Morphology and ecology of the poorly known dinoflagellate *Prorocentrum arcuatum* (Dinophyceae) from the Medulin Bay (eastern Adriatic Sea)

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The unusual and prolonged occurrence of rare and poorly known dinoflagellate Prorocentrum arcuatum in the phytoplankton of the shallow aquaculture site in Medulin Bay was recorded from July 2013 to October 2014. This enabled us to investigate changes in abundance and environmental drivers of P. arcuatum population dynamics in natural conditions, and to provide first detailed description of P. arcuatum from field samples based on SEM images. During 15 months of observations we also recorded seasonal variability in P. arcuatum cell size. The optimum physical conditions for P. arcuatum proliferation were reached in autumn 2013, during the narrow temperature range between 19.6°C – 20.4°C, and salinity between 36.7 - 37.7. Despite the general similarity in physical conditions in autumn 2014, this increase in the abundance of P. arcuatum was not repeated, which might be connected to higher competition due to observed interannual changes in phytoplankton population structure.

Key words: Adriatic Sea, planktonic dinoflagellates, Prorocentrum, *Prorocentrum arcuatum*

INTRODUCTION

The dinoflagellates of genus *Prorocentrum* are morphologically different from other dinoflagellate species, most evidently in the absence of sulcus and cingulum. The cell is protected by two opposing valves, with some species

containing an anterior spine located by the periflagellar region. Surface markings vary from pores to areolae (STEIDINGER & TANGEN, 1996). Genus *Prorocentrum* was first described by EHRENBURG (1834), with *Prorocentrum micans* as the type species. Since then, about 60 species have been described (BURSA, 1959; DODGE, 1975;

Fig. 1. Map of Adriatic Sea (a) and sampling site in Medulin Bay (b)

COHEN-FERNANDEZ *et al.*, 2006; MURRAY *et al.*, 2009; HOPPENRATH *et al.*, 2013). They are nearly exclusively marine and can be either benthic or pelagic. Significant attention today is devoted to the investigations of benthic *Prorocentrum* species since many of them have the ability to produce toxins (IGNATIADES & GOTSIS-SKRETES, 2010; HOPPENRATH *et al.*, 2013), while the ecology of planktonic species is in many cases poorly known. Planktonic *Prorocentrum* species are commonly bloom-forming, and sometimes also toxin-producing as *P. cordatum* (GRZEBYK *et al.*, 1997; DENARDOU-QUENEHERVE *et al.*, 1999) and *P. arabianum* (MORTON *et al.*, 2002).

Prorocentrum arcuatum (Issel) has been reported from coastal locations in the Atlantic, Pacific and Mediterranean waters (HERNÁNDEZ-BECERRIL *et al.*, 2000; GIL-RODRÍGUEZ *et al.*, 2003; FELDER & CAMP, 2009; ODEBRECHT, 2010; LAKKIS, 2011; GUIRY & GUIRY, 2013) and is sometimes known to proliferate to bloom conditions (VILIČIĆ *et al.* 1997, MARASOVIĆ & NINČEVIĆ, 1998, BURIĆ *et al.*, 2009), but had never reported to produce toxins. Due to insufficient research, the majority of *P. arcuatum* records provide only the annotation of species appearance, whereas its morphology and population dynamics are scarcely analyzed.

So far, the only previous record of *P. arcuatum* in the Adriatic Sea originates from the small marine meromictic Lake Rogoznica (VILIČIĆ *et al.*, 1997; MARASOVIĆ & NINČEVIĆ, 1998; BARIĆ *et al.*, 2003; BURIĆ *et al.*, 2009), where its abundance

was investigated from 1995-1998. In 2013 and 2014, we recorded the continuous occurrence of *P. arcuatum* at aquaculture site in Medulin Bay which enabled us to (1) investigate the population dynamics and environmental drivers of abundance fluctuations during 15-month period and (2) to provide first detailed morphological description of *Prorocentrum arcuatum* based on SEM microscopy.

MATERIALS AND METHODS

Study area

Medulin Bay is situated along the karstic coast of Istrian peninsula in the northeastern part of the Adriatic Sea (Fig. 1). The maximum depth is approximately 8 meters, and there is limited freshwater influence from the land. The inner shielded part of the Bay is the location for small boats anchorage and hosts several shellfish aquaculture sites. Due to its shallow depth, hydrographic parameters in the inner part of the Bay are highly variable and largely dependent on current weather conditions.

Sampling and water analyses

Water samples were taken at a station situated in the inner part of the Medulin Bay (44° 49,407' N, 13° 54,955'E). For phytoplankton analyses samples were collected fortnightly in the period from July 2013 to October 2014. Due to the station's shallow depth, samples were collected at two discrete depths of the water column; surface and bottom (6m), using 1.6 L Niskin bottles. For environmental parameters (T, S, oxygen saturation) samples were also taken fortnightly concurrently with phytoplankton samples, while nutrient analyses were performed on a monthly basis.

Salinity and temperature were determined by YSI 63 probe. Dissolved oxygen was determined by Winkler titration and oxygen saturation was calculated from the solubility of oxygen in seawater as a function of the corresponding temperature and salinity (UNESCO, 1986). Nutri-

ent concentrations (NO_3^- , NO_2^- , NH_4^+ , TIN, NTOT, NORG, HPO_4^{2-} , PTOT, PORG, SiO_4^{4-}) were determined following the standard methods using the Perkin-Elmer Lambda 15 UV/VIS spectrophotometer (STRICKLAND & PARSONS, 1972; DEGOBBIS, 1990).

For analysis of phytoplankton community, samples were preserved in a 2% (final concentration) neutralized formaldehyde solution. Cell counts were obtained by the inverted microscope Olympus IX 51 according to Utermöhl method (UTERMÖHL, 1958). Subsamples of 25 ml were analysed microscopically after 24 h sedimentation. Microphytoplankton cells (MICRO) defined as $>20\ \mu\text{m}$ were counted under magnification of 400 X (1-2 transects), as well as 200 x magnification. The precision of the counting method was $\pm 10\%$. For *P. arcuatum*, half of the chamber was counted in order to improve accuracy.

The morphometric analysis was carried out measuring at least 5 cells in each phytoplankton sample (N of measured cells = 243) to determine variation of *P. arcuatum* in length during the investigated period. Cells were measured directly using light microscope equipped with ocular micrometer or photographed. Measured parameters included: valve length (l), length with spine=total length (tl) and maximum width of the cells (w).

Ultrastructural analysis of *P. arcuatum* was performed with the scanning electron microscope (SEM). A drop of the sample was air-dried overnight on aluminium stubs and coated with chromium. SEM observations were made at the Warsaw University of Technology, Faculty of Materials Science and Engineering, using a Hitachi S-8000 and SEM/ STEM S-5500 (Hitachi, Tokyo, Japan).

Data analysis

GRAPHPAD PRISM 6 program was used for the statistical analysis and graphs.

For the analysis of *P. arcuatum* distribution, D'Agostino -Pearson test for data normality was used. To determine whether there is a statistically significant difference in the abundance

distribution of this species between the sampling layers (surface and bottom) we used Mann-Whitney U test. Spearman rank correlations were performed to test environmental parameters with abundance of *P. arcuatum*.

By performing Wicoxon-mached pair test we analysed the difference in phytoplankton community structure between two investigated layers. Data weretested for normality (distribution) with D'Agostino-Pearson test.

RESULTS

Description of *Prorocentrum arcuatum* by light and SEM microscopy

Cells are asymmetrical, elongated and lanceolate in form, medium to large in dimensions (length range 40–74 μm ; width range 20–36 μm) (Fig. 2a-b, 3a-b). Length/width ratio is usually around 2 or slightly larger. In anterior view cell is broadest in the middle part and tapering towards at the posterior end (Fig. 2; 3a-b, d). Marked torsion of the cells visible in lateral

Fig. 2. Microphotographs of *Prorocentrum arcuatum* cells from Medulin Bay taken by LM. Scale bars (a, b, c) = 20 μm

Fig. 3. General features of *P. arcuatum* cells taken by SEM. Cell in valval view (Fig. 3a, b). Characteristic torsion of the cell in lateral view (Fig. 3c); Antapical end of the cell (Fig. 3d); Intercalary band (Fig. 3e); Anterior apical spine (Fig. 3, f). Peritragellar area (Fig. 3g, h) with adjacent small spine (arrowed). Scale bars are as follows: 40 μ m, 30 μ m, 30 μ m, 5 μ m, 10 μ m, 10 μ m, 5 μ m, 3 μ m, respectively

view, (Fig. 2c, 3c) with cells appearing narrow and twisted (Fig. 2c, 3c). Surface abundantly covered with shallow depressions and scattered pores. The anterior apical spine is long and sharp (length $15.6 \pm 4.80 \mu\text{m}$, $N=243$), broadest at the base (Fig. 3f). Next to the long massive aliform spine, there is a much smaller spine adjacent to the periflagellar area (arrowed in Fig. 3f, g, h). The periflagellar area is a relatively shallow, broad triangular depression situated apically on the right valve off-center (Fig. 3g, h).

The valves are bounded with horizontally and transversally striated intercalary band (Fig. 3e).

The average cell length (l) was $58.1 \pm 7.10 \mu\text{m}$ ($N=243$), while average total length (with apical spine) (tl) was $73.7 \pm 11.40 \mu\text{m}$. Average width (w) at the broadest point of the valve was $w=30.1 \pm 3.23 \mu\text{m}$ ($N=243$).

Seasonal variability of *P. arcuatum* abundance and cell length

P. arcuatum in Medulin Bay was continuously present during 15 months of research period. Among 60 samples, only in three of them *P. arcuatum* was not observed (in bottom layer:

Table 1 Most common phytoplankton taxa (frequency >20%) recorded in Medulin Bay during the research period ($N=60$)

Phytoplankton taxa	Max (cells L^{-1})	F %
<i>Prorocentrum arcuatum</i>	13200	95
<i>Pseudonitzschia</i> spp.	92610	58
<i>Tripos furca</i>	4960	53
<i>Alexandrium minutum</i>	1520	48
<i>Proboscia alata</i>	4410	46
<i>Thalassionema nitzschioides</i>	14700	43
<i>Cyclotella</i> sp.	49980	38
<i>Cylindrotheca closterium</i>	8820	35
<i>Pleurosigma angulatum</i>	400	31
<i>Tripos fusus</i>	240	26
<i>Syracosphaera pulchra</i>	2940	23
<i>Gymnodinium</i> sp.	2940	21
<i>Prorocentrum scutellum</i>	800	21
<i>Protoperidinium divergens</i>	1470	21
<i>Hemiaulus hauckii</i>	4410	20

Fig. 4. Abundance of *P. arcuatum* in surface and bottom layer in the investigated period (July 2013–September 2014)

Fig. 5. Monthly means of *P. arcuatum* cell length (l) from September 2013 to October 2014

May, July, September 2014), that is 5% of all analyzed samples (Table 1).

Average monthly abundances per layer are demonstrated in Fig. 4. The cell density varied from 80 cells L^{-1} (Sept. 2014) to 13 200 cells L^{-1} (Sept. 2013).

The abundances of *P. arcuatum* between surface and bottom layer were highly correlated ($r=0.6322$, $p<0.001$) without statistical differences between these layers ($H=317.5$, $p>0.05$).

Seasonal variation in cell length (l) was noticeable (Fig. 5); the smallest cells length (calculated for month average) of 49–52 μm were recorded in winter (December to March), while larger cells occurred in May–July period (62–67 μm). Neither average cell length (l) or total cell length (tl) were significantly correlated with temperature, but positive correlation for both parameters was recorded with salinity ($r=0.501$ and $r=0.587$, $p<0.05$, respectively).

Table 2 Range of nutrients: nitrates NO_3^- ($\mu\text{mol L}^{-1}$), nitrites NO_2^- ($\mu\text{mol L}^{-1}$), ammonia NH_4^+ ($\mu\text{mol L}^{-1}$), total dissolved nitrogen NTOT ($\mu\text{mol L}^{-1}$), organic dissolved nitrogen NORG ($\mu\text{mol L}^{-1}$), orthophosphates HPO_4^{2-} ($\mu\text{mol L}^{-1}$), organic dissolved phosphorus PORG ($\mu\text{mol L}^{-1}$) and orthosilicate SiO_4^{4-} ($\mu\text{mol L}^{-1}$) during the investigated period

Environmental parameters	Range [$\mu\text{mol L}^{-1}$]	Average $\pm$ SD
NO_3^-	0.17 – 13.34	4.19 ± 2.88
NO_2^-	0.06 – 0.38	0.19 ± 0.07
NH_4^+	0.05 – 3.12	1.15 ± 0.93
TIN	0.87 – 14.64	5.53 ± 2.77
NTOT	6.51 – 31.13	6.51 ± 5.48
NORG	0.92 – 26.17	5.65 ± 5.26
HPO_4^{2-}	0.02 – 0.14	0.07 ± 0.03
PTOT	0.10 – 0.68	0.20 ± 0.14
PORG	0.00 – 0.57	0.13 ± 0.13
SiO_4^{4-}	0.15 – 5.40	2.30 ± 1.22

Fig. 6. Oxygen saturation (%), temperature ($^{\circ}\text{C}$) and salinity for surface and bottom layers at investigated sites

Environmental conditions during *P. arcuatum* proliferation

The temperature fluctuations at the sampling site in Medulin Bay were similar at both investigated depths and followed the regular

seasonal pattern with the lowest value measured in December at the surface (9.3°C) and the highest in July (24.3°C) (Fig. 6a). Absolute salinity values ranged from 33.7 to 37.8, but with majority of values in the 36–38 range (Fig. 6b). Higher fluctuations occurred in the surface layer. The water column was generally well oxygenated, with oxygen saturation between 96 and 154%, and the average value of $115 \pm 18\%$ (Fig. 6c). Higher variability was also noticed in the surface layer.

At the peak of *P. arcuatum* abundance in September, the temperature of the water column was 20.4°C , while the salinity was 37.7.

The ranges of nutrients concentration are presented in Table 2. There was no significant correlation between abundance of *P. arcuatum* and nutrients concentration.

Interactions of *P. arcuatum* and phytoplankton community

The list of most frequent phytoplankton taxa (frequency $>20\%$) recorded in Medulin Bay during the research period are presented in Table 1. Wicoxon-mached pair test showed no significant difference in phytoplankton community structure between two investigated layers ($W = -842.0$, $p = 0.191$).

Prorocentrum arcuatum occurred with the frequency of 95%. Maximal contribution of *P. arcuatum* abundance to total microphytoplankton community was recorded in June 2014 (69.5%).

The phytoplankton population of the Medulin Bay was generally dominated by Bacillariophyceae (49 taxa) throughout the research period; *Chaetoceros curvisetus*, *Cyclotella* sp., *Proboscia alata*, *Pseudo-nitzschia* sp. and *Thalassionema nitzschioides* were the species with highest abundance and frequency of occurrence. Dinoflagellates were also taxonomically diverse (57 taxa), but with significantly lower abundances.

All of these species were particularly numerous from July-October 2013 when the significant proliferation of *P. arcuatum* was recorded (33.9% of total microphytoplankton). This proliferation was accompanied with high density of diatoms *Cyclotella* sp. *Pseudo-nitzschia* spp. and *Thalassionema*, and low concentrations of dinoflagellates *Prorocentrum scutellum*, *Triplos* spp. and *Hermesinum adriaticum*. However, this community structure changed notably after sudden cooling of the water column in November, when the temperature dropped to 9°C. Consequently, *P. arcuatum* (and *Pseudo-nitzschia* spp.) densities considerably decreased, while cells of *Cyclotella* sp. vanished completely till next June.

DISCUSSION

Despite the long tradition in phytoplankton taxonomical research in the eastern Adriatic Sea (PUCHER-PETKOVIĆ & MARASOVIĆ, 1982; REVELANTE & GILMARTIN, 1983; VILIČIĆ *et al.*, 2002; MARIĆ *et al.*, 2012; GODRIJAN *et al.*, 2013), so far *Prorocentrum arcuatum* has only been detected in the meromictic Lake Rogoznica (VILIČIĆ *et al.*, 1997; BARIĆ *et al.*, 2003; BURIĆ *et al.*, 2009). Even on global basis, there is scarce information about this planktonic dinoflagellate. First description was provided by ISSEL (1928) and amended by DODGE (1975), based on cells mainly from the field samples, indicating the distinct morphological characteristics of *P. arcuatum* as a separate species. In particular, those authors emphasized the significant torsion of *P. arcuatum* cells, claiming it as a unique feature among *Prorocentrum* species.

The pronounced torsion of *P. arcuatum* cells from Medulin Bay that we observed is in

accordance with the original description. By using SEM, we also observed the characteristic large anterior spine in periflagellar area and an additional small spine featuring in the description by SCHILLER (1933).

Despite the long date since discovery, taxonomy of this species is still incompletely resolved. Recent description of *P. arcuatum*, (WOOD, 1963; DODGE, 1975; BURIĆ *et al.*, 2009; SPATHARIS *et al.* 2009; MUNIR *et al.*, 2013) recognizes the same morphological features of *P. arcuatum* as we have described in this study. However, TOLOMIO (1988) distinguishes between *P. arcuatum* and *P. gibbosum* based on the position of small apical spine, observing that in *P. gibbosum* “spine and tiny tooth (*i.e.* small spine) seem to belong to the same valve and not the opposite valves, as in accordance with the description from ISSEL (1928) for *P. arcuatum*.” Considering that this opinion is not repeated by any recent investigation, we think that insufficient evidence exists for assigning our specimens to anything other than *P. arcuatum*, until species’ thorough revision.

The size range of *P. arcuatum* cells measured during this study is consistent with the length ranges reported by WOOD (1963) and DODGE (1975) from the Mediterranean. Small discrepancies were found when compared to MUNIR *et al.* (2013), who observed cells in range of 45-50 µm in north Arabian Sea, and TOMAS (1996) who indicated the general range of 60-70 µm. Cells from Medulin Bay were slightly larger than those reported by former authors, but we have also noticed some seasonal variability in cell length in Medulin Bay.

Given that there are no previous analyses on influence of environmental conditions to length variation of *P. arcuatum* cells, we were not able to draw more definite conclusions. Despite the lack of significant correlation with temperature conditions, due to small dataset the influence of temperature is not to be excluded. In their paper, BURIĆ *et al.* (2009) reported positive correlation of *P. arcuatum* with temperature. We have also compared the morphology of *P. arcuatum* from Medulin Bay with the cells described from the Lake Rogoznica (BARIĆ *et al.*, 2003; BURIĆ *et al.*,

2009). Variations of those morphotypes from two locations were especially expressed in torsion of the cell, which was significantly more pronounced in the cells from Medulin Bay. Additionally, some discrepancies in the cell length were also determined, with *P. arcuatum* from Medulin Bay being slightly larger than those reported from Lake Rogoznica.

In Medulin Bay *P. arcuatum* was observed continuously through 15-month period, with significant proliferation from September to October 2013. Similar prolonged appearance was noticed by SPATHARIS *et al.* (2009) and MUNIR *et al.* (2013) in the eastern Mediterranean and northern Arabian Sea, respectively. Species maximum occurred in the September 2013 after which *P. arcuatum* abundance decreased, but its presence remained constant until the end of the investigation. However, the expected high abundance of *P. arcuatum* in the autumn of the next investigated year (2014) was not recorded. Instead, *P. arcuatum* abundance was barely 80 cells per liter. Similar interannual variability was recorded by MUNIR *et al.* (2013), who found unequal seasonal distribution of *P. arcuatum* during the two-year period research, despite the fact that environmental conditions in both investigated years were quite similar.

Presumably, temperature and salinity are major factors controlling the increased abundance of *P. arcuatum* (MUNIR *et al.*, 2013; SAHRAOUI *et al.*, 2013). Based on our results from Medulin Bay and the results from Lake Rogoznica (BARIĆ *et al.*, 2003; BURIĆ *et al.*, 2009), the optimum physical conditions for *P. arcuatum* proliferations in the water column seem to be the temperature of about 20°C, and salinity of at least 37. Considering that both areas are characterized with increased productivity (the aquaculture location in Medulin Bay and naturally

eutrophicated Lake Rogoznica), the eutrophic conditions might also provide an incentive to population growth. Cells of *P. arcuatum* have never been recorded in oligotrophic waters.

The observed absence of *P. arcuatum* proliferation in 2014, despite the considerable similarity in environmental conditions, might be attributed to increased competition due to changes in phytoplankton population structure. The major change in phytoplankton assemblage of Medulin Bay in autumn 2014 with respect to autumn 2013, was the increase in the abundance of *Tripes* species.

The study from the Lake Rogoznica by BURIĆ *et al.* (2009) has demonstrated the ability of *P. arcuatum* to proliferate successfully in more restrictive conditions, characterized with nitrate deficiency in the water column, if within temperature and salinity optimum. However, suppression of *P. arcuatum* proliferation in 2014 in Medulin Bay could be explained by the competitive mechanism of *Tripes* species that respond quickly to nutrient abundance, especially if organic compounds of nitrogen prevail (SMALLEY & COATS, 2002).

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Morfologija i ekologija slabo poznatog dinoflagelata *Prorocentrum arcuatum* (Dinophyceae) iz Medulinskog zaljeva (istočni dio Jadranskog mora)

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SAŽETAK

Na području Medulinskog zaljeva, u razdoblju od srpnja 2013. do listopada 2014. godine, zabilježena je pojava rijetke i slabo poznate vrste dinoflagelata *Prorocentrum arcuatum*. Prisutnost vrste *P. arcuatum* u fitoplanktonskoj zajednici Medulinskog zaljeva tijekom 15- mjesecnog razdoblja omogućila je istraživanje promjene u brojnosti vrste i okolišnih parametara koji uvjetuju dinamiku populacije vrste *P. arcuatum* u prirodnim uvjetima, a ujedno nam je omogućilo i prvi detaljni opis *P. arcuatum* temeljen na SEM metodologiji. Tijekom istraživanja zabilježili smo sezonsku varijabilnost u veličini stanica *P. arcuatum*. Optimalni fizikalni uvjeti za povećanu brojnost *P. arcuatum* zabilježeni su u jesen 2013. pri rasponu temperature od 19.6°C do 20.4°C i salinitetu 36.7 - 37.7. Unatoč činjenici da su slični okolišni uvjeti zabilježeni i naredne godine, u jesen 2014, povećanje brojnosti *P. arcuatum* se nije ponovilo, što može biti povezano s većom kompetitivnošću drugih vrsta uslijed godišnjih promjena fitoplanktonske zajednice.

Ključne riječi: Jadransko more, planktonski dinoflagelati, *Prorocentrum*, *Prorocentrum arcuatum*