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Dynamics of *Lingulodinium polyedra* development in the Bulgarian part of Black Sea (1992-2022)

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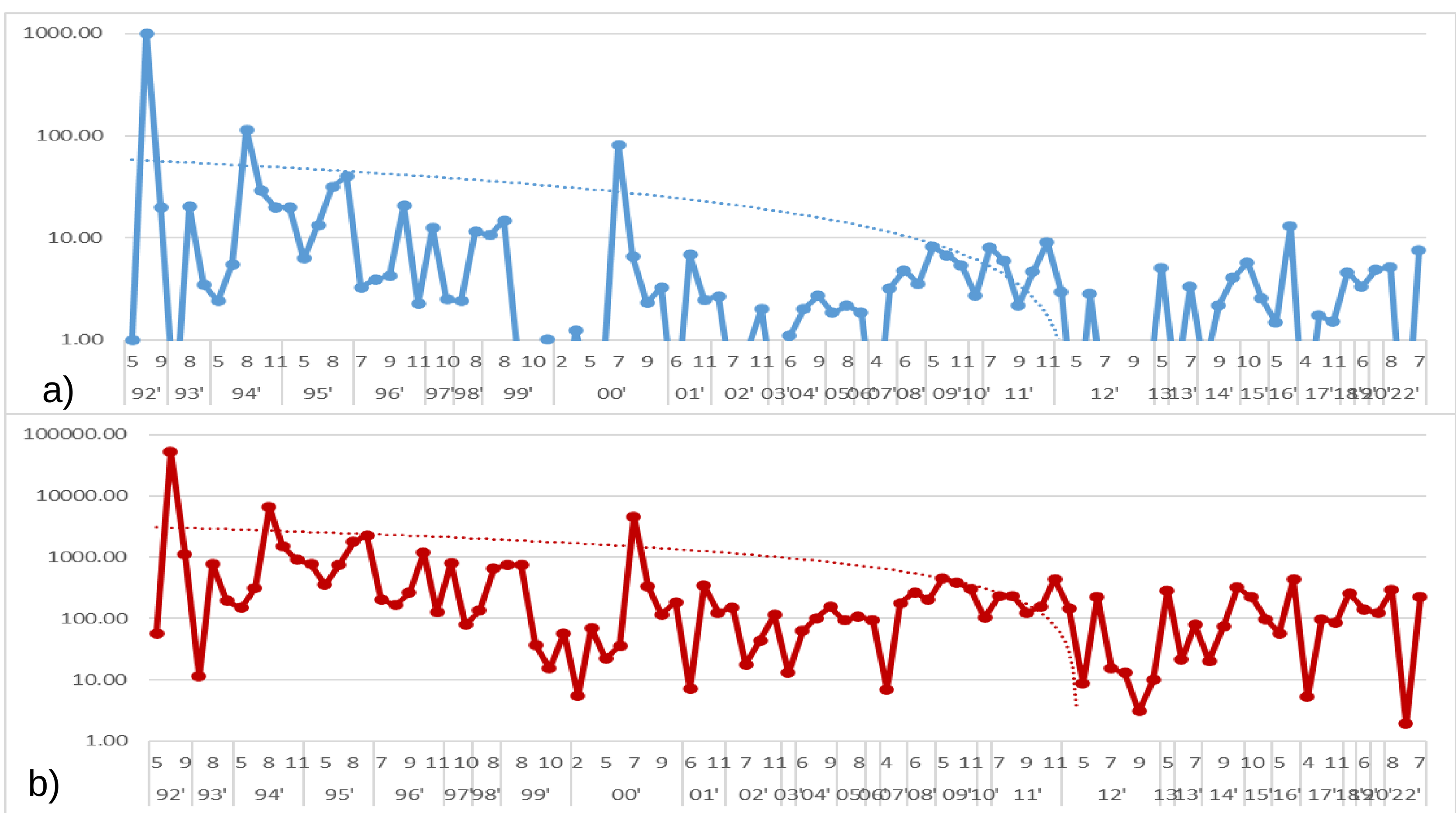
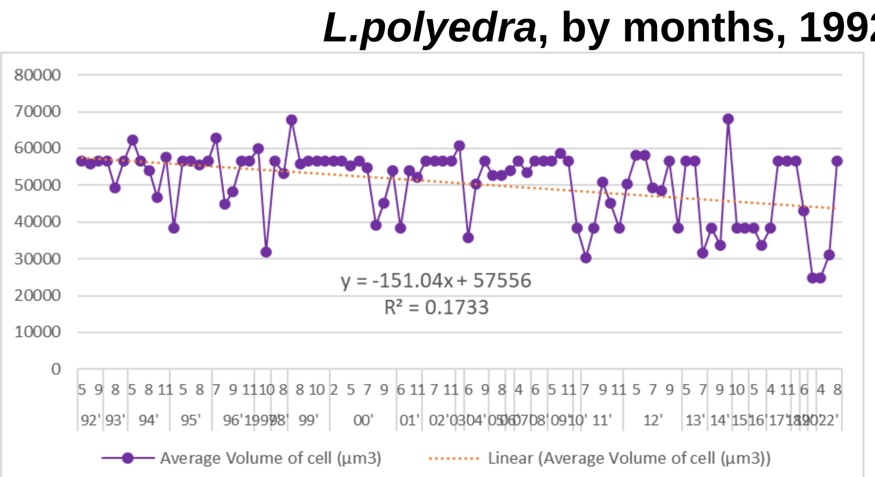
INTRODUCTION & AIM

The warm-water species *Lingulodinium polyedra* (F.Stein) J.D.Dodge 1989 is a red tide former that has been associated with fish and shellfish mortality events. *L.polyedra* is an armoured, marine, bioluminescent dinoflagellate species, with dark, orange-brown chloroplasts. Cells of *L.polyedra* are angular, roughly pentagonal and polyhedral-shaped, 40-54 µm in length and 37-53 µm in transdiameter width. The species was first described in the Black Sea by Zernov (Zernov, 1913). In October 1909, he observed the appearance of rusty-red stripes caused by *L.polyedra* in the coastal zone of Sevastopol (Terenko and Krakhmalnyi, 2022). Long-term data on the phytoplankton of the Bulgarian Black Sea (BBS) coast describe three states for the ecosystem: a “pristine”, reference phase (1954 - 1970); an intensive anthropogenic eutrophication (1970 - 1992) phase; and a post-eutrophication phase after the early 1990s of the 20th century. The eutrophication period is characterised with degradation of the BBS ecosystem and high biomass and abundance of the phytoplankton as well as the frequency, duration, and intensity of the phytoplankton blooms (some of which are formed by the potentially toxic species, like *L.polyedra*, which sharply increased during the beginning of the study period). In the 1980s, *L.polyedra* reached the highest biomass of 84403.20 $mg.m^{-3}$ in Varna Bay, BBS. During this period of development of the Black Sea ecosystem, from 1970 to 1992, *L.polyedra* was recorded as an accompanying species during the red tides formed by the dinoflagellate *Prorocentrum cordatum*, typically in spring and summer. The species is included in the taxonomic reference list of harmful microalgae (Lundholm et al., 2009). *L.polyedra* is associated with the production of yessotoxins (YTX), and the impact of YTX on human health continues to be studied. In the 1990s, during blooms of *L.polyedra*, the presence of paralytic shellfish poison (like saxitoxin) was reported. However, there are no recent studies confirming the production of saxitoxin by *L.polyedra*.

The aim of this long-term study (1992-2022) was to provide a comprehensive overview of the development of *L.polyedra* in the phytoplankton biocoenosis in the Bulgarian Black Sea when under anthropogenic stress and during the ecosystem recovery.

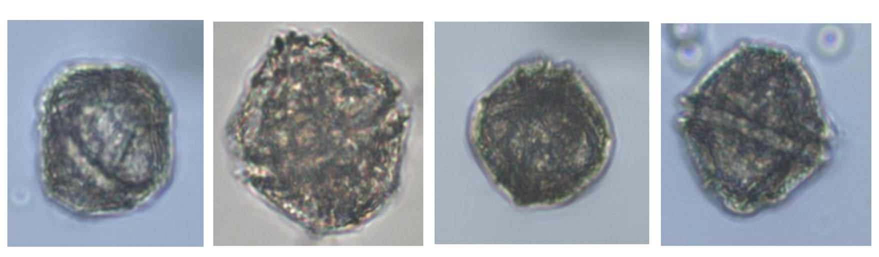


Maximum abundance (mln. cells.m^{-3}) (a) and maximum biomass ($mg.m^{-3}$) (b) of *L.polyedra*, by months, 1992-2022 (logarithmic scale)



Average volume of the cells of *Lingulodinium polyedra*, by months, 1992-2022

The abundance and biomass of *L.polyedra* has declined from 1992 to the present. with an increasing frequency of individuals exhibiting smaller cell sizes.



METHOD

A total of 5126 phytoplankton samples were collected monthly and seasonally of each year during the research period (1992–2022). The sampling was conducted from 316 stations (as part of routine monitoring programs of the Institute of Fish Resources (IFR)-Varna, Bulgaria beginning in the 1990s, and national and international projects) with Niskin bottles (5l) at the standard depths (0, 5, 10, 25, 50, 75, 100 m). The phytoplankton samples (500 ml) were fixed with 37% formaldehyde solution at a final concentration of 2% on board the research vessels (RV) and concentrated by the settling method. The information on the maximum concentrations of *L.polyedra* for the period 1987–1992 is based on literature data (Velikova et al., 1999). The qualitative and quantitative analyses of the samples were performed with a conventional light microscope in counting chambers of Palmer–Maloney—0.05 ml and Sedgwick Rafter—1 ml, using standard protocols for the Black Sea (Moncheva and Parr, 2010). The cell volume of phytoplankton was calculated using geometric formulas. Frequency of occurrence was determined by dividing the number of phytoplankton samples in which the species was found by the total number of samples for a given time period and multiplying the result by 100.

RESULTS & DISCUSSION

The maximum values for the species in Bulgarian waters between 1987 and 2022: offshore (to 3 n.m.), open sea (>3 n.m.)

№	Regions	Distance from the shore	Max of Abumdance (mln. cells x m^{-3})	Max of Biomass (mg x m^{-3})
1	**Biala**	offshore	3.35	70.96
2	**Albena**	offshore	0.16	8.79
3	**Balchik**	offshore	4.91	121.48
4	**Beloslav lake**	offshore	11.59	799.35
5	**Burgas bay**	offshore	40.08	2114.22
6	**cape Emine**	offshore	18.29	965.08
	cape Emine	open sea	14.67	674.27
7	**cape Galata**	offshore	81.44	4603.07
	cape Galata	open sea	9.43	532.90
8	**cape Kaliakra**	offshore	9.01	191.15
	cape Kaliakra	open sea	1.19	67.49
9	**cape Maslen**	offshore	0.55	31.25
10	**Dvoinitca**	offshore	0.43	21.41
11	**Ilandzhik**	offshore	1.51	85.36
12	**Kamchia**	offshore	0.34	19.00
13	**Krapec**	offshore	0.26	9.91
14	**Rusalka**	offshore	2.43	93.32
15	**Shabla**	offshore	5.67	160.28
16	**Sozopol**	offshore	2.72	104.73
17	**Trawling**	open sea	4.61	260.82
18	**Varna bay**	offshore	1600.00	84403.20
19	**Varna lake**	offshore	8.14	460.18
20	**Varvara**	offshore	0.66	37.58
21	**Veleka**	offshore	1.94	87.86

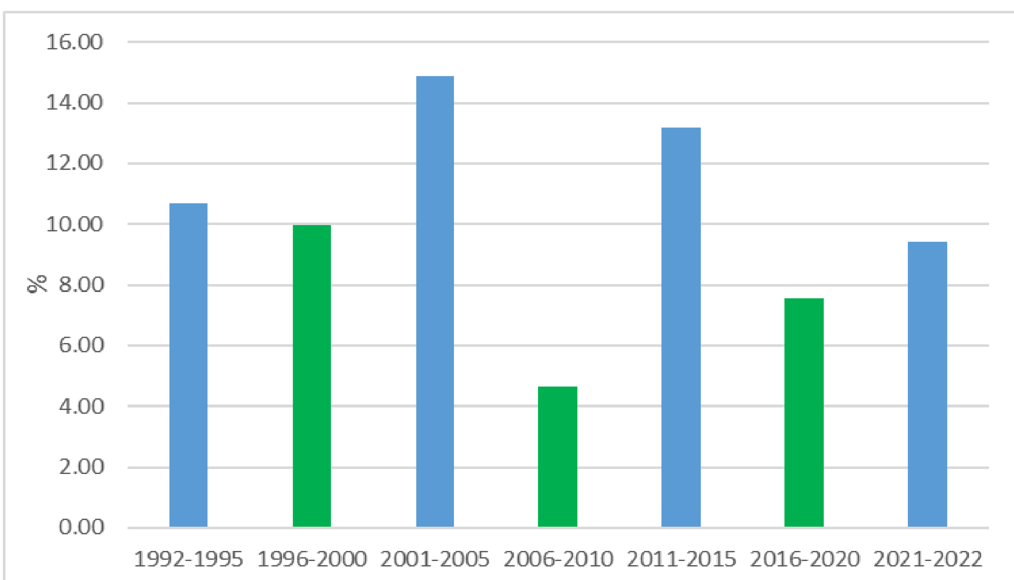
Blooms of *L. polyedra* (≥1000 mln. cells.m^{-3}) in Bulgarian marine waters were observed exclusively in the late 1980s and early 1990s. In the 1980s, the highest recorded abundance was 1600.00 mln. cells.m^{-3} and biomass of 84403.20 $mg.m^{-3}$ (September 1987, Varna Bay). In the 1990s, a single bloom of the species was recorded with abundance of 1000.00 mln. cells.m^{-3} and biomass of 52752.00 $mg.m^{-3}$ (August 1992, Varna Bay) (Velikova et al., 1999). Since the early 1990s, blooms of *L.polyedra* have not been observed, and higher concentrations of the species have been recorded during the warmer months only. *L.polyedra* developed most intensively in the eutrophicated waters of Varna Bay, Burgas Bay, and Cape Galata, up to 3 miles.

In all other water areas, the species was with abundance of 18.29 mln. cells.m^{-3} and biomass - 965.08 $mg.m^{-3}$.

Monthly development of *L. polyedra*, for the period 1992-2022

*during March the species has been recorded only twice

Month	Max Abundance (mln cells.m^{-3})	Average Abundance (mln cells.m^{-3})	Max Biomass ($mg.m^{-3}$)	Average Biomass ($mg.m^{-3}$)
II	0.10	0.08	5.42	4.55
III	20.00	11.46	769.30	458.21
IV	1.24	0.39	70.24	21.09
V	8.14	2.29	460.18	130.29
VI	13.33	2.60	762.13	146.11
VII	81.44	3.11	4603.07	151.94
VIII	1000.00	13.15	52752.00	686.72
IX	40.08	5.45	2265.52	285.76
X	28.98	5.86	1528.81	298.17
XI	20.00	2.40	906.83	128.27



Percent frequency of occurrence of *L.polyedra* in the phytoplankton samples (average of 5 year periods), 1992-2022.

The frequency of occurrence of *L. polyedra* over a 30-year period is lower during the second half of each decade that the study covers. This periodicity in the species' occurrence correlates with the generally established pattern for the entire Black Sea (Oguz et al., 2006), indicating that cold winters with increased levels of nutrients in the photic layer are usually recorded during the first half of each decade, creating conditions for the intensive development of *L.polyedra*.

CONCLUSION

- The highest concentrations of *Lingulodinium polyedra* in the Bulgarian Black Sea waters were recorded during the 1980s.
- The species basically developed in the layer up to 10 meters in depth during the July, August, and September, when the seawater was well-warmed and tempered.
- The highest abundance and biomass of the species were observed in coastal marine waters, subjected to intensive anthropogenic eutrophication.
- The decreasing tendency of abundance and biomass of the dinoflagellate *L. polyedra* was established in investigations waters.
- The frequency of occurrence of *L. polyedra* is characterized by regular periodic changes, likely associated with variations in hydrometeorological and hydrological conditions in the Black Sea.

FUTURE WORK / REFERENCES

Lundholm, N., Churro, C., Escalera, L., et al., (Eds) (2009 onwards). IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. *Lingulodinium polyedra* (F.Stein) J.D.Dodge, 1989; Moncheva, S., Parr, B., 2010. Manual for Phytoplankton Sampling and Analysis in the Black Sea, 68p.; Oguz, T., Dippner, J.W., Kaymaz, Z., 2006. Climatic regulation of the Black Sea hydro-meteorological and ecological properties at interannual-to-decadal time scales. Journal of Marine Systems, 60, 235–254; Terenko, G., Krakhmalnyi, A. 2022. Red tide of the *Lingulodinium polyedrum* (Dinophyceae) in Odessa Bay (Black Sea). Turkish Journal of Fisheries and Aquatic Sciences, 22(4);Velikova, V., Moncheva, S., Petrova, D., 1999. Phytoplankton dynamics and Red Tides (1987-1997) in the Bulgarian Black Sea. Water Science and Technology, vol 39(8), pp. 27–36; Zernov S.A., 1913. About the study of the life of the Black Sea. West Imp. Academic sciences in physics and mathematics 32(1). 299 p. (in Russian).