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Ephemeral parasitism on blooming diatoms in a temperate estuary

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Abstract. Parasites of phytoplankton influence phytoplankton bloom dynamics and may severely affect the type of food available for higher trophic levels. The incidence of parasitic infections generally is expected to increase across ecosystems worldwide under the scenario of global change. Herein we report on a massive parasite infection on two dominant diatoms of the austral winter bloom, namely *Thalassiosira pacifica* and *Chaetoceros diadema*, recorded during an extreme precipitation period in the Bahía Blanca Estuary, Argentina. The parasite infection was concomitant with a marked drop in water salinity and affected more than 40% of host cells. Although the parasite on *C. diadema* was not identified, the parasite on *T. pacifica* was most likely *Pirsonia* sp., a nanoflagellate with high host specificity. After the intense rainy period and the parasitic infection, the phytoplankton biomass dropped (by more than 80%) and the community structure shifted to one with smaller species (i.e. *Thalassiosira curviseriata, T. hibernalis* and *T. minima*). We discuss the implications that these modifications may have on the food web dynamics and the potential relationship between precipitation-driven modifications in water properties and the emergence of parasitism in coastal eutrophic environments.

Additional keywords: parasitic protists, phytoplankton bloom, precipitation, species turnover.

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Introduction

Phytoplankton blooms are triggered by species life history and the optimal use of essential resources (i.e. light and nutrients), whereas biomass loss is shaped by cell sinking, autolysis, zooplankton grazing or viral and parasite infections (Sommer *et al.* 2012). Parasites of phytoplankton play a crucial role in controlling plankton population dynamics, thereby affecting the type of food available for higher trophic levels (Frenken *et al.* 2016). However, parasitism on primary producers is difficult to document in field investigations (e.g. Tillmann *et al.* 1999), probably because of the relatively small size of parasites, their short generation time and the intermittence and fleeting occurrence of infection events (Park *et al.* 2004). Parasitic protists are small unicellular eukaryotic heterotrophs that comprise diverse taxonomic groups (i.e. euglenozoa, dinoflagellates, cercomonads, plasmodiophorids, oomycetes and chytrids; Hanic *et al.* 2009; Mazzillo *et al.* 2011; Alves-de-Souza *et al.* 2012). Parasitism often occurs on large phytoplankton (e.g. diatoms and dinoflagellates; Kagami *et al.* 2007) and is reported highly host specific (e.g. Kühn 1998). In addition, rates of infection increase with host population density, which is markedly reduced when conditions allow epidemic outbreaks of disease (Tillmann *et al.* 1999; Salomon *et al.* 2009).

Empirical investigations have documented parasitism on phytoplankton linked with enhanced temperature, changes in pH, salinity and turbulence (Kühn and Hofmann 1999; Park *et al.* 2004; Kühn and Köhler-Rink 2008). These studies indicate strong regulation by environmental forces on the incidence of infection and that parasites can significantly impair carbon transfer at the base of the food web (Frenken *et al.* 2016). Yet, growing uncertainties regarding the potential effects global warming and extreme atmospheric events may have on

outbreaks of parasitic infection are currently a matter of concern in global change ecology (Brooks and Hoberg 2007; Burge *et al.* 2014).

In the Bahía Blanca Estuary, field surveys of phytoplankton community composition and seasonality have been performed for more than three decades. Long-term changes in bloom phenology and structure have been related to climate- and anthropogenic-driven environmental modifications (e.g. temperature, salinity and dissolved nutrients; Guinder *et al.* 2010; Spetter *et al.* 2013) and shifts in zooplankton species (Berasategui *et al.* 2009). However, thus far no records of parasites on phytoplankton have been reported from field surveys. In the present study we investigated the occurrence of a host-specific parasitism episode on dominant diatoms of the austral winter bloom concurrent with an intense precipitation period and quantified consequent changes in phytoplankton community structure.

Materials and methods

Field sampling and laboratory analysis

Sampling was performed biweekly or weekly during high tide from a pier at Puerto Cuatreros (38°50′S, 62°20′W), a shallow station (mean depth 7 m) in the eutrophic inner zone of the Bahía Blanca Estuary, Argentina. In the present study we focused on the period August–October 2012, which corresponds to the blooming season (winter–early spring; Guinder *et al.* 2010). Discrete records of surface water temperature, salinity, dissolved oxygen (DO) and pH were measured *in situ* using a digital multisensor (Horiba U-10, Miyanohigashi, Kisshoin, Minami-ku, Kyoto, Japan). Local precipitation data were obtained from SATELMET (Bahía Blanca, Argentina).

For phytoplankton assessment, samples were collected using a phytoplankton net (30-μm size pore) and first examined without fixatives under an optical microscope for rapid evaluation of cell condition (e.g. colour, chloroplasts, aggregate formation). Thereafter, samples were preserved in formaldehyde (final concentration 0.4%). Phytoplankton was identified to species level using a Zeiss (Jena, Germany) Standard R microscope and a Nikon (Kanagawa, Japan) Eclipse microscope at a magnification of 1000× and phase contrast. Taxonomy was largely done following Round *et al.* (1990) and Tomas (1997), among other specific references regarding phytoplankton from the Bahía Blanca Estuary (i.e. Guinder *et al.* 2010 and references therein). Parasites were identified according to Kühn *et al.* (1996, 2004). Phytoplankton densities were estimated from surface water samples collected with a plastic bottle (250 mL) and fixed with acidified Lugol's solution. The total abundance of phytoplankton (cells L^{-1}) was determined using a Sedgwick-Rafter chamber (1 mL) (Graticules S50, Tonbridge, UK), whereby the entire chamber was examined and each cell >3 μm was counted as a unit (McAlice 1971). It was not possible to determine the density of parasites because some host cells were severely covered. Thus, only the prevalence of infection was estimated, which indicates the proportion of infected hosts among all hosts examined. Biomass was estimated using cell volumes assigning simple geometric shapes to the species (Hillebrand *et al.* 1999) and converted into carbon content (μg C L^{-1}) according to Menden-Deuer and Lessard (2000).

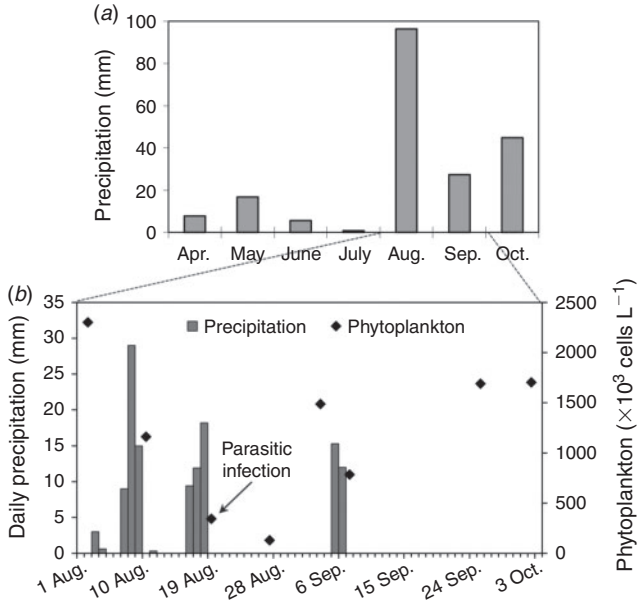

Fig. 1. (*a*) Monthly variability in precipitation (mm) in 2012 in the inner zone of the Bahía Blanca Estuary from April to September. (*b*) Daily precipitation and phytoplankton abundance during the period 1 August–2 October. The date when parasite infection was detected (19 August) is indicated.

Statistical analysis

Ordination with non-metric multidimensional scaling (MDS) was used to assess phytoplankton composition during bloom succession. Only species with abundances >10% of total phytoplankton abundance on at least one sampling date were considered in the MDS analysis. Raw data were homoscedastic and thus analysed without transformation to avoid downplaying the species dominance (Clarke and Warwick 1994). A matrix of similarities between each pair of samples was calculated using the Bray–Curtis similarity index. Organisms with a higher contribution to differences among groups were identified by means of similarity percentage (SIMPER) analysis. Statistical analyses were performed using PRIMER software, ver. 6 (PRIMER-E, Plymouth, UK) (Clarke and Gorley 2006).

Results and Discussion

Environmental variables, phytoplankton and parasitism

Intense monthly precipitation, 96.4 mm, was registered in August 2012 (Fig. 1*a*). This unusual period contrasted markedly with the low rainfall in previous months of the year (Fig. 1*a*). Moreover, the monthly precipitation in August 2012 was fourfold higher than the monthly mean of the previous decade (2000–11), namely 23.2 ± 24.3 mm (±s.d.) (Guinder *et al.* 2010), and was mostly concentrated between 3 and 18 August 2012 (Fig. 1*b*), when salinity dropped from 36.5 to 30.0.

During the rainy period in August, total phytoplankton abundance dropped markedly from 2304×10^3 to a minimum of 129×10^3 cells L^{-1} (Fig. 1*b*). On 19 August, after 3 days of intense rain (Fig. 1*b*), a massive parasite infection was noticed on the dominant diatoms *Thalassiosira pacifica* Gran and Angst

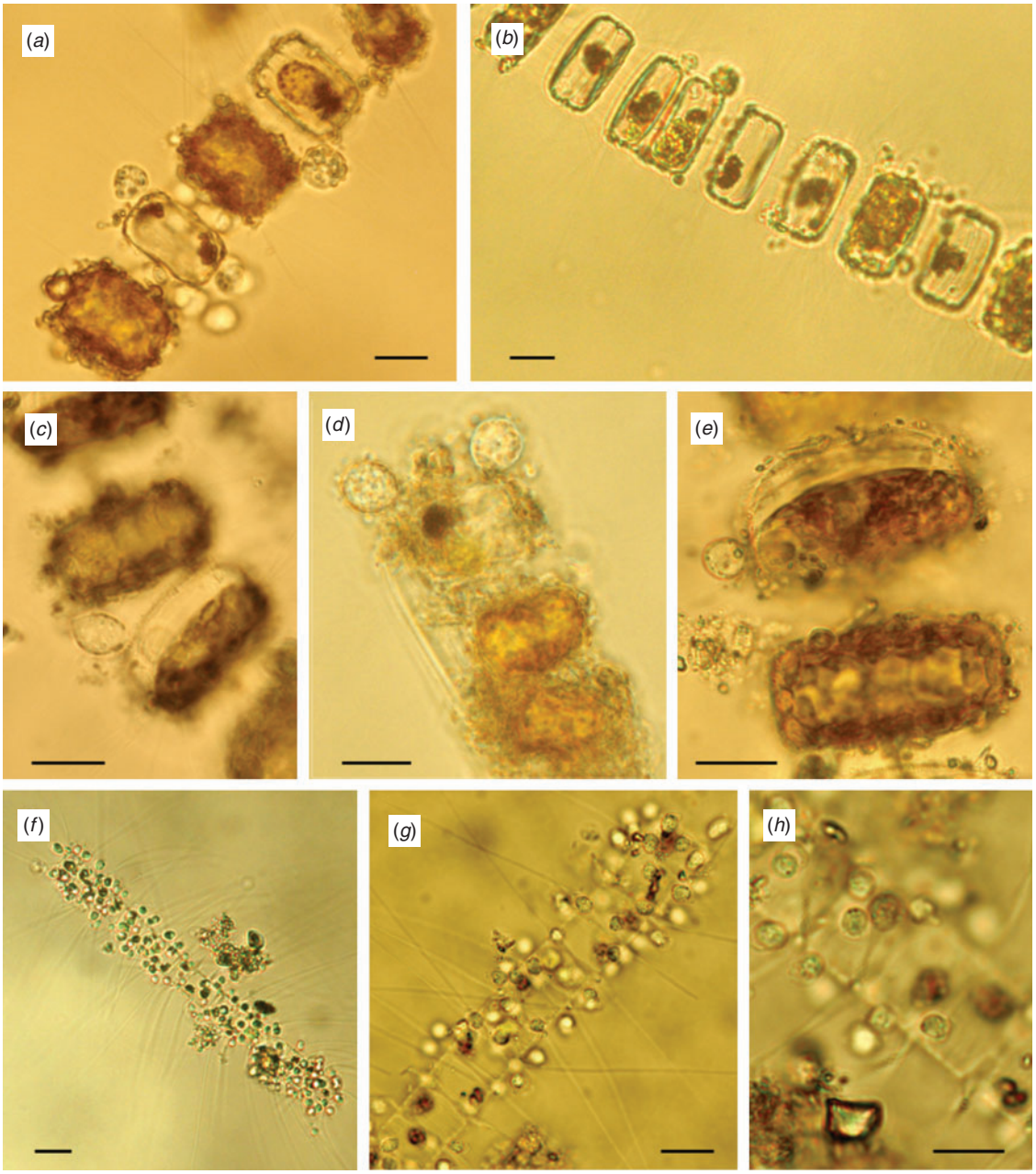

Fig. 2. Parasites attached to the diatoms *Thalassiosira pacifica* (*a–d*) and *Chaetoceros diadema* (*f–h*). (*e*) The cytoplasm of a cell of *T. pacifica* can be seen coming out of the diatom frustule, likely in response to a drop in water salinity. (*a*, *b*) The parasite on *T. pacifica* is presumably the nanoflagellate *Pirsonia* sp., which penetrates the host cell with a pseudopod and then phagocytoses and digests portions of the diatom protoplast. (*c*) At the beginning of the infection, the protoplast of the host is contracted away from the attached parasite. (*f–h*) Parasites on *C. diadema* were not identified and, in infected cells, only dark brown remnants of the protoplast remained. Scale bars: 10 μm.

and *Chaetoceros diadema* (Ehrenberg) Gran (Fig. 2), with >40% of cells infected in each population. *T. pacifica* was infected by a nanoflagellate, presumably *Pirsonia* sp. (Fig. 2*a–d*), whereas the cells of *C. diadema* were completely covered by a smaller unidentified parasite (Fig. 2*f–h*). However, a similar parasite has been observed in the North Sea in the German Bight (S. Kühn, unpubl. data). Populations of both diatom populations (i.e. *C. diadema* and *T. pacifica*) were reduced by 80% after the infection (Fig. 3*a*). Injured cells showed either constrained protoplasm (Fig. 2*c*) or trophosome remains (Fig. 2*a*, *b*), and completely empty cells in chains were often observed (Fig. 2*f–h*), likely caused by parasitic attack. In the following 10 days, the parasitic infection declined, and phytoplankton abundance recovered towards October (Figs 1*b*, 3*a*).

Changes in the water chemistry can disable plankton defences against predators and pathogens (Riessen *et al.*

2012). In particular, phytoplankton species have different tolerance ranges to salinity and physiological mechanisms for counterbalancing changes on the osmotic gradient between the cytoplasm and environment (Flöder *et al.* 2010). However, abrupt changes in salinity may overcome the physiological plasticity of phytoplankton cells and affect their physiological condition (e.g. transmembrane diffusion of ions and nutrients, silicification of diatom frustules and cell motility; Balzano *et al.* 2011). For example, the drop in salinity of 6.5 units registered in the hypersaline inner zone of the estuary (Guinder *et al.* 2012) likely affected the osmotic regulation of *T. pacifica* cells. Indeed, *T. pacifica* cells were observed with cytoplasm coming out of the frustules (Fig. 2*e*), a common consequence of abrupt changes in osmotic gradient (Kirst 1996). Therefore, it is plausible that such environmental stress may have increased the vulnerability of phytoplankton species to parasite infection.

Along with these changes, surface water temperature increased gradually from August to October (from 10.1 to 14.8°C), whereas pH and DO fluctuated between 8.1 and 9.4 and from 7.8 to 9.6 mg L^{-1} respectively. It is worth noting that our observations differ from previous studies showing that diatom susceptibility to infection can be reduced by water turbulence (Kühn and Hofmann 1999). Empirical evidence shows that turbulence may destroy the chemical gradient around diatom cells, which is composed of exudates of organic substances, and thus the microparasites are unable to find their hosts through chemosensory detection (Kühn and Hofmann 1999). However, in the present study, the parasitic infection occurred in the highly turbid and well-mixed inner zone of the Bahía Blanca Estuary (Guinder *et al.* 2012), even during an extreme rainy event. In addition, the high pH (9.4) measured in the estuary did not prevent parasitism on blooming diatoms as observed experimentally with *Pirsonia diadema* Kühn, 1996 on *Coscinodiscus* spp., where pH conditions over 8.7 increased diatom survival by repressing parasitic infections (Kühn and Köhler-Rink 2008).

Although the dataset does not allow identification of the drivers of the host-specific parasitism in blooming diatoms, it does provide useful information on transient parasitic infections in the phytoplankton community while stressing the importance of high-frequency field plankton surveys to track the potential consequences of mounting extreme weather events on epidemic outbreaks in marine systems.

Changes in phytoplankton bloom succession and community structure

Diatoms were the dominant phytoplankton group over the entire study period, whereas phytoflagellates were highly abundant from 10 to 27 August with maximum abundance on 19 August (156×10^3 cells L^{-1}), when levels reached up to 52% of total phytoplankton abundance (Fig. 3*a*). The MDS plot (Fig. 3*b*) and SIMPER analysis (Table 1) revealed segregation of the dates into three distinct groups according to the species composition, namely before, during and after infection, with six species contributing most to the differences between these periods. Initiation of the winter bloom was dominated by relatively large diatoms of the genera *Thalassiosira* (diameter 21–48 µm) and *Chaetoceros* (diameter 10–36 µm), primarily *Chaetoceros debilis* Cleve, *C. diadema*, *Thalassiosira pacifica* and *T. rotula*

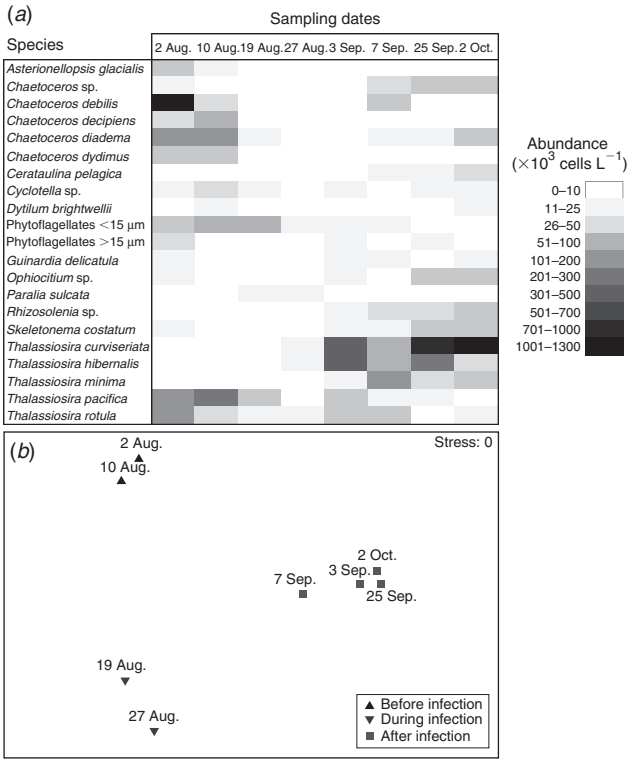

Fig. 3. (*a*) Phytoplankton succession of the most abundant species ($>10 \times 10^3$ cells L^{-1} on at least one sampling date) during the late winter–early spring bloom in the Bahía Blanca Estuary from 2 August to 2 October 2012. Note the marked shift in species dominance after the intense precipitation and the detection of parasites on 19 August. (*b*) Non-metric multidimensional scaling plot for phytoplankton samples (species abundance) during the winter–early spring bloom. Data separated into three groups (i.e. before, during and after infection) based on the specific parasitic event on phytoplankton cells.

Table 1. Phytoplankton species contributing most to differences among groups before (BI), during (DI) and after infection (AI) as identified by similarity percentage (SIMPER) analysis

Entries in bold are diatom species responsible for the shift in the structure of the phytoplankton community from BI to AI

Species	Mean abundance ($\times 10^3$ cells L^{-1})		
	BI	DI	AI
Chaetoceros sp.	0.0	0.0	52.3
Chaetoceros decipiens	75.5	0.0	0.00
Chaetoceros diadema	**239.0**	**9.0**	**24.5**
Chaetoceros didymus	77.0	0.0	0.0
Chaetoceros debilis	**631.0**	**4.5**	**12.8**
Flagellates <15 µm	92.0	84.5	8.5
Ophiocytium sp.	0.0	2.0	42.3
Rhizosolenia sp.	0.0	0.0	34.3
Skeletonema costatum	0.0	0.0	49.8
Thalassiosira curviseriata	**0.0**	**10.5**	**688.5**
Thalassiosira hibernalis	**0.0**	**7.0**	**251.3**
Thalassiosira minima	**0.0**	**0.0**	**90.0**
Thalassiosira pacifica	**305.5**	**30.5**	**24.5**
Thalassiosira rotula	119.5	22.0	39.5

Meunier (altogether accounting for ~80% of total phytoplankton abundance; Fig. 3*a*). These species were replaced by smaller diatoms of the genera *Thalassiosira* (diameter 3–22 µm; *Thalassiosira curviseriata* Takano, *T. hibernalis* Gayoso and *T. minima* Gaarder) and *Chaetoceros* sp. (diameter 3–8 µm), with levels reaching up to 75% of total phytoplankton abundance after rainy events and parasite infection (Fig. 3*a*).

Total phytoplankton biomass (carbon content) exhibited the same pattern as phytoplankton abundance; specifically, it dropped markedly from 326 to 23 µg C L^{-1} during the rainy period in August, and then increased to 330 µg C L^{-1} towards October. The biomass recovered after the parasitic infection due to an increase in the density of codominant large-sized diatoms such as *Rhizozolenia* sp. (diameter 10–25 µm), *Cerataulina pelagica* (Cleve) Hendey (diameter 17–30 µm) and *Guinardia delicatula* (Cleve) Hasle (diameter 8–25 µm) and the xanthophyte *Ophiocitium* sp. (diameter 6.5–15.5 µm; Fig. 3*a*). Despite their relatively low abundances, these represented a significant proportion of phytoplankton carbon.

The results of the present study further showed that compound effects of intense precipitation, salinity changes and parasitic protists affected phytoplankton species succession and impaired biomass accumulation during the growing season. The highly host-specific infection on *T. pacifica* and *C. diadema* resulted in a severe reduction of their populations and allowed the development of small, fast-growing opportunistic species able to exploit open niches (i.e. *T. curviseriata, T. hibernalis* and *T. minima*). Changes in species composition comprised a restructuring of the community by cell sizes and shapes. Conversion of the biomass of large and abundant host diatoms into biomass of small parasites can shift the flow of matter away from the classic 'small-to-large' trophic direction (Salomon *et al.* 2009), because food quality encompasses not only chemical stoichiometry, but also species morphology. In agreement with previous findings (Dunne *et al.* 2013; Gsell *et al.* 2013), the results of the present study suggest that mass host mortalities as a result of specific parasitism altered the phytoplankton bloom succession and community size structure, eventually enhancing species diversity and trophic chain complexity (Salomon *et al.* 2009).

Parasitism and emergence of a key phytoplankton species

It is worth noting that *T. curviseriata* was the dominant diatom of the winter bloom in the Bahía Blanca Estuary during the period 1978–2002 (Guinder *et al.* 2010), but the population decreased substantially and has been replaced by other species of *Thalassiosira*, such as *T. pacifica*, *T. eccentrica*, *T. rotula* and *Chaetoceros* spp. over the previous warmer and drier decade (Guinder *et al.* 2010, 2017; Spetter *et al.* 2013). The sudden extreme rains in winter 2012 and the emergence of massive parasitism on blooming diatoms likely released species competition and allowed the development of *T. curviseriata*. Concerning the ephemeral nature of parasitic episodes, the consequences on phytoplankton community structure are difficult to track because cell abundance is rapidly re-established after the infection and changes in the community structure cannot be easily discriminated from natural succession patterns. Nevertheless, in the present study, it is likely that the growth of *T. curviseriata* appeared as a compensatory response to the severe reduction of the community biomass due to environmental stress (Flöder *et al.* 2010) rather than from temporal succession of phytoplankton species.

Conclusions

Species-specific parasitism appears to be a significant factor related to bloom collapse, further affecting the structure of the phytoplankton community in the Bahía Blanca Estuary, in addition to the effects of nutrients and grazers. Hence, future work will aim to integrate parasitism in estuarine food web models as a potential prominent vector of phytoplankton biomass loss and species turnover. Further field research and thorough examinations of live plankton are required to evaluate the effects of environmental stress (i.e. changes in salinity) on the vulnerability of phytoplankton cells to parasitism.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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