

Composition and distribution of diatoms on mussel farms in Boka Kotorska Bay

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ABSTRACT

In this study, it was presented composition and distribution of diatoms on mussel farms in Boka Kotorska Bay.

Samplings were conducted one per month, from January to June 2013, on surface layer. Samples were taken on eleven positions, including Kotor Bay, Risan Bay and Tivat Bay. Maximum of phytoplankton abundance (microplankton) was noticed in June and it was 8.94×10^5 cells/l, while minimum value was recorded in March (6.18×10^4 cells/l). Diatoms prevailed almost all investigated period, except in June when dinoflagellates prevailed on diatoms and made maximum of microplankton. Highest value of dominant group during investigation period was in February when almost all microplankton abundance was consisting of diatoms. Among dominant diatoms, prevailed species which preferred nutrient enriched area, such as: Cerataulina pelagica, Chaetoceros affinis, Navicula spp., Pseudo-nitzschia spp, Thalassionema nitzschioides.

Due to increase of human impact at recent time, this study is useful for getting new information and for further studding, especially knowing that there is a lack of information related to distribution of diatoms on mussel farms in Boka Kotorska Bay.

Keywords: Diatoms, phytoplankton, Boka Kotorska Bay

INTRODUCTION

Gap between consumer demand and seafood production from traditional capture fisheries started to grow. The only way to fill this gap is worldwide expansion of aquaculture and industry development needs to be promoted and managed to minimized negative impact on environment (FAO, 2008).

Mussel farming in Boka Kotorska Bay started in the 1980s and today there are 16 farms using the system of the floating buoys and ropes. As mussels are filter feeders which accumulate phytoplankton, the problem can occur if there is a presence of toxic phytoplankton, mostly are dinoflagellates and potentially toxic diatoms which can caused negative human health consequences.

Phytoplankton is the main representative of primary production in estuarine ecosystems (Williams, 1981) and the major component of the shellfish diet (Viličić *et al.* 1994). A number of factors define the role of phytoplankton in estuarine production such as salinity, temperature, light (influenced by turbidity), nutrients, water dynamics and the configuration of the water basin.

Eutrophication triggers various physical and chemical changes in the marine environment and exerts a pressure on algal populations, allowing the intensive growth of certain harmful-toxin producing species or nuisance blooms that may create problems in the structure of the ecosystem and public health. These blooms are collectively called Harmful Algal Blooms (HABs). The greatest number of toxic species is found among dinoflagellates, but evidence has been provided for several species of other taxa (diatoms, flagellates, cyanobacteria, prymnesiophytes, raphidophytes) suggesting that they belong in this category (Codd 1999; Vershinin 2008; Moestrup 2010).

The mucilage phenomenon of the Adriatic Sea had been usually related to an extracellular organic matter production, of phytoplanktonic origin, without the identification of a specific causative organism (Mingazzini & Thake, 1995). Historically, diatoms have always been considered as the algal group mainly involved in this event due to their abundant presence in mucilaginous aggregates (Rinaldi *et al.*, 1995) and for their known extracellular release of polysaccharidic substances (Mykkestad, 1974, 1977, 1995; Mykkestad *et al.*, 1989).

In area of investigation, domination of diatoms was noticed in previous studies (Drakulović & Vuksanović 2010; Drakulović *et al.*, 2011; 2012, Bosak *et al.* 2012).

The aim of this paper is to show distribution of diatoms on mussel farms in Boka Kotorska Bay and trying to emphasize on consequences which can be results of high diatoms growth and presence of mucilage aggregates.

MATERIALS AND METHODS

Area of investigation in this paper is Boka Kotorska Bay. Bay is situated in southeastern part of Adriatic Sea and it consists of four small Bays: Kotor Bay, Risan Bay, Tivat Bay and Herceg Novi Bay. The Bays of Herceg Novi and Tivat are connected by Kumbor strait and the Kotor and Tivat Bays are joined by Verige strait (width 340 m, length 2300 m) (Magaš 2007). Verige strait is the narrowest section of the Bay, which separate the inner Bay from the rest of the Bay. Current study was conducted in inner and middle part of Boka Kotorska Bay where are situated mussels farms (Kotor and Tivat Bay). The freshwater influx from 5 small rivers, numerous streams and submarine springs greatly affects the hydrological and chemical properties of water column (Milanović 2007)..

Sampling was conducted one per month, from January to June 2013, on surface layer. Samples were taken on eleven positions, including Kotor Bay, Risan Bay and Tivat Bay (Figure 1, Table 1).

Six positions were located in the Kotor Bay, one position in Risan Bay and four positions in the Bay of Tivat.

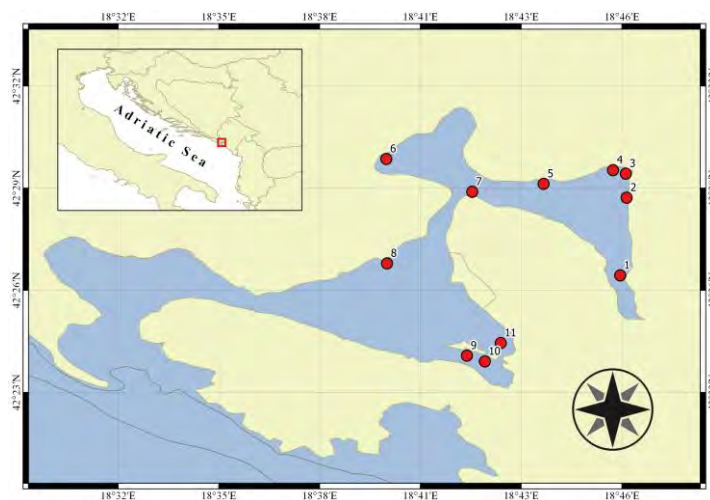


Figure 1. Investigated area

Table 1. Investigated positions

Number	Position	N	E
1	IMB	42° 26' 11"	18° 45' 49"
2	Ljuta	42° 29' 02"	18° 45' 50"
3	Orahovac	42° 29' 13"	18° 45' 53"
4	COGI	42° 29' 09"	18° 44' 44"
5	Dražin vrt	42° 29' 01"	18° 43' 35"
6	Lipci	42° 29' 53"	18° 39' 38"
7	Stoliv	42° 28' 39"	18° 41' 41"
8	Sveta Nedelja	42° 27' 31"	18° 40' 21"
9	Obala Đuraševića	42° 24' 03"	18° 41' 44"
10	Ostrvo cvijeća	42° 24' 15"	18° 42' 27"
11	Kalardovo	42° 24' 60"	18° 42' 39"

Samples were taken with Niskin sampler of 5l. Physical parameters such as temperature, salinity and dissolved oxygen concentration were measured in situ using a universal meter (Multiline P4; WTW). Nutrient (nitrates, nitrites, silicates and phosphates) concentrations were determined by standard colorimetric method (Strickland *et al.*, 1972) using a spectrophotometer type Perkin Elmer χ 2. Phytoplankton samples were moved to 250 ml bottles and preserved in a 3% neutralized formaldehyde solution. After 24 h of sedimentation in sediment chambers, cells were enumerated using Leica inverted microscope according to Utermöhl method (1958). For determination of phytoplankton species, it was used appropriate key for the specified field of investigation (Hustedt 1959; Hasle & Syvertsen 1997; Round *et al.*, 1990; Taylor 1987 and Thronsen *et al.*, 2007).

RESULTS AND DISCUSSIONS

Physical parameter, temperature showed typical distribution with maximum value in June 2013. (29.1°C), while minimum value (9°C) was recorded in January 2013 on position Stoliv (Figure 2.).

Salinity varied during investigation period, decreasing to a minimum value of 3.9 at surface layer (Figure 2.) in March on position IMB. This lower salinity usually means a stronger freshwater inflow and this minimum was result of winter and early spring precipitation events. Oxygen concentration ranged from 4.41 to 8.31 mg/l (Figure 2.). Lower concentration of dissolved oxygen is attributed to the oxidation of organic matter and higher water temperatures prevailing in summer (Dorgham *et al.*, 2004).

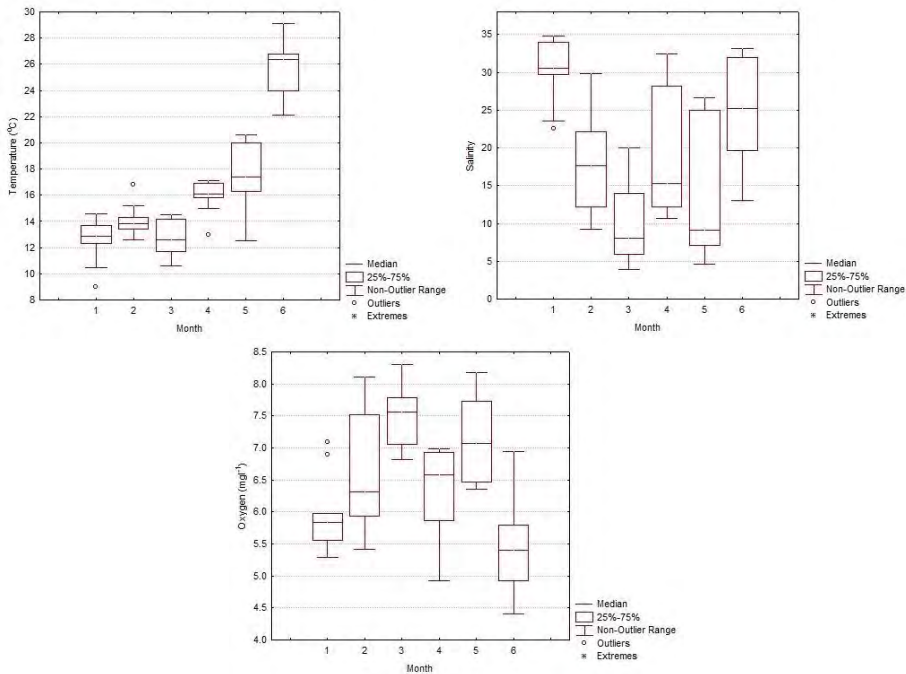


Figure 2. Variation of temperature, salinity and oxygen concentration during investigated period

Concentration of nutrients in the Boka Kotorska Bay was generally high during researching period and favorable for phytoplankton growth. The maximum concentration of nitrate was 22.98 $\mu\text{mol/l}$ and it was recorded in January 2013. Peak of phosphate concentration was noticed in June (0.53 $\mu\text{mol/l}$), while peak of silicate concentration was 34.03 $\mu\text{mol/l}$ in March 2013 (Figure 3). The recorded values were similar to values noticed by Krivokapić *et al.* (2011) for Boka Kotorska Bay. Noticed results were higher in comparison to the values reported for the Zrmanja river estuary (Viličić *et al.*, 2008, Svensen *et al.*, 2007), where phosphorous was limiting factor throughout the whole year and nitrogen sporadically in the summer. For SE

Mediterranean (Krom 1991) and middle Adriatic (Vukadin & Stojanoski 2001), it was noticed that phosphate is known as the limiting nutrient instead of nitrate.

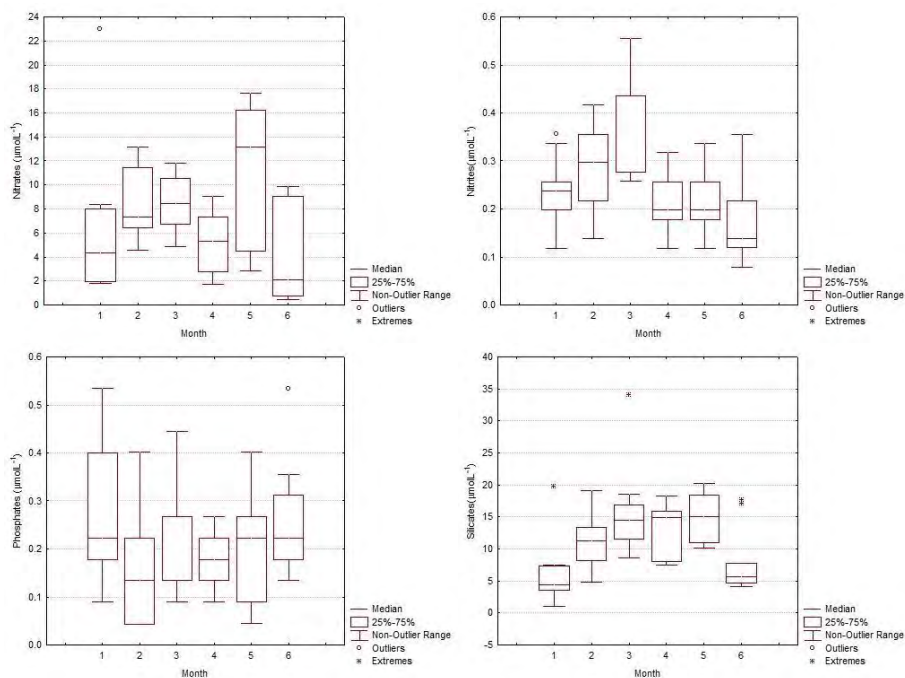


Figure 3. Variation of nutrients concentration (nitrates, nitrites, phosphates and silicates) during investigated period

Microplankton fraction showed maximum abundance in June 2013 and reached value of 8.94×10^5 cells/l on position Orahovac. Abundance up to 10^5 cells/l was also noticed in January, February and May (3.45 , 6.16 and 2.28×10^5 cells/l) while in other periods of investigation (March and April) values were up to 10^4 cells/l (Figure 4.).

Smaller fraction-nanoplankton was maximum in February 2013 (6.35×10^5 cells/l), while minimum abundance was in March 2013 (2.12×10^5 cells/l) (Figure 5.).

Diatoms were dominant group almost during entire investigated period except June 2013 when prevailed dinoflagellates. Values of diatoms reached up to 10^5 cells/l and maximum was noticed in February 2013 on position Stoliv (6.13×10^5 cells/l). This high values can be result of enough nutrients supply by rivers which caused higher phytoplankton growth (Figure 6.).

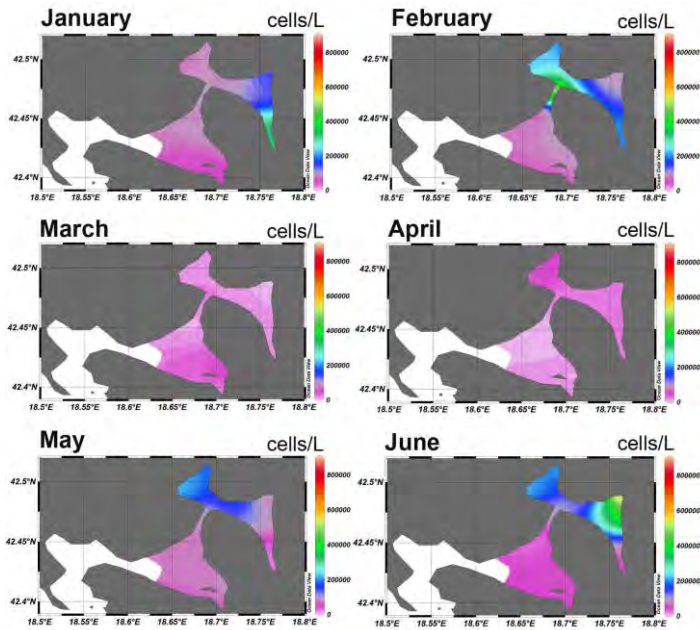


Figure 4. Abundance of microplankton on investigation position from January to June 2013.

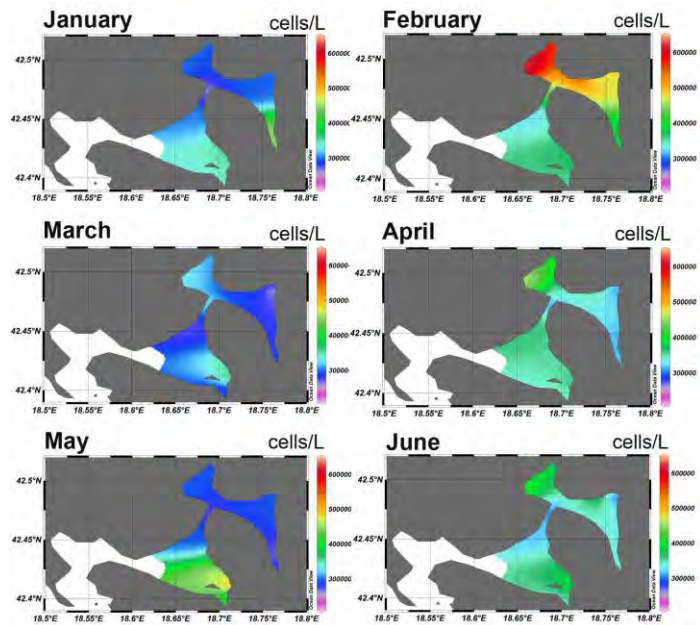


Figure 5. Abundance of nanoplankton on investigation position from January to June 2013.

Domination of diatom was already noticed in Boka Kotorska Bay by Drakulović *et al.* 2010, 2011, 2012 and Bosak *et al.* 2011. Some authors noticed most abundant of diatoms in late winter–early spring, which has been previously recorded in the northern Adriatic Sea (Viličić *et al.* 2009) and in the southwestern part of Adriatic (Caroppo *et al.* 2005). Diatom domination in late winter-early spring most likely reflects their particular ability to survive relatively more turbulent conditions.

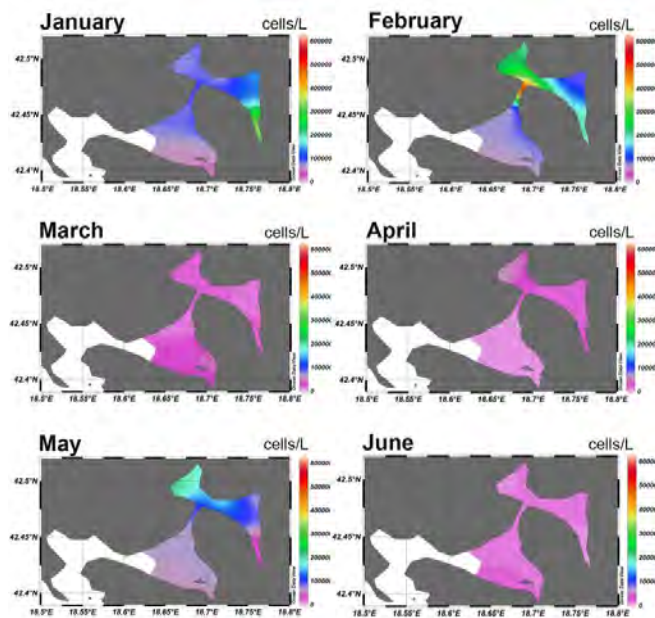


Figure 6. Abundance of diatoms on investigation position from January to June 2013.

Diatom dominance is often described in connection with the appearance of large mucilaginous aggregates that often occur during late spring/early summer in the northern basin (Totti *et al.* 2005). The exact role of diatoms remains elusive, but some studies indicate the importance of the intensive production of extracellular polysaccharide by several diatom species (e.g. *Skeletonema marinoi*, *Chaetoceros* spp., *Ceratoneis closterium*) commonly found during mucilage occurrences (Totti *et al.* 2005).

Dominant diatoms during investigated period were: *Bacteriastrum hyalinum*, *Chaetoceros affinis*, *Chaetoceros diversus*, *Guinardia striata*, *Navicula* spp., *Proboscia alata*, *Pseudo-nitzschia* spp., *Thalassionema nitzschioides*.

Mostly of this dominant diatom species (*Cerataulina pelagica*, *Chaetoceros affinis*, *Leptocylindrus danicus*, *Pseudo-nitzschia* spp., *Thalassionema nitzschioides*) are characterized as nutrient preferred species in Adriatic Sea (Revelante & Gilmartin 1985; Pucher-Petković & Marasović 1980).

Table 2. List of dominant and abundant diatoms in Boka Kotorska Bay

Species	Abundance cells/l
Diatoms	
<i>Bacteriastrum hyalinum</i>	10 ⁵
<i>Chaetoceros affinis</i>	10 ⁵
<i>Ch. curvisetus</i>	10 ⁴
<i>Ch. diversus</i>	10 ⁴
<i>Cerataulina pelagica</i>	10 ²
<i>Cocconeis scutellum</i>	10 ²
<i>Leptocylindrus danicus</i>	10 ²
<i>Melosira nummuloides</i>	10 ³
<i>Navicula spp.</i>	10 ³
<i>Proboscia alata</i>	10 ³
<i>Pseudo-nitzschia spp</i>	10 ⁵
<i>Skeletonema spp.</i>	10 ⁴
<i>Thalassionema nitzschioides</i>	10 ³

Thalassionema nitzschioides was dominant species in this study and also is frequent species in the Adriatic Sea (Viličić *et al.* 1995) and in the Krka estuary usually is present in summer period (Cetinić *et al.* 2006).

In current study frequently noticed diatom genus was potentially toxic *Pseudo-nitzschia* spp. Diatoms of the genus *Pseudo-nitzschia* are dominant in the phytoplankton composition in the middle (Burić *et al.* 2008) and southern Adriatic (Burić *et al.* 2008, Caroppo *et al.* 2005).

Diatoms belonging to the genus *Pseudo-nitzschia* are generally considered to be dominant in the phytoplankton of the Adriatic Sea (Viličić *et al.* 1995, 2009).

CONCLUSION

Presence of potentially toxic diatom genus *Pseudo-nitzschia* spp. and frequency and higher abundance of species which prefer nutrients enriched waters show on necessary monitoring of this area. Especially, knowing that investigated area is mussel farms and that presence of potentially toxic organisms and possible blooms of diatoms can cause serious problems to mussel farms and humans health.

REFERENCES:

- Bosak, S., T. Šilović, Z. Ljubešić, G. Kušpilić, B. Pestorić, S. Krivokapić & D. Viličić (2011): Phytoplankton size structure and species composition as an indicator of trophic status in transitional ecosystems: the case study of a Mediterranean fjord-like karstic bay. *Oceanologia* **54**(2): 1-32, doi:10.5697/oc.54-2.xxx.
- Burić, Z., D. Viličić, K. Caput Mihalić, M. Carić, K. Kralj & N. Ljubešić (2008): *Pseudo-nitzschia* blooms in the Zrmanja River estuary (Eastern Adriatic Sea). *Diatom Res.* **23**: 51-63.

- Caroppo C., R. Congestr, L. Bracchini & P. Albertano (2005): On the presence of *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle and *Pseudonitzschia delicatissima* (Cleve) Heiden in the Southern Adriatic Sea (Mediterranean Sea, Italy), *J. Plankton Res.*, 27: 763–774
- Codd, G. A. (1999): Cyanobacterial toxins: Their occurrence in aquatic environments and significance to health. In *Marine Cyanobacteria*; Charpy, L., Larkum, A.W.D., Eds.; Bulletin De L'Institut Oceanographique: Paris, France; pp. 483–500.
- Dorgham, M., N.E. Abdel-Aziz, K.Z. El-Deeb & Okbah M. A. (2004): Eutrophication problems in the Western Harbour of Alexandria, Egypt. *Oceanologia* 46, 25-44.
- Drakulović, D. & N. Vuksanović (2010): Phytoplankton assemblages and density in the Montenegrin coastal sea. *Rapp. Comm. Int. mer Médit. (CIESM)*, 39: pp.351.
- Drakulović, D., N. Vuksanović & D. Joksimović (2011): Dynamics of phytoplankton in Boka Kotorska Bay. *Studv Mar.*, 25(1): 1-20.
- Drakulović, D., B. Pestorić, M. Cvijan, S. Krivokapić & N. Vuksanović (2012): Distribution of phytoplankton community in Kotor Bay (south-eastern Adriatic Sea). *Cent. Eur. J. Biol.*, 7(3): 470-486.
- European Commission (2008): Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Official Journal of the European Communities, L164.19.p.21.
- Hustedt, F. (1959): *Die Kieselalgen Deutschlands, Österreichs und der Schweiz*. Otto Koeltz, Scientific Publishing, Koenigstein.
- Hasle, G. R. & E. E. Syvertsen (1997): Marine diatoms. In: Tomas, C.R. (ed.), *Identifying marine diatoms and dinoflagellates*. Academic Press, San Diego. pp. 5–385.
- Krivokapić, S., B. Pestorić, S. Bosak, G. Kušpilić & Wexels Riser, C. (2011): Trophic state of Boka Kotorska Bay (South-Eastern Adriatic Sea). *Fresenius Environ. Bull.* 20 (8): 1960-1969.
- Krom, M.D., N. Kress & S. Brenner (1991): Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.* 36 (3), 424-432.
- Magaš D. (2002): Natural-geographic characteristics of the Boka Kotorska area as the basis of development, *Geoadria*, 7, 51-81.
- Moestrup, Ø.; R. Akselman, G. Cronberg, M. Elbraechter, S. Fraga, Y. Halim, G. Hansen, M. Hoppenrath, J. Larsen, N. Lundholm, L.N. Nguyen & A. Zingone (2010): IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (HABs). <http://www.marinespecies.org/hab/>, accessed 3 March 2010.
- Milanović, S. (2007): Hydrogeological characteristics of some deep siphonal springs in Serbia and Montenegro karst. *Environmental Geology* 51: 755-759.
- Mingazzini M & B. Thake (1995): Summary of the workshop on marine mucilages in the Adriatic Sea and elsewhere. *Sci Total Environ* 1995;165:9 – 14.
- Myklestad S. M. (1974): Production of carbohydrates by marine planktonic diatoms: I Comparisons of nine different species in culture. *J Exp Mar Biol Ecol.*, 15:261– 74.
- Myklestad S.M. (1977): Production of carbohydrates by the marine planktonic diatoms: II Influence of the N:P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis* var *willei* (Gran) Hustedt and *Skeletonema costatum* (Grev) Cleve. *J Exp Mar Biol Ecol.*, 29:161– 79.
- Myklestad S.M. (1995): Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Sci Total Environ.*, 165:155– 64.
- Myklestad S.M., & A. Haug (1972): Production of carbohydrates by the marine diatom *Chaetoceros affinis* var *willei* (Gran) Hustedt: I Effect of the concentration of nutrients in the culture medium. *J Exp Biol Ecol.*, 9:125 – 36.

- Myklestad S.M., O. Holm-Hansen, K.M. Vaerum & B.E. Volcani (1989): Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*. *J Plankton Res.*, 11: 763–73.
- Pucher-Petković, T. & I. Marasović (1980): Développement des populations phytoplanktoniques caractéristiques pour un milieu eutrophisé (Baie de Kastela, Adriatique centrale). *Acta Adriat.* 21: 79-93.
- Revelante, N. & M. Gilmartin (1985): Possible phytoplankton species as indicators of eutrophication in the northern Adriatic Sea. *Rapp.Comm. int. Mer Médit.*(CIESM), 11-19 October, Lucern, Switzerland, 29:89–91
- Round, F.E., R. M. Crawford & D. G. Mann (1990): The diatoms. Biology and morphology of the genera. Cambridge University Press, Cambridge.
- Rinaldi A, R.A. Vollenweider, G. Montanari, C.R. Ferrari & A. Ghetti (1995): Mucilages in Italian Seas: the Adriatic and Tyrrhenian Seas, 1988–1991. *Sci Total Environ.*, 165:165–83.
- Strickland J. D. H & T. R. Parsons (1972): A Practical Handbook of Seawater Analysis, *Bull. Fish. Res. Board Can.*, 167, 1–310.
- Svensen, C., D. Viličić, P. Wassmann, E. Arashkevich, & Ratkova T. (2007): Plankton distribution and vertical flux of biogenic matter during high summer stratification in the Krka estuary (Eastern Adriatic). *Estuar. Coast. Shelf S.* 71, 381- 390.
- Taylor, F.J.R. (1987): The Biology of Dinoflagellates. Botanical Monographs. Blackwell Scientific Publications, Oxford.
- Thingstad, T.F. & E. Sackshaug (1990): Control of phytoplankton growth in nutrient recycling ecosystems. Theory and terminology. *Mar. Ecol. Prog. Ser.* 63: 261– 272.
- Thronsen, J., G. R. Hasle & K. Tangen (2007): Phytoplankton of Norwegian coastal waters. Almatel Forlag As, Oslo.
- Totti C., Cangini M., Ferrari C., Kraus R., Pompei M., Pugnetti A., *et al.* (2005): Phytoplankton size distribution and community structure in relation to mucilage occurrence in the northern Adriatic Sea, *Sci Total Environ.*, 353, 204–217.
- Viličić, D., D. Mušin & N. Jasprica (1994): Interrelations between hydrographic conditions, nanoplankton and bivalve larvae in the Mali Ston bay (the southern Adriatic). *Acta Adriatica*, 3, 55–64.
- Viličić, D., N. Leder, Z. Gržetić & N. Jasprica (1995): Microphytoplankton in the Strait of Otranto (eastern Mediterranean). *Mar. Biol.* 123: 619-630.
- Viličić, D., S. Terzić, M. Ahel, Z. Burić, N. Jasprica, M. Carić, C. K. Mihalić & G. Olijić (2008): Phytoplankton abundance and pigment biomarkers in the oligotrophic, eastern Adriatic estuary. *Environ. Monit. Assess.* 142, 199-218.
- Viličić D., T. Dakovac, Z. Burić & S. Bosak (2009): Composition and annual cycle of phytoplankton assemblages in the northeastern Adriatic Sea, *Bot. Mar.*, 52, 291–305.
- Vershinin, A.O. & Orlova, T.Y.(2008): Toxic and harmful algae in the coastal waters of Russia. *Mar. Biol.*, 48, 524–537.
- Vukadin, I. & L. Stojanoski (2001): Phosphorus versus nitrogen limitation in the middle Adriatic Sea. *Rapp. Comm. int Mer Médit.* 36, 174.
- Williams, P. J. (1981): Primary productivity and heterotrophic activity in estuaries. In Martin, J. M., J. D. Burton & D. Eisma (eds) *River inputs to Ocean Systems*. UNESCO: p 384.
- Utermöhl, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton Methodik, *Mitt. Int. Ver.Theor. Angew. Limnol.* 9: 1–38.