance and bacterial production in the water column during 24-hour cycle at the station near the fish cages (station SL) and the station 200m away from the cages (station CL) in September 2001.
Sl. 3: Gostota bakterij in bakterijska produkcija v vodnem stolpcu (5 m, 8 m, 11 m) na postaji blizu ribjih kletk (postaja SL) in na postaji, oddaljeni 200m od ribjih kletk (postaja CL) 24-mesečnem ciklu v septembru 2001.

Tab. 1: Average bacterial abundance, bacterial production (BP) and P/B ratio in the water column at the station near the fish cages (station SL) and the station located 200 m away (station CL) during 24-hour measurement from 26 to 28 September and from 27 to 29 November 2001.
Tab. 1: Povprečne gostote bakterij, bakterijske produkcije (BP) in P/B razmerja v vodnem stolpcu na postaji blizu ribjih kletk (postaja SL) in na postaji, oddaljeni 200 m (postaja CL) v 24-urnih meritvah od 26. do 28. septembra in od 27. do 29. novembra 2001.

Station	CL			SL			SL/CL
Date	No.	Average	± SD	No.	Average	± SD	%
26/28 September 2001							
Abundance (cells l^{-1})	11	1.57×10^9	3.75×10^8	11	1.49×10^9	4.57×10^8	95
BP (^{3}H-Thy) (µgC $l^{-1}d^{-1}$)	15	3.78	1.63	15	3.97	1.86	105
BP (^{3}H-Leu) (µgC $l^{-1}d^{-1}$)	15	4.80	2.27	15	5.84	3.09	122
P/B (d^{-1})	11	0.178		11	0.247		
27/29 November 2001							
Abundance (cells l^{-1})	8	1.15×10^9	1.29×10^8	8	1.10×10^9	7.64×10^7	96
BP (^{3}H-Leu) (µgC $l^{-1}d^{-1}$)	15	3.55	0.66	15	3.92	1.43	110
P/B (d^{-1})	8	0.154		8	0.175		

Based on laboratory results, we decided to examine the possible effect of fish farm on bacterioplankton dynamic in the field. During the study of diurnal dynamics, five samples at three different depths (5 m, 8 m and 11 m) were analysed for each station (Fig. 3). Vertical distribution of heterotrophic bacteria was similar at all sampled depths, and the abundance varied from 1.41×10^9 cells l^{-1} to 2.3×10^9 cells l^{-1}. The bacterial production measured as ^{3}H-leucine incorporation varied from 0.2 to 1.9 µg C $l^{-1}h^{-1}$ at the station CL and from 0.2 to 1.1 µg C $l^{-1}h^{-1}$ at the station SL, with the highest values at 5m depth over the 24 hour experiment (Fig. 3). Comparison between both sampling locations is presented in Table 1, based on the results of bacterial abundance and production rates during the 24-hour measurements in September and November. The average number of bacteria was 1.49×10^9 cells^{-1} (±3.8×10^8, n=11) at the station near the fish cages, compared to the average number of 1.57×10^9 cells^{-1} (±4.6×10^8, n=11) at the station located 200 m away. Bacterial production measured as ^{3}H-leucine incorporation was 5.84 µg C $l^{-1}d^{-1}$ (±3.1 µg C l^{-1} d^{-1}, n=15) and 4.8 µg C $l^{-1}d^{-1}$ (±2.3 µg C l^{-1} d^{-1}, n=15) at the station SL and CL, respectively. Similar were results of the bacterial production measured as ^{3}H-thymidine incorporation (Tab. 1). No difference between both stations was recorded in November. The average number of bacteria was 1.15×10^9 cells l^{-1} at the station CL and 1.10×10^9 cells l^{-1} at the station SL. The bacterial production was 3.92 µg C $l^{-1}d^{-1}$ at the station SL and 3.55 µg C $l^{-1}d^{-1}$ at the station SL (Tab. 1). The P/B ratios for the heterotrophic bacteria were from 0.070 to 0.342 for the station CL and from 0.071 to 0.642 at the station SL. Although the average biomass of bacteria was higher at the station located 200 m away from the fish farm, bacterial production was higher (5-22%) at the station near the fish cages. However, the abundance and production rates were in the range reported for the Gulf of Trieste (Turk *et al.*, 2001). Similar results were reported from other areas in the Mediterranean (Pitta *et al.*, 1999), when the plankton community structures and abundance were more dependent on seasonal environmental characteristics and locations than by the presence of fish farming.

In contrast to the results in the water column that did not show a significant difference, the results obtained in the enclosures were different, thus eliminating currents and tides. *In situ* mesocosm experiments were performed in order to relate microbial dynamics to sessile biota on bio-filter. The experiment was performed in

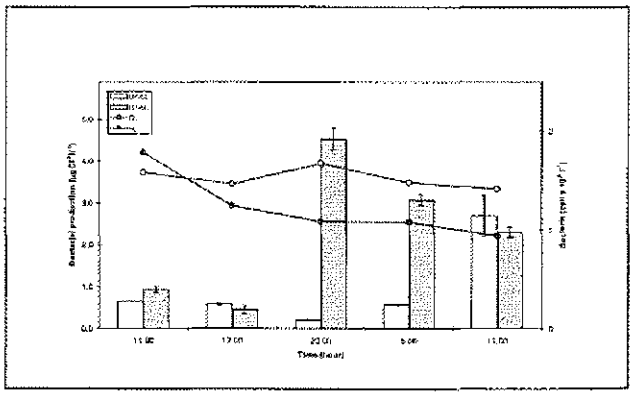

Fig. 4: Results of bacterial abundance and bacterial production in the mesocosm experiment at the station near the fish cages (station SL) and at the station location above 200 m away (station CL) during 24-hour cycle in September 2001.
Sl. 4: Gostota bakterij in bakterijska produkcija v mezokozemskem poizkusu na postaji blizu ribjih kletk (postaja SL) in na postaji, oddaljeni 200 m (postaja CL) v 24-urnem ciklu, septembra 2001.

September using the bio-filter, submerged for 3 months near the fish cages at a depth of 5 m. Dominating fouling community on the enclosed bio-filter was bryozoan *(Schizobrachiella sanguinea)* (Frumen *et al., this volume).* Over a diel cycle, bacterial numbers constantly decreased throughout the experiment from 2.2 x 10^9 cells l^{-1} to 1.1 x 10^9 cells l^{-1} at the station SL (Fig. 4). Contrary to bacterial abundance, production showed an increase from midnight and early morning up to 4.5 µg C l^{-1} h^{-1} in the enclosure with bio-filter at the station near the fish farm (station SL). According to high bacterial production and constant decrease in number during the night, it is assumed that the majority of the bacteria produced were consumed, presumably due to grazing of sessile organisms on bio-filters. Studies on bryozoans feeding indicate that small particles are principal food source (Hudges, 2001).

Our preliminary study did not reveal any large-scale eutrophication or significant differences between stations near the fish farm cages and open water stations. However, a pronounced response of bacterial community was observed after addition of enriched water from the fish farm in laboratory experiment and for *in situ* mesocosm experiment. Results from mesocosm experiment showed that filter feeding could remove significant amount of attached bacteria on suspended particles and thus deplete bacterioplankton in the water column. However, bacteria within the pelagic ecosystem recycle the excreted nutrients and additional investigations should be considered in the future.

VPLIV MARIKULTURE NA BAKTERIOPLANKTON V PIRANSKEM ZALIVU

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POVZETEK

Avtorici sta preučevali vpliv gojenja rib v kletkah na bakterioplankton v morski vodi z naravnim plnaktonom, vzeti z lokacij s kletkami in 200 m od njih v Piranskem zalivu. S hranilnimi snovmi obogatena morska voda spodbuja produkcijo in gostoto bakterij. Raziskave sta opravili tudi v naravnem okolju, v katerem je bil za 3 mesece potopljeni biofilter s prevladujočo vrsto Schizobrachiella sanguinea *obdan z mezokozmom. Glede na rezultate produkcije in gostote bakterij avtorici domnevata, da bi filtratorski organizmi s hranjenjem lahko odstranili precejšnjo količino bakterij na suspendiranih delcih in tako zmanjšajo vlogo bakterioplanktona v vodnem stolpcu.*

Ključne besede: marikultura, okoljski vplivi, bakterioplankton, cianobakterije, Jadransko morje

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