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PHYTOPLANKTON ASSEMBLAGES AT FISH FARM IN MASLINOVA BAY (THE ISLAND OF BRAČ)

S. Skejić, I. Marasović, Ž. Ninčević Gladan[1]

Summary

The aim of this study was to establish phytoplankton composition at the sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) fish farm in the middle Adriatic Sea. The investigation was performed from September 2005 to September 2006 at a station located in Maslinova Bay at the island of Brač. Considering the whole research period, diatoms generally prevailed in terms of abundance while dinoflagellates were particularly abundant in June. Number of species of diatoms in comparison to dinoflagellates through the investigated period was similar. From 111 species of phytoplankton found, there were 55 species of Bacillariophyceae (diatoms), 47 species of Dinophyta (dinoflagellates), 5 species of Prymnesiophyceae, 3 Chrysophyceae and 1 Euglenophyta. Among the diatoms, the majority of species belonged to genus *Chaetoceros*. The most represented dinoflagellate genera were *Oxytoxum* and *Gymnodinium*. There were no considerable differences in phytoplankton composition with respect to different depths, but seasonal influence was significant. Biodiversity and abundance ranges of phytoplankton species indicated good water conditions and there were no evident alterations induced by the increased release of nutrients.

Key words: fish farm, phytoplankton, taxonomic composition, Maslinova Bay

INTRODUCTION

Mariculture in Croatia mostly comprises production of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) in floating cages located in sheltered bays as well as tuna fish (*Thunnus thynnus*) in floating offshore cages (Katavić, 2003). Each farm produces significant amount of waste consisting of feed residue, feces and other metabolism products as well as certain chemicals, microorganisms and parasites. The degree of farm impact on water biota and sediments depends on the dimensioning of fish farm production, the number of deployment cages and water dynamics in a particular area (Karakassis et

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al., 1998). Although the mariculture is developing rapidly along the eastern Adriatic coast and plans are made for a multiple increase of production, little research has been done on the influence of fish farms on the biotic characteristics of the water column, especially on phytoplankton (Katavić, 2003).

Each ecosystem has specific factors that define a community within it (Harris, 1980). Which parameters will affect phytoplankton community depends on the interaction of all factors in the environment. Although phytoplankton grows autotrophically, utilising mineral nutrients, vitamins and trace elements, the heterotrophic properties of phyto-planktonic cells are also important, and much use is made by some phytoplankton species of organic molecules (Flynn, 1990; Arzul et al., 1996). Some experiments showed that effluent water from fish farms may regulate growth in some phytoplankton species. As such additions act differently on the growth of different species, fish farming is likely to markedly modify species composition in its vicinity (Arzul et al., 2001).

Therefore, the changes in phytoplankton taxonomic composition could be a good indicator of eutrophication process and the stability of an ecosystem.

The aim of this work was to determine the taxonomic composition of phytoplankton and to compare seasonal influence and fish farm influence on composition and abundance of the resident phytoplankton community.

MATERIAL AND METHODS

Study area

The present study focused on the sea bream and sea bass farm located in the semi enclosed Maslinova Bay on the Brač Island. The Bay is elongated in shape and extended in the north-south direction. The entrance of the Bay is about 350 m wide, while the length from the entrance to the northern part is 1.2 km. The Bay is the deepest at the entrance area (50 m), while in the northern part the depth decreases significantly to about 5 m. The geographical position of the Bay and sampling station (coordinates 43º 18’20.2”N, 16º 28’14.2”E) is shown in Figure 1.

Description of farming process

The fish farm was established in 1993 in the northern part of Maslinova Bay. Over the years, production increased and reached the production level of 200-300 tons per year. During the study period at the farm there were 32 cages for juvenile fish (volume 250 m^3) and 30 cages for commercial size fish (22 cages of 900 m^3 and 8 cages of 3000 m^3 volume). The maximum density of fish in cages prior to harvesting had been 13 kg m^{-3}. According to the volume of the cages on the farm, the growing fish biomass is likely to reach up to 400 tons. One breeding cycle of sea bass and sea bream takes up to 24 months, so every other year new fry is inoculated in cages and reared to the commercial size of 250 – 350 g.

The fry are fed 3 to 6 times a day, depending on the sea water temperature. Feeding is performed manually by throwing pressed and extruded pellets on the surface. Juvenile sea bream hatches in April, while sea bass hatches from April to August.

Figure 1. Map of the Adriatic Sea (A) and Maslinova Bay (B) with the sampling station
Slika 1. Karta Jadranskog mora (A) i uvala Maslinova (B) s istraživanom postajom

Composition and abundance of the phytoplankton community
The field work was conducted on monthly basis from September 2005 to September 2006. Water samples for the phytoplankton analyses were collected using 1.7 L Nansen bottles at the depth of 0, 5, 10, 20 and 30 m. At each of the depths, 250 ml of water were fixed in 2% glutaraldehyde solution. Sub-samples were sedimented for 24 hours in the 25 ml chambers. Determination of phytoplankton composition and cells counts were carried out by an inverted microscope "Olympus IX 50" (Utermöhl, 1958). The cells were counted in two transects under magnification of 200 and 400 times. The obtained data were converted into the number of phytoplankton cells per litre of seawater.

To determine the taxonomic composition of phytoplankton species we used the following references: Hustedt, 1930, 1931; Schiller, 1933, 1937; Dodge, 1982; Tomas, 1993, 1996; Viličić et al., 2002 and Viličić, 2004.

Numerical abundance data were analyzed by the software package PRIMER 6 (Clark and Gorley, 2006) and PERMANOVA (Anderson et al., 2008).

RESULTS AND DISCUSSION

Considering the seasonal distribution of diatoms in relation to dinoflagellates, there was a significantly higher proportion of diatoms during the colder part of the year. The succession model was similar to the expected one for this area in correspondence with to the temperature zone. However, some discrepancies were found due to high proliferation of diatoms in August, when they dominated the phytoplankton community with maximum abundance of 3.9×10^5 cells L^{-1} recorded at the depth of 5 m. Diatoms have also prevailed over dinoflagellates at the end of July, due to the strong north wind episode, which cooled

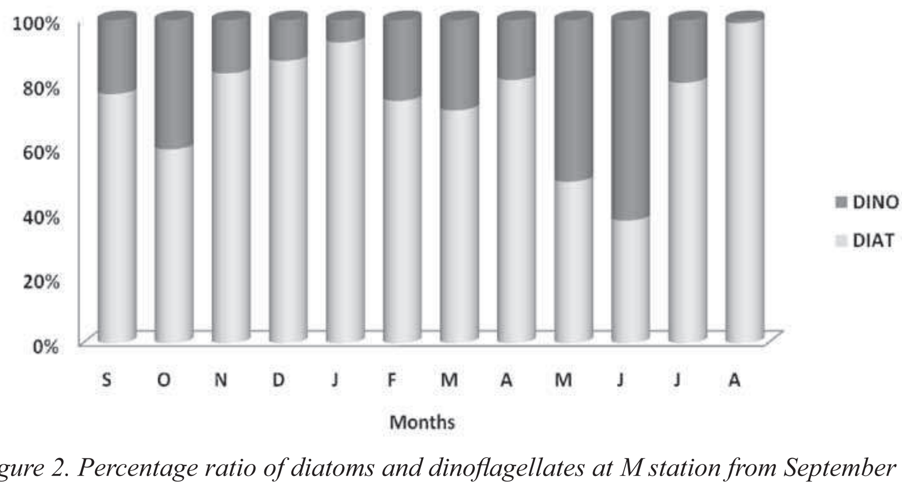

Figure 2. Percentage ratio of diatoms and dinoflagellates at M station from September 2005 to September 2006

Slika 2. Postotna zastupljenost dijatomeja i dinoflagelata na postaji M od rujna 2005. do rujna 2006 the surface layer and caused intensive mixing of the water column (Meteorological bulletin, 2006). Among phytoplankton groups, the diatoms adapted better to the turbulent environmental conditions than dinoflagellates (Casas et al., 1999), while stratified conditions in the water column favoured proliferation of microflagellates and dinoflagellates. Although the abundance of dinoflagellate was relatively low and did not exceed 10^3 in the colder part of the year, they increased in May, June and July (Figure 2). The maximum abundance of dinoflagellates comprised 9.3 x 10^4 cells L^{-1} in June which was mostly due to high numbers of gymnodinial dinoflagellates smaller than 20 µm.

Considering the whole research period, it was found that diatoms generally prevailed in number of species and in terms of abundance. The same was recorded in previous studies in Maslinova Bay (SUO IOR, 2000), as well as on other sea bream and sea bass farms (Tomec, 2004; Tomec, 2006). These findings have been in accordance with the results of Marasović and Pucher-Petković (1991) and Ninčević Gladan et al. (2009), who based their research on long term data series from Kaštela Bay (middle Adriatic), where diatoms were mostly dominant in the phytoplankton community.

However, with regard to the number of species, we have recorded approximately the same number in diatoms and dinoflagellates.

Qualitative analysis of the phytoplankton composition at the investigated site revealed the presence of 111 phytoplankton taxa: 55 diatoms, 47 dinoflagellates, 5 Prymnesiophyceae, 3 Chrysophyceae and 1 Euglenophyta (Table 1).

Most diatoms belonged to the Centrales order (33 species) out of which the most common genus *Chaetoceros* was represented with 17 species. Among pennatae diatoms (order Pennales) 22 taxa were recorded. The most abundant diatom was *Pseudo-nitzschia x,* an unidentified species morphologically different from other *Pseudo-nitzschia* species, which made 36.9% of total phytoplankton abundance, but occurred only in August.

Regarding dinoflagellates, the most represented genera were *Oxytoxum* (8 species) and *Gymnodinium* (7 species).

Species that significantly contributed to the total phytoplankton density at this station were *Pseudo-nitzschia* spp. (7.35%) *Cylindrotheca closterium* (6.50%), *Leptocylindrus danicus* (2.41%), *Chaetoceros curvisetus* (4.56%) and *Gymnodinium* spp. (3.06%). Small dinoflagellates made up to 10.74% of the total abundance.

Species that showed the most frequent appearance were: *Cylindrotheca closterium* (65.52%), *Pseudo-nitzschia* spp. (60.34%), *Leptocylindrus danicus* (31.03%), *Proboscia alata* (39.66%) and gymnodinial dinoflagellates (62.07%). Coccolithophorids were presented in small abundances. A single recorded euglenophyta species had a very low frequency of appearance.

The number of phytoplankton species that have been observed in the water column of investigated fish farm during the whole research period, was relatively high. Also, relatively high indices of biodiversity were calculated. The average number of species was 10 species per sample. Calculated Shannon's index of diversity (H'), Margalef's (d) and Pielou's index (J') indicated high biodiversity in the phytoplankton during the study period (Table 2). Similar results regarding biodiversity were obtained from the studies conducted at other sea bass and sea bream farms in the Adriatic Sea. For instance, in the Kaldonta Bay (Cres Island) 161 species of phytoplankton were identified, among which 98 diatoms and 55 dinoflagellates (Tomec, 2004).

Table 1. Taxonomic phytoplankton composition, frequency distribution (F), average values (AVG) and maximal values (MAX) of abundances (cell L^{-1}) at the investigated station.

Tablica 1. Taksonomski sastav fitoplanktona, učestalost pojavljivanja (F), srednja (AVG) i maksimalna vrijednost (MAX) brojnosti (stanica L^{-1}) na istraživanoj postaji.

Phytoplankton taxa	F%	AVG	MAX
BACILLARIOPHYCEAE (DIATOMS)			
1. *Achnantes longipes* Agardh	1.72	735	735
2. *Asterionellopsis glacialis* (Castracane) Round	1.72	8085	8085
3. *Bacteriastrum delicatulum* Cleve	1.72	1470	1470
4. *Cerataulina pelagica* (Cleve) Hendey	5.17	982	1470
5. *Chaetoceros affinis* Lauder	15.52	3852	12580
6. *C. atlanticus* Cleve	1.72	2220	2220
7. *C. compressus* Lauder	1.72	19240	19240
8. *C. costatus* Pavillard	5.17	9640	8820
9. *C. curvisetus* Cleve	8.62	23667	62005
10. *C. dadayii* Pavillard	3.45	1850	2220
11. *C. danicus* Cleve	3.45	735	735
12. *C. decipiens* Cleve	5.17	3438	4410
13. *C. didymus* Ehrenberg	1.72	2940	2940
14. *C. gracilis* Pantocsek	5.17	735	735
15. *C. holsaticus* Schütt	1.72	20580	20580
16. *C. laciniosus* Schütt	1.72	13230	13230
17. *C. peruvianus* Brightwell	3.45	735	735
18. *C. pseudocurvisetus* Mangin	1.72	20580	20580
19. *C. teres* Cleve	1.72	15430	15430
20. *Chaetoceros* spp.	13.79	3959	11760
21. *C. wighamii* Brightwell	8.62	7355	22900
22. *Climacosphaenia moniligera* Ehrenberg	5.17	980	1470
23. *Cyclotella* sp.	3.45	2940	2940
24. *Cylindrotheca closterium* (Ehr.) Reimann et Lewin	65.52	4445	33075
25. *Dactyliosolen fragilissimus* (Bergon) Hastle	5.17	1230	1470
26. *Diploneis* sp.	5.17	735	735
27. *Ditylum* sp.	1.72	735	735
28. *Grammatophora oceanica* (Ehrenberg) Grunow	3.45	1108	1470
29. *Guinardia flaccida* (Castracane) Peragallo	8.62	1327	2220
30. *G. striata* (Stolterfoth) Hasle	22.41	1873	3700
31. *Hemiaulus hauckii* Grunow	5.17	1960	2940
32. *H. sinensis* Greville	3.45	1108	1470
33. *Leptocylindrus danicus* Cleve	31.03	34,82	11760
34. *L. mediterraneus* (Peragallo) Hastle	5.17	1230	1470
35. *L. minimus* Gran	1.72	735	735
36. *Licmophora abbreviata* Agardh	17.24	1030	2205
37. *L. flabellata* (Greville) Agardh	6.90	920	1470
38. *Licmophora* sp.	8.62	884	1470
39. *Melosira* spp.	3.45	5510	7350
40. *Navicula* spp.	10.34	858	1470
41. *Navicula directa* (Smith) Ralfs	1.72	1470	1470
42. *Nitzschia longissima* (Brelb.) Ralfs	15.52	1146	2205
43. *Odontella mobiliensis* (Bailey) Grunow	1.72	735	735
44. *Pleurosigma angulatum* (Quekett.) Smith	3.45	735	735
45. *Pleurosigma* sp.	6.90	735	735
46. *Pseudo-nitzschia* spp. Peragallo	60.34	5451	23520
47. *Pseudo-nitzschia* x	8.62	191700	386084
48. *Proboscia alata* (Brightwell) Sundstrom	39.66	1440	4410
49. *Rhizosolenia hebetata* Bailey	1.72	735	735

50. *R. imbricata* Brightwell	1.72	735	735
51. *R. styliformis* Peragallo	3.45	1470	1470
52. Thalassionema frauenfeldii	15.52	1968	5920
53. *Thalassionema nitzschioides* Schrader	27.59	1565	3680
54. *Thalassiosira* sp.	24.14	1158	2205
55. *Toxarium undulatum* Bailey	1.72	735	735
DINOPHYTA (DINOFLAGELLATES)			
1. *Alexandrium* sp	1.72	735	735
2. *Amphidinium acutissimum* Schiller.	17.24	883	1470
3. *A. globosum* Schröder	3.45	1105	1470
4. *Amphidinium* sp.	3.45	735	735
5. *Ceratium symmetricum* Pavillard	1.72	735	735
6. *Cochlodinium* sp.	1.72	1470	1470
7. *Cochlodinium adriaticum* Schiller	3.45	735	735
8. *Dinophysis rotundata* Claparède & Lachmann	1.72	735	735
9. *Dissodinium elegans* Pavillard	1.72	735	735
10. *Gonyaulax spinifera* (Claparède et Lachmann) Diesing	1.72	735	735
11. *Gymnodinium abbreviatum* Kofoid et Swezy	1.72	735	735
12. *G. agiliforme* Schiller	3.45	2208	3680
13. *G. grammaticum* (Pouchet) Kofoid et Swezy	1.72	735	735
14. *G. heterostriatum* Kofoid et Swezy	1.72	735	735
15. *G. neglectum* Schiller	3.45	1103	1470
16. *G. ostenfeldii* Schiller	3.45	1470	1470
17. *G. simplex* Lohmann	17.24	2058	4410
18. G. uberimum	3.45	735	735
19. *Gymnodinium* sp.	62.07	2209	8820
20. *Gyrodinium ascendens* Kofoid	1.72	735	735
21. *G. fusiforme* Kofoid et Swezy	25.86	834	1470
22. *G. obtusum* (Schüett) Lebour	1.72	735	735
23. *G. pingue* (Schuett) Kofoid et Swezy	3.45	735	735
24. *Gyrodinium* sp.	15.52	735	735
25. *Hermesinum adriaticum* Zacharias	1.72	735	735
26. *Oxytoxum adriaticum* Schiller	3.45	735	735
27. *O. gladiolus* Stein	1.72	735	735
28. *O. laticeps* Schiller	1.72	1470	1470
29. *O. ovale* Schiller	1.72	735	735
30. *O. viride* Schiller	1.72	735	735
31. *Oxytoxum* sp.	6.90	735	735
32. *O. scolopax* Stein	1.72	735	735
33. *O.sphaeroideum* Stein	3.45	735	735
34. *Pronoctiluca spinifera* (Lohmann) Schiller	8.62	885	1470
35. *Prorocentrum compressum* (Bailey) Abe et Dodge	1.72	1470	1470
36. *P. micans* Ehrenberg	5.17	735	735
37. *P. minimum* (Pavilard) Schiller	1.72	368	735
38. *P. scutellum* Schröder	8.62	735	735
39. *P. triestinum* Schiller	12.07	2625	8820
40. *Protoperidinium bipes* (Paulsen) Balech	1.72	735	735
41. *P. brevipes* (Paulsen) Balech	3.45	735	735
42. *P. divergens* (Ehrenberg) Balech	1.72	735	735
43. *P. tuba* (Schiller) Balech	5.17	980	1470
44. *Phyrophacus steinii* (Schiller) Wall et Dale	5.17	735	735
45. *Scrippsiella trochoidea* (Stein) Loeblich	8.62	1177	2205
46. *Warnowia* sp.	1.72	735	735
47. *Warnowia altra* Schiller	1.72	1470	1470
PRYMNESIOPHYCEAE			
1. *Calyptrosphaera sphaeroidea* Schiller	5.17	1960	2940
2. *Rhabdosphaera claviger* Murray et Blackman	1.72	1470	1470
3. *Syrachosphaera apsteinii* Lohmann	1.72	1470	1470

4. *Syracolithus dalmaticus* (Kampt.) Loeblich et Tappan	1.72	735	735
5. *Syracosphaera pulchra* Lohmann	6.90	1108	1470
CHRYSOPHYCEAE			
1. *Dictyocha fibula* Ehrenberg	10.34	735	735
2. *D. speculum* Ehrenberg	1.72	370	370
3. Dynobrion sp.	1.72	735	735
EUGLENOPHYTA			
1. *Eutreptia lanowii* Steuer	5.71	5880	16170

N= 58

Table 2. Average (AVG), maximal (MAX) and minimal (MIN) values for diatoms (cell L^{-1}), dinoflagellates (cell L^{-1}), number of species (S), Margalef index of biodiversity (d), Pielou index (J') and Shannon index (H').

Tablica 2. Srednja (AVG), maksimalna (MAX) i minimalna (MIN) vrijednost dijatomeja (stanica L^{-1}), dinoflagelata (stanica L^{-1}), broj vrsta (S), Margalef-ov indeks biodiverziteta (d), Pielou-ov indeks (J') i Shannon-ov indeks (H').

	AVG	MAX	MIN
Diatoms	34804	417689	1470
Dinoflagellates	8797	92610	0
S	10	17	4
d	0.88	1.49	0.33
J'	0.80	0.97	0.18
H'(log2)	2.58	3.53	0.59

These results are not in accordance with the study of Acūna et al. (2008) since these authors claimed that fish farm causes reduced phytoplankton biodiversity. However, the difference is probably due to the natural features of the particular area (depth, ventilation, oligotrophy) that determines sustainable capacity of the area in relation to productivity of the fish farm. Seasonal effect of aquaculture waste on phytoplankton is important in connection to the release of the nutrients (Pitta et al., 1999). In warm temperate areas such as the Mediterranean, the highest densities of phytoplankton occur in the spring and autumn as a combined result of availability of nutrients (due to the mixing of the water column) and favourable light conditions (Siokou-Frangou et al., 2002). During the summer, thermal stratification causes a decrease of nutrients on the surface and results in low phytoplankton growth despite the favourable light conditions. In contrast, the release of nutrients at fish farm is an ongoing process throughout the year, reaching maximum values during the summer months when high temperatures accelerate fish metabolism and feeding process, while water dynamics is significantly weakened. Although this process was also typical for fish farm in Maslinova Bay, our study is in accordance with the results of Pitta et al. (1999) who stated that changes in the phytoplankton community were more the result of seasonal variability than the fish farm influence. This is also ascertained in the paper by Skejić et al. (2011) when ecological parameters and biota of Maslinova Bay were investigated in terms of characterization of trophic status.

Our results obtained by PERMANOVA analysis showed that there was no significant difference in phytoplankton composition with respect to the different depths *(Pseudo-*

F=0.96092, p=0.5544), while seasonal influence was very important (*Pseudo-F=3.7012, p=0.0001*) (Table 3).

Table 3. Results of PERMANOVA test
Tablica 3. Rezultati PERMANOVA testa

Factor	Abbrev.	Type	Levels
Depth	de	Fixed	5
Season	se	Fixed	4

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
de	4	10799	2699.7	0.96092	0.5544	9861
se	3	31195	10398	3.7012	0.0001	9871
dexse	12	24278	2023.2	0.72012	0.9932	9777

CONCLUSION

Throughout the research period, phytoplankton composition in the water column near fish cages showed seasonal distribution typical for middle Adriatic waters. Biodiversity and abundance range of phytoplankton species indicated good water conditions, with no evident alterations induced by the increased release of the nutrients.

Sažetak

FITOPLANKTONSKI SASTAV NA UZGAJALIŠTU RIBA U UVALI MASLINOVA (OTOK BRAČ)

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Cilj ovog rada je utvrditi sastav fitoplanktona na uzgajalištu komarči (*Sparus aurata*) i lubina (*Dicentrarchus labrax*) u srednjem Jadranu. Istraživanje je provedeno u razdoblju od rujna 2005. do rujna 2006. godine na postaji smještenoj u uvali Maslinova na otoku Braču. Promatrajući cijelo istraživano razdoblje, dijatomeje su općenito prevladavale svojom brojnošću, dok su dinoflagelati bili brojniji u lipnju.

Broj vrsta dijatomeja i dinoflagelata kroz istraživano razdoblje bio je sličan. Zabilježeno je 111 vrsta fitoplanktona, od kojih je 55 vrsta iz skupine Bacillariophyceae (dijatomeja), 47 vrsta Dinophyta (dinoflagelata), 5 vrsta Prymnesiophyceae, 3 vrste Chrysophyceae

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te 1 vrsta Euglenophyta. Većina vrsta dijatomeja pripada rodu *Chaetoceros*. Najznačajnije zastupljeni dinoflagelati pripadaju rodovima *Oxytoxum* i *Gymnodinium*. Nije bilo značajne razlike u sastavu s obzirom na dubine postaje, dok je sezonski utjecaj bio značajan. Biodiverzitet i brojnost fitoplanktona ukazuju na povoljne uvjete vodenog stupca u blizini uzgajališta; nema promjena uvjetovanih povećanim ispuštanjem nutrijenata.

Ključne riječi: uzgajalište ribe, fitoplankton, taksonomski sastav, uvala Maslinova

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