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RESEARCH PAPER

Red tide of *Lingulodinium polyedrum* (Dinophyceae) in Odesa Bay (Black Sea)

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Abstract

One of the most massive red tides in Odessa Bay was observed in September-October 2020. It was caused by a toxic dinoflagellate *Lingulodinium polyedrum* (Stein) Dodge. The maximum abundance (56.1x10^6 cells L^{-1}) of *L. polyedrum* was registered at the Odessa port area on October 6, when the water temperature and the salinity were 19.7°C and 14.3‰, respectively. The red tide was so huge and dense that the water glowed at night due to the bioluminescence characteristic of this species. The article briefly describes the history of the study of *L. polyedrum* in this area and provides a detailed morphological description with original photographs of this species sampled from the bloom. We associate the appearance of the red tide with an increased temperature of sea water and air, a high content of nutrients, the presence of viable *L. polyedrum* cysts, and a slight decrease in salinity in the bay during the period of a mass development of the species in autumn of 2020. The red tide was accompanied by *Protoperidinium steini, P. divergens, Prorocentrum cordatum, P. minimum, P. micans, Gonyaulas scrippsae, Diplopsalis lenticula, Azadinium spinosum, Dinophysis rotundata, D. acuminata, Oblea rotunda, Scrippsiella trochoidea,* and *Tripos furca.*

Introduction

Red tides refer to colour changes of seawater due to the massive development of microscopic planktonic algae. There are more than 300 species of microorganisms that can cause this phenomenon (Harmful Algal Bloom, HAB) and about 80 of them produce highly poisonous toxins (Hallegraeff, 2003). To date, twenty-three toxic and potentially toxic species of dinoflagellates have been found in the Black Sea. *Lingulodinium polyedrum* is one of them (Ryabushko, 2003; Vershinin et al., 2005).

Lingulodinium polyedrum is a widespread species, and it is capable of yessotoxins production (YTX; Faust & Gulledge, 2002; Paz et al., 2004; Paz at al., 2008; Peter at al., 2018). Cases of mass mortality of marine animals in red tide zones caused by *L. polyedrum* have been reported by several authors (Bruno et al., 1990; Turabo et al., 1998).

Red tides caused by mass growth of *L. polyedrum* are periodically found in different parts of the World Ocean. (Amorim et al., 2000; Bennouna et al., 2001; Morales-Ramirez et al., 2001; Salgado et al., 2011).

The species was originally described as *Gonyaulax polyedrum* (Stein, 1883: Organ. Flagell. 3(2): T. 4, Figure. 7–9). In 1989, it was transferred to the genus *Lingulodinium* (Wall) Dodge (Dodge, 1989) with the thecal formula: Po, 3', 3a, 6'', 6C, S, 6''', 1p, 1''''. To date, a new reinterpretation of the thecal formula of *L. polyedrum* has been proposed as APC (Po), Q, 5', 6'', 6C, 6S, 5''', 2'''' (Kim et al., 2005).

The first note of *Lingulodinium polyedrum* in the Black Sea (as *Gonyaulax polyedra*) belongs to E. A. Zernov. In October 1909, he observed the appearance of rusty-red stripes caused by *L. polyedrum* in the coastal zone of Sevastopol (Zernov, 1913).

The abundance of *L. polyedrum* in the Black Sea has been steadily increasing since the 1970s, *L. polyedrum* and other resistant to eutrophication species of dinoflagellates, such as *Prorocentrum cordatum, P. micans, P. compressum, Scrippsiella acuminata, Heterocapsa triquetra* have become the dominant species (Krakhmalnyi et al., 2018).

The first records of red tides in the Black Sea were associated with *Prorocentrum cordatum* (Skolka & Gautis, 1969; Nesterova, 1979, 1985, 2001). Since the 1990s, *Akashiwo sanguinea, Gymnodinium simplex, Gyrodinium cornutum, G. instriatum, Heterocapsa triquetra, Scrippsiella trochoidea, Prorocentrum cordatum, P. micans* and, finally, *L. polyedrum* have been identified as the main species responsible for red tides in the region (Terenko & Kurilov, 2001; Terenko & Terenko, 2009).

The first HAB of *L. polyedrum* in Odesa Bay was observed near the mouth of the Dnieper-Bug Estuary in October 1999. The abundance of the species was $0,8x10^6$ cells L^{-1}. This phenomenon was followed by the mass death of fish (Terenko & Terenko, 2008). HAB of *L. polyedrum* with abundance of $1,3x10^6$ cells L^{-1} was registered off the Bulgarian coast in the late 1980s to early 1990s (Velikova et al., 1999).

In September-October 2020, one of the biggest red tides in the entire history of the Black Sea studies, caused by L. polyedrum, was observed in Odesa Bay. This dangerous phenomenon was so significant and spectacular that it is still being discussed by the general public (URL-1).

The glow of the water was observed in the dark due to the bioluminescence characteristic of this species, which was repeatedly reported in social media and news (URL-2).

Mass deaths of some marine animals (fish, mollusks) were observed in the red tide zone Odesa Bay, along with the intense coloration of the water and bioluminescence at night. However, *Rhizostoma pulmo* Macri, *Atherina boyeri* Risso и *A. hepsetu* Linnaeus were alive and active inside of the bloom.

Materials and Methods

Phytoplankton samples were collected from the surface water near the shore at 11 stations located at the Odesa Bay in September-October 2020 (Figure.1). The reverse filtration method was used to concentrate water samples (1.5-2L) and nucleopore filters with a pore size of 1.5 µm were applied (Fedorov, 1979; Sorokin, 1979). All samples were condensed to 40-45 mL and then fixed with a 40% solution of neutralized formaldehyde to the final concentration of 4%. Subsequently, repeated concretion was carried out, bringing the sample volume to 20–30 mL. Preliminarily, dinoflagellate samples were examined under microscope before fixation with formaldehyde. Quantitative count of cells was carried out in a Nageotte counting chamber with a volume of 0.05 mL using a light microscope "Mikmed-2" with 300-600X magnification. Calculations of an abundance and a biomass were performed using the DCT software (data collection template) developed in Microsoft Excel, using a Visual Basic script at the Ukrainian Scientific Center of Ecology of the Sea (Odesa).

The morphology of *L. polyedrum* was studied at the Institute for Evolutionary Ecology National Academy of Sciences of Ukraine, (Kiev) using an Olympus-BX51 microscope. *L. polyedrum* cells were examined under transmitted light, differential interference contrast (DIC) mode, and fluorescence mode with preliminary staining of thecal plates with Calcofluor White Stain 18909-100ML-F (Fritz & Triemer, 1985). Lenses UPlanFLN 40 x / 0.67, Plan 40 x / 0.65 Ph 2 and Uplan FLN, 100 x /1.30 Oil were used. Cell sizes were determined using the AmScope software. Pictures were taken on Canon EOS 1000D camera. Images were processed in AdobePhotoshop CS5 Extended. The Statistica program was used to calculate the average cell size and standard deviation. The plates were numbered according to A. Kofoid's system (Kofoid, 1909, 1911), taking into account the reinterpretation of the thecal formula of *L. polyedrum* (Kim et al., 2005).

Results

Morphological Observation

Lingulodinium polyedrum (Stein) Dodge, 1989. Bot. Mar. 32: 289, pl. 29, Figure. 1–6. (Figure. 1–12).
Basionym: *Gonyaulax polyedra* F. Stein 1883. Organ. Infus. 3 (2): Tafel. 4, Figure. 7–9.
Synonyms: *Lingulodinium machaerophorum* (Deflandre & Cookson) Wall, *Hystrichosphaeridium machaerophor um* Deflandre & Cookson.
References: Paulsen, 1908: 31, Figure 40; Schiller, 1937: 291, Figure 299 a–f; Kiselev, 1950: 222, Figure 386 a–g; Dodge, 1982: 211, Figure. 25 D–F, Pl. VI a; Balech, 1988: 170, Figure 17–24; Dodge, 1989: Figure 1 H, I, 37–39; Steidinger & Tangen 1996: 510, Pl. 43; Faust & Gulledge, 2002: 45, Pl. 29, Figure 1–6; Konovalova & Selina, 2010: 122, Tabl. XIII, 8 a–b; Krakhmalnyi, 2011: 128, Табл. 32: *1–13*; Al-Yamani & Saburova, 2019: 250, Pl. 136.
Description: Cells are angular, pentagonal, with distinct ridges along the intercalary sutures. Epicone and hypocone are almost equal. Epicone has a pointed tip. The plates are strong, with pores and ridges at the seams. The apical pore plate (Po) is oval, surrounded by an elevated ridge. There are five apical plates, 3'–5' separated from Po by the Q plate (equivalent to the 3'

plate according to the Kofoidean system). The Q plate is narrow, C-shaped, located between Po and 4' (Figure. 2.3, 2.9–2.10). The first apical plate (1') contacts with Po (Figure 2.10). A cingulum is equatorial, deep, displaced downward by one or two of its width (Figure. 2.2, 2.7), and composed of plates (6C). A sulcus widens towards the antapex and consists of six plates, the largest being Sp (Figure. 2.4). The hypocone consists of 5''' and two antapical (2''''). Thecal formula is Po, Q, 5', 6'', 6C, 6S, 5''', 2''''. The nucleus is in the middle of the cell. Chloroplasts are numerous, oval, yellow-brown in colour, diverging from the center (Figure. 2.1, 2.11). Cells are 33.9–44.5 (39.8±2.7) µm long and 33.3–43.7 (38.9±3.1) µm wide; n=21.

Distribution: *L. polyedrum* is a neritic species commonly occurring in warm temperate to tropical waters of the coastal areas worldwide, bloom-forming species.

Nomenclatural notes: In the Kofoidean system, 1'''' is interpreted as 1p (Kofoid, 1909, 1911). We used a reinterpretation of the thecal formula of *L. polyedrum* (Kim et al., 2005). The cell sizes of *L. polyedrum* from the Odesa Bay were in good agreement with the data obtained from Deukryang Bay, Korea (Kim et al., 2005): 32.7–46.2 µm long and 30.4–42.9 µm wide; from Kuwait's waters (Al-Yamani & Saburova, 2019): 37–42 µm long and 35–39 µm wide and from Caribbean Sea and adjacent area (Wood, 1968): 30–50 µm long.

Under laboratory conditions, vegetative cells produced cysts after storage of samples in the dark at +5°C for 6 days (Figure. 2.12). Cysts are spherical, with a hairy surface, and a noticeable red-brown colour area in the center. Sizes of cysts varied from 43.4 to 45.9 µm in diameter.

Quantitative Parameters of the Red Tide of *L. polyedrum*

The first detection of *L. polyedrum* in the plankton of Odesa Bay was recorded in early July 2020 (abundance of $1.2x10^3$ cells L^{-1}), at a seawater temperature of 22.1°C and a salinity of 16.71 ‰. The species had een presented in the plankton for 14 weeks from July to November 2020.

A small growth of *L. polyedrum* was noted at two weekly monitoring areas (Arcadia-Ar and Maliy Fontan-MF) on September 23. The development was insignificant due to intense water exchange with the open sea at these locations. The maximum abundance was recorded in Arcadia ($51.4x10^3$ cells L^{-1}) at a seawater temperature of 20.1°C and a salinity of 16.4‰.

A red tide of *L. polyedrum* was found in Koblevo area (31.17389; 46.62750) during a comprehensive study of Odesa Bay (Figure. 1), conducted from October 1 to October 3, 2020. The abundance of the species was

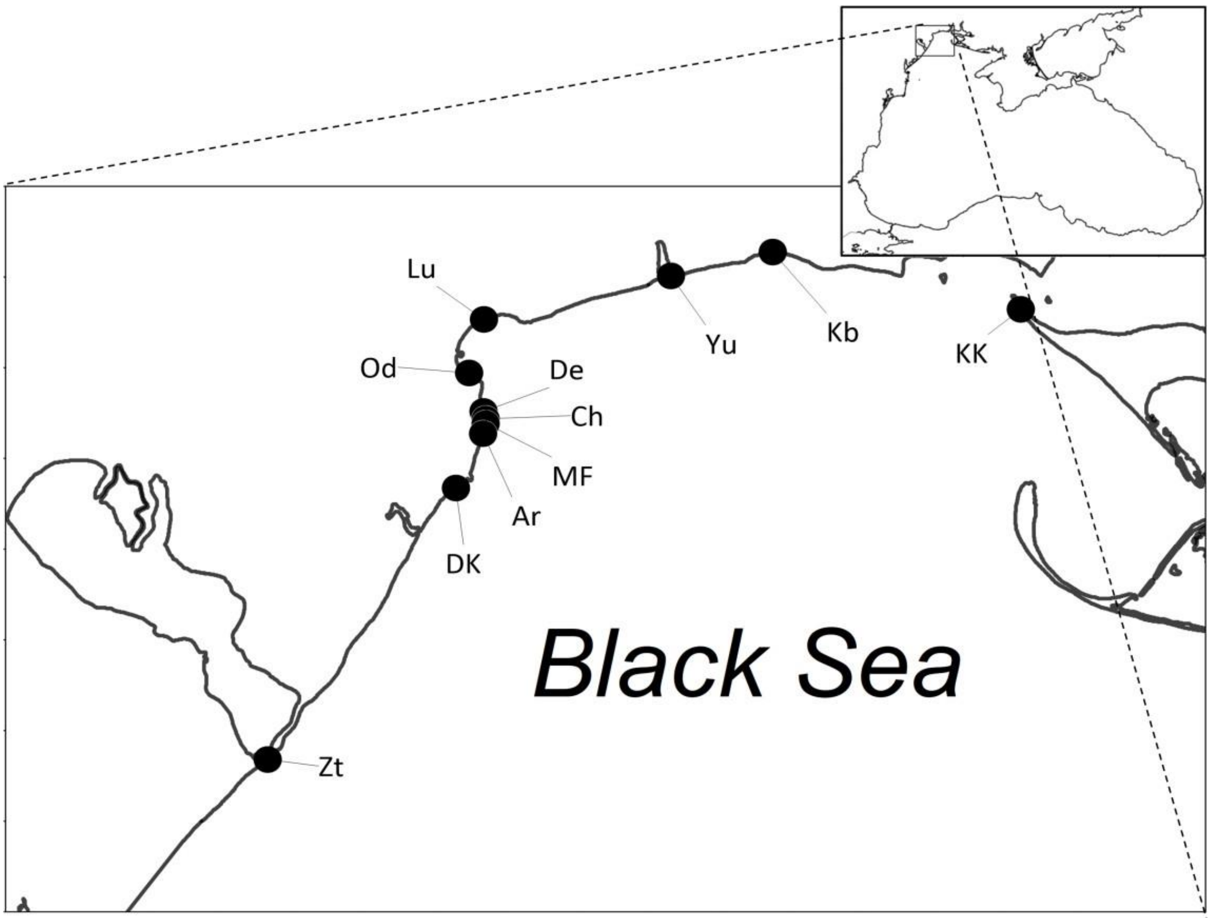

Figure 1. Map of sampling stations at the Odesa Bay in September-October 2020: KK, Kinburn Split; Kb, Koblevo; Yu, Yuzhnoe; Lu, Luzanovka; Od, Port; De, Delphin; Ch, Chkalovskiy; MF, Maliy Fontan; Ar, Arcadia; DK, Dacha Kovalevskogo; Zt, Zatoka

1.45x10^{6} cells L^{-1}, at a temperature of 20.0°C and salinity 13.9 ‰.

The maximum abundances of *L. polyedrum* were recorded in the Odesa Port (30.74917; 46.49417) on September 16 with an abundance 9.2x10^{6} cells L^{-1} at a water temperature of 21.7°C and salinity 15.9‰, and October 6 with an abundance 56.1x10^{6} cells L^{-1} at a water temperature of 19.7°C and a salinity of 14.3‰. The presence of such high abundances is primarily associated with the "closeness" of this area of the Odesa Bay. It is characterized by a limited water exchange with the open water area of the bay, which contributes to an increase of a trophic state of water.

Discussion

The red tide of *L. polyedrum* in the Odesa Bay was provoked by a combination of hydrological and hydrochemical conditions that took place in September–October 2020. Thus, from September 27 to October 2, 17 mm of precipitation fell in the area of the bay that was half of the October monthly norm (Hydrometeorological Center of the Black and Azov Seas, Odesa, Ukraine). Average monthly air and water temperatures in September–October 2020 significantly exceeded the corresponding average values for the last 30 years (from 1981 to 2010). This excess was 3.2°C in

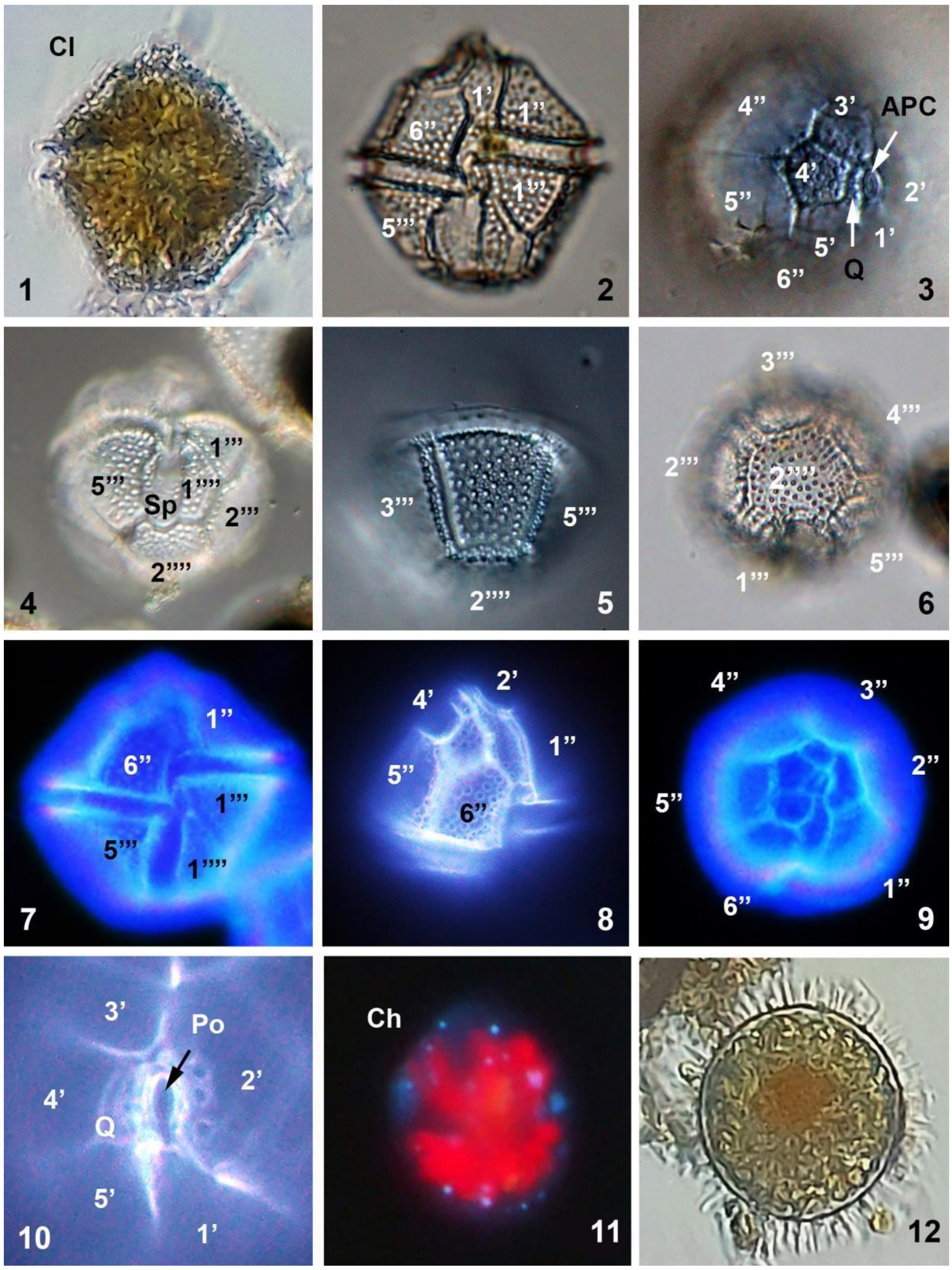

Figure 2. 1–12: *Lingulodinium polyedrum* (Stein) Dodge. 1–2, 4, 7–8 – ventral view; 6– antapical view (antapex); 3, 9–10– apical view (apex); 5– dorsal view; *3–5*– is the differential interference contrast microscopy (DIC); 7–10– a mode of fluorescence with preliminary staining of cells using Calcofluor White M2R. 11– luminescence of chloroplasts in ultraviolet light 12- cyst. Designations: APC – the apical pore complex; Ch– chloroplasts; Po, Q, 1'–5', 1''–6'', 1''' –5''', 1'''' –2''''– thecal plates; Sp–sulcal plate. 1, 12– "Mikmed–2" microscope, 2–11– Olympus BX51 microscope.

September and 5.0°C in October for the air temperature. In October, the average monthly air temperature reached a record value of 16.7°C for the entire time of observations. In September and October 2020, the mean monthly water temperature in the bay was also higher by 3.4°C and 4.4°C, respectively. Meanwhile, the average monthly water temperature reached a record value of 18.7°C in October. In recent years, the coastal regions of the Black Sea have experienced an increase in the air and water temperatures that influence the local ecosystem.

The second important factor contributing to the spread of *L. polyedrum* in Odesa Bay was the wind. Thus, from October 4 to October 6, a southeasterly wind (6–7 m x s^{-1}) blew in the bay contributing to the surge of the upper layer of seawater with a large number of *L. polyedrum* cells, into the bay. Wave heights reached 1 m and they were able to disturb the sediments to a depth of 10 m. This facilitated the migration of dormant *L. polyedrum* cysts and nutrients from the bottom to the upper layers of the water accelerating the formation of the red tide throughout the bay.

A significantly lower oxygen saturation compared to the previous years was one of the characteristics of the water in autumn 2020. It was about 70%, which is most likely associated with a long period of high temperatures until mid-October (over 20°C) and active destructive processes in the water column.

During our research, *L. polyedrum* developed in a range of water temperature between 12.5–24.0°C, salinity between 13.9–16.7 ‰, pH between 7.69–8.46, $P(PO_4)^{3-}$ between 5.43–44.4 µg x L^{-1}, $N(NO_2)^{2-}$ between 1.51–11.4 µg x L^{-1}, $N(NO_3)^{2-}$ between 8.24–146.0 µg x L^{-1}, $N(NH4)^{+}$ between 0.26–17.3 µg x L^{-1}, Si between 129–1003 µg x L^{-1}.

A high abundance of *L. polyedrum* cysts was revealed by earlier studies of the upper layer of silty sediments in Odesa Bay (Nikonova, 2008; Nesterova, 2010). In our opinion, this fact provided a high rate of biomass growth, even during periods of lower illumination, which took place in early October. As a result, by the end of the active bloom of *L. polyedrum* (October 6), water samples had a rich dark amber color, which could be explained by the high number of cells. Greasy spots were observed on the surface due to the accumulation of waste products in the water, including lipids of *L. polyedrum.*

Conclusions

The phenomenon of the red tide had a mosaic nature, with varying degrees of coloration of water in different areas of the bay from light amber to dark amber (amber colours).

As noted above, the red tide in the bay was observed from September 16 to October 6, 2020. At the same time, it was most intense in early October, at a water temperature of 19.7–20.0°C and salinity 13.9–15.9‰.

The development of a red tide in September–October 2020 in Odesa Bay was associated with an increased air and water temperature in the autumn of 2020, a sufficient supply of nutrients, winds from the open sea, the presence of viable cysts and a slight decrease in the salinity of water areas where blooming spots were formed.

Other species of dinoflagellates, such as *Protoperidinium steinii, P. divergens, Prorocentrum cordatum, P. minimum, P. micans, Gonyaulas scrippsae, Diplopsalis lenticula, Azadinium spinosum, Dinophysis rotundata, D. acuminata, Oblea rotunda, Scrippsiella trochoidea,* and *Tripos furca* were found in smaller numbers in the red tide.

Ethical Statement

This article does not contain any experiments on humans and animals.

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Author Contribution

G.T. contributed to the ecology study of the species

A.K. contributed to the morphological study of the species

Writing of the paper was done by both authors in equal part.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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