

SHORT COMMUNICATION

Dominance of the planktonic diatom *Thalassiosira minima* in recent summers in the Bahía Blanca Estuary, Argentina

VALERIA ANA GUINDER^{1*}, JUAN CARLOS MOLINERO², CECILIA ANGELINES POPOVICH³,
JORGE EDUARDO MARCOVECCHIO¹ AND ULRICH SOMMER²

¹ÁREA DE OCEANOGRAFÍA QUÍMICA, INSTITUTO ARGENTINO DE OCEANOGRAFÍA (IADO-CONICET), CC 804 B8000FWB, BAHÍA BLANCA, ARGENTINA,

²FB3, MARINE ÖKOLOGIE, LEIBNIZ-INSTITUT FÜR MEERESWISSENSCHAFTEN, IFM-GEOMAR, DÜSTERNBROOKER WEG 20, KIEL 24105, GERMANY AND

³LABORATORIO DE FICOLOGÍA Y MICOLOGÍA, UNIVERSIDAD NACIONAL DEL SUR (UNS), SAN JUAN 670, BAHÍA BLANCA 8000, ARGENTINA

*CORRESPONDING AUTHOR: vguinder@criba.edu.ar

Received April 2, 2012; resubmitted on August 7, 2012; accepted in principle August 11, 2012

Corresponding editor: Zoe Finkel

The diatom *Thalassiosira minima* was first recorded in the Bahía Blanca Estuary in 1992. In 1992–1993 it exhibited a broad seasonal occurrence. A recent survey (2006–2007) showed a seasonal appearance restricted mainly to summer together with a greater relative abundance within the phytoplankton. A close connection was found with warmer, more saline and highly turbid conditions experienced in recent summers in the estuary. Whether these changes will impact the estuary trophic dynamics remains an open question.

KEYWORDS: *Thalassiosira minima*; summer blooms; temperature; salinity; sediment resuspension; mesozooplankton; temperate estuary

In coastal shallow systems, phytoplankton structure is shaped by modifications in the benthic–pelagic environment and abundance changes of specific taxa may be indicative of ecosystem-state changes (Paerl *et al.*, 2007). Phytoplankton seasonal events (e.g. phenology) and community structure (e.g. species niches, size-structure and diversity) are regulated by modifications in water temperature, salinity, light availability, nutrients and pelagic trophodynamics (Litchman and Klausmeier, 2008). In particular, the cell size follows biophysical rules (e.g. nutrient uptake, motion, sinking rates, kinetics of metabolism), affecting growth rates and consequently

the biogeochemical cycling of elements and trophic interactions (Finkel *et al.*, 2010).

We report here that in the Bahía Blanca Estuary, Argentina, recent summer phytoplankton blooms (2006–2008) have been dominated by the small diatom *Thalassiosira minima* Gaarder 1951. This cosmopolitan species is common in temperate brackish to marine planktonic waters (Hoppenrath *et al.*, 2007) and shows tolerance ranges for high water temperature and salinity in coastal and estuarine systems (Muylaert and Sabbe, 1996; McQuoid, 2005). For instance, in the Tagus Estuary, Portugal, this species was reported only in summer

(salinity: 16–35 PSU and temperature: 20–25°C) (Gameiro *et al.*, 2004) and on the coast of Hong Kong within salinity ranges between 31 and 34 PSU and temperatures of 25–26.5°C (Gao *et al.*, 2003). We investigated changes in *T. minima* abundance in the Bahía Blanca Estuary in relation to changing physico-chemical conditions and trophic interactions. We discuss possible ecological traits and trade-offs that might allow the emerging dominance of this small species in recent summers and the implications for the carbon pathway in the food web.

The Bahía Blanca Estuary (38°42′–39°25′S, 61°50′–62°22′W) is located on the southwestern Atlantic coast of Argentina, in a temperate climate region. The estuary is mesotidal with a semidiurnal cycle, shallow, turbid and eutrophic. The inner zone of the estuary has undergone severe anthropogenic perturbations enhancing both eutrophication and turbidity as a result of growing industrial activities, maritime traffic, dredging and pollution since the late 1980s (Perillo *et al.*, 2001; Hoffmeyer, 2004). Sampling was performed in the inner zone of the estuary (mean depth: 6 m) at Puerto Cuatros station (38°50′S; 62°20′W). Physico-chemical parameters were sampled on a continuous fortnightly basis from a pier at high tide and around noon time

since 1978. Here, we examined the years in which species-specific phytoplankton cell counts were made over the annual cycle: 1978–1982, 1992–1993 and 2006–February 2008. Abundance data from the years 1988–1991 were also analyzed (Gayoso, 1998, 1999), but raw data were not available for further analyses. Nevertheless, the species composition during this 3-year period (Gayoso, 1999) was considered in the present work.

Surface water temperature (in °C), salinity (in PSU) and turbidity (in nephelometric turbidity units, n.t.u.) were measured *in situ* using a digital multi-sensor Horiba U-10. For dissolved inorganic nutrients, water samples collected with a Van Dorn Bottle (2.5 L) and were filtered through Whatman GF/C filters and frozen in plastic bottles until analysis. Dissolved nitrate (NO₃⁻), phosphate (PO₄³⁻) and silicate (SiO₄⁴⁻) concentrations were determined by standardized methods (Technicon, 1973; Treguer and Le Corre, 1975; Eberlein and Kattner, 1987) using a Technicon AA-II Autoanalyzer. The surface samples for phytoplankton quantitative analysis were also taken using a Van Dorn Bottle (2.5 L) and preserved in acidified Lugol's solution. The phytoplankton abundance (in cells L⁻¹) was determined using a Sedgwick-Rafter chamber (1 mL). Cells >3 μm were counted. The entire chamber was examined at ×200, and each cell was counted as a unit. For species identification, samples were collected using a Nansen 30-μm net and preserved with formaldehyde (final concentration 0.4%). Phytoplankton was identified at the species level using a Zeiss Standard R microscope and a Nikon Eclipse microscope, with a magnification of ×1000 and phase contrast. To avoid misidentification of *T. minima*, we used scanning electron microscopy (SEM) following Hasle and Fryxell (Hasle and Fryxell, 1970).

The phytoplankton abundance in summer during the years 1978–1982, 1992–1993 and 2006–2008 varied between 0.003 × 10⁶ and 5 × 10⁶ cells L⁻¹, and notably increased in recent summers, from a minimal summer mean of 12 × 10³ cells L⁻¹ in 1980 to a maximal summer mean of 2239 × 10³ cells L⁻¹ in 2008. Between 1978 and 1993, the main phytoplankton groups in summer were phytoflagellates (unidentified Cryptophyceae, 10–20 μm) dinoflagellates (unidentified Gymnodinians, *Scripsiella trochoidea*, *Protoperidinium punctatum* and *Prorocentrum* sp.) and recurrent diatoms (diameter range: 5–90 μm) such as *Cerataulina pelagica*, *Leptocylindrus minimus*, *Thalassiosira hendeyi*, *Cyclotella striata*, *Paralia sulcata* and *Coscinodiscus* spp. (Gayoso, 1989, 1998, 1999; Popovich, 1997; Popovich and Marcovecchio, 2008). *Thalassiosira minima* was first documented in 1992 (Popovich, 1997). During 1992–1993, *T. minima* was present practically all year around with an abundance

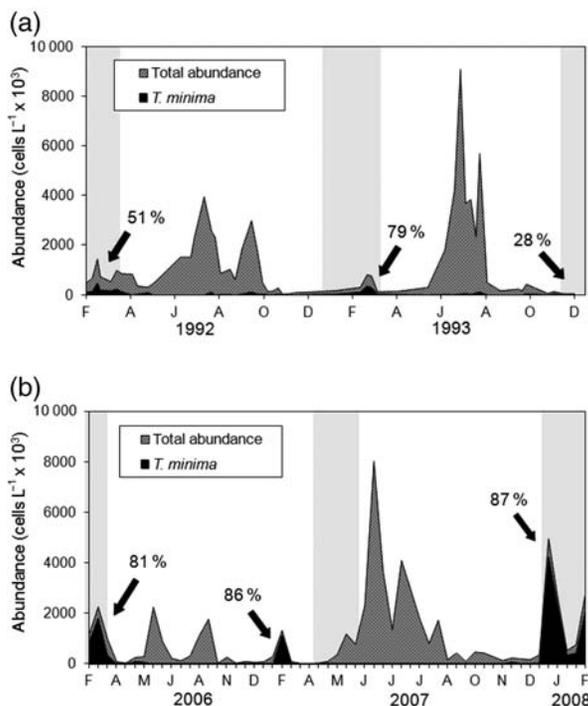


Fig. 1. Seasonal variability in the abundance of total phytoplankton and *Thalassiosira minima* during the years (a) 1992–1993 and (b) 2006–February 2008 at Puerto Cuatros station. The arrows indicate the maximal percentage reached by *T. minima* of the total phytoplankton abundance during summer periods (grey columns).

under 0.5×10^6 cells L^{-1} (Fig. 1a). Later, during 2006–February 2008, the presence of *T. minima* was mainly restricted to summer where the species reached high concentrations, with maximum values over 1.2×10^6 cells L^{-1} (Fig. 1b). The observed increase in phytoplankton summer blooms was driven mostly by the *T. minima* population, which represented more than the 81% of the total phytoplankton abundance (Fig. 1b). For instance, in January 2008, the population density was maximal, up to 4.3×10^6 cells L^{-1} , corresponding to 87% of the summer phytoplankton.

The cells of the centric diatom *T. minima* were rectangular shaped in the girdle view with diameters of 5–15 μm , and arranged in linear chains of 3–15 cells (Fig. 2). At low population abundance, at the beginning of the blooms, the cells of *T. minima* showed sediment grains attached to their walls (Fig. 2c and d). However,

at higher abundances, only clean vegetative cells were observed (Fig. 2b).

Changes in physico-chemical conditions were observed in the estuary during the years studied. Turbidity in summer increased over the period 1978–2008, with a minimum of 30 ± 27 n.t.u. in 1981 and a maximum of 107 ± 36 n.t.u. in 2007; dominated by resuspended sediments (Guinder *et al.*, 2009; Perillo *et al.*, 2001). The mean turbidity during the years 1992–1993 and 2006–Feb 2008 (78.2 ± 19.5 n.t.u.) was significantly higher (the non-parametric Mann–Whitney test, $P < < 0.01$) than the mean of the years 1978–1982 (46.2 ± 10.5 n.t.u.). In addition, there was a significant increase between the years 1992–1993 and 2006–2007 (the non-parametric Mann–Whitney test) in salinity (30.6 ± 2.4 and 33.3 ± 2.5 PSU; $P < < 0.01$) and phosphate (1.6 ± 0.8 and 3.2 ± 1.4 μM ; $P < < 0.01$).

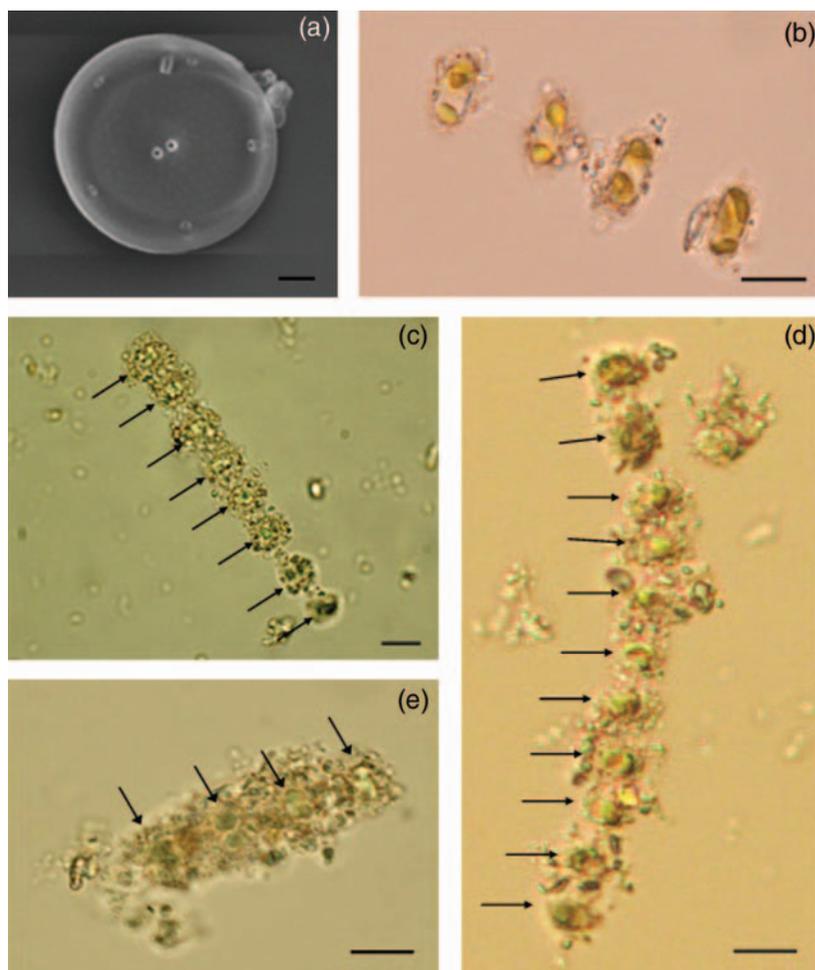


Fig. 2. Pictures of *Thalassiosira minima* using (a) scanning electron microscopy and (b–e) light microscopy. (a) The internal view of the valve showing two central processes and the labiate process close to one marginal strutted process. (b–e) Chains of *T. minima* showing different levels of sediment cover. The arrows indicate the location of the cells in the chains which have fine sediment grains attached to the walls. Scale bars: (a) 1 μm ; (b–e) 10 μm .

To identify possible covariations with environmental factors (temperature, turbidity, salinity, silicate, phosphate and nitrate), *T. minima* abundance was examined using multiple regression (stepwise procedure, variables entered the analysis if $P < 0.1$). This analysis showed both temperature ($P < 0.00$) and turbidity ($P < 0.06$) as the most explanatory factors for the abundance of *T. minima* over the period 1992–2007. These factors were followed by silicate ($P < 0.02$), which was also linked to the abundance changes although not during the whole period. Potential changes in the habitat preferences of *T. minima* were assessed by a non-parametric test of association between environmental factors (i.e. temperature, salinity and turbidity) and *T. minima* abundance. Habitat association curves were obtained from the maximum absolute difference between the cumulative frequency distribution (CFD) of an environmental factor and the abundance-weighted CFD of that environmental factor. After determining the maximum

absolute difference between the two CFD curves, we compared it with the distribution of the maximum absolute differences from 5000 randomizations of the Monte Carlo resampling for *T. minima* and the environmental variable. Details of the method are shown in Molinero *et al.* (Molinero *et al.*, 2009). Habitat associations showed specific environmental windows in the distribution of the *T. minima* population when we contrasted the periods 1992–1993 and 2006–2007 (Fig. 3). In the later years, the mode of the abundance frequency distribution was associated with higher water temperature (22.2°C), salinity (35.4) and turbidity (102.5 n.t.u.) compared with the former period temperature (18.3°C), salinity (31.2) and turbidity (45 n.t.u.), although the species occurred within a large range of these variables (Fig. 3).

In temperate coastal environments, the phytoplankton community in summer is characteristically dominated by nano- and pico-planktonic taxa. Diatoms are adapted to live in turbid and well-mixed environments and small-sized cells with a high surface area to volume ratios, such as *T. minima*, have additional competitive skills to harvest light and nutrients (Finkel *et al.*, 2010). The observed dominance of *T. minima* in the late summer in the Bahía Blanca Estuary might be explained by high cell division rates as well as suitable growth conditions in the water column. The first record of *T. minima* in the early 1990s coincided with the initiation of major dredging operations in the inner zone of the Bahía Blanca Estuary to deepen the navigation channel and harbour (Perillo *et al.*, 2001). During the year 2000, a new dredging procedure was adopted in the semi-enclosed sites to allow larger vessels to enter the estuary. This procedure was based on water injection into the bed sediments, followed by the resuspension and dispersion of the suspended sediments by tidal currents (Perillo *et al.*, 2001). The occurrence of *T. minima* in this shallow estuarine system and the sediments attached to the cell walls during the start phase of blooms suggest that this species might have benthic stages. In fact, *T. minima* produces resting spores able to remain as dormant stages in the bottom sediments for >2 years (McQuoid, 2005; Hoppenrath *et al.*, 2007) and able to germinate after resuspension in the water column. The combined environmental stimuli that promote spore germination are high temperature, salinity and light availability (Eilertsen *et al.*, 1995; McQuoid, 2005). The notable increase in the abundance of *T. minima* in recent summers (mean water temperature: $22.1 \pm 1.7^\circ\text{C}$ and salinity: 35.5 ± 2.3) could be in part associated with the release of benthic resting stages into the pelagic habitat. Indeed, the high turbidity and phosphate concentration observed in recent

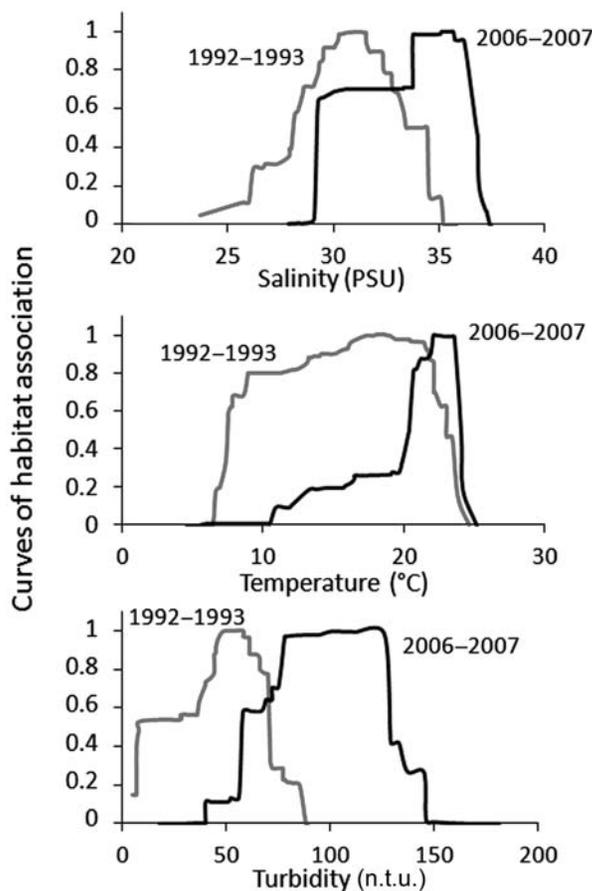


Fig. 3. The distribution of *Thalassiosira minima* abundance (standardized values between 0 and 1) within the ranges of water temperature, salinity and turbidity during the periods 1992–1993 and 2006–2007.

years might support the hypothesis of increasing resuspension of material from the bottom. Dissolved phosphate is consumed during phytoplankton growth, and in shallow, eutrophic estuaries sediments play an important role in the regeneration and the internal load of this nutrient (Coelho *et al.*, 2004). In addition to dredging activities, the complex patterns of winds and tides characteristic of the inner part of the estuary could be important causal factors of resuspension processes and release of phosphate from bottom sediments (Guinder *et al.*, 2009). It is worth noting that despite the high water turbidity in the inner zone of the estuary, the mean light (PAR) intensity in the mixed layer I_m (total depth of the water column), reached maximal values between 150 and 252 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in summer (Guinder *et al.*, 2009). These light levels are over the threshold to promote the germination of resting stages in the bottom layer in shallow systems and trigger phytoplankton summer blooms (e.g. Shikata *et al.*, 2008). Further ecological strategies related to the dominance of *T. minima* are not excluded. For instance, the presence of agglutinating substances and attached sediments around the cells of *T. minima* could protect the cells against photodamage and/or photoinhibition under the high solar radiation during summer (e.g. Waring *et al.*, 2007) and/or provide a substratum for bacteria development that could facilitate the absorption of nutrients (e.g. Grossart *et al.*, 2006).

Finally, the observed changes in the mesozooplankton community in the Bahía Blanca Estuary over the last 20 years likely favour the increase in *T. minima* in recent summers. In the late 1980s to early 1990s, the invasive copepod *Eurytemora americana* was introduced into the estuary probably via ballast waters (Hoffmeyer, 2004). After its arrival, the abundance of *E. americana* has increased during winter–spring and has constrained the temporal distribution of the native copepod *Acartia tonsa* to summer periods (Hoffmeyer, 2004; Hoffmeyer *et al.*, 2009). Empirical studies on grazing activity of *A. tonsa* isolated from the Bahía Blanca Estuary in summer, have shown that this omnivorous species feeds preferentially on microzooplankton (mostly tintinnids, 88%) and detritus, both widely available in this turbid environment. *Acartia tonsa* feeds on phytoplankton cells $>10 \mu\text{m}$ (Diodato and Hoffmeyer, 2008). It is therefore plausible that released predation pressure on *T. minima* and a relaxation of competition with bigger phytoplankton species are two of the factors that promoted its outburst in recent summer. Additionally, we further hypothesize that the dominance of *T. minima* might cause a shift in the pelagic food web away from the biological pump dominated by copepods and rapid sedimentation of particulate matter towards rapid carbon

cycling in the microbial loop dominated by smaller zooplankton (Finkel *et al.*, 2010). Indeed, in the Bahía Blanca Estuary, aloricate ciliate and tintinnid communities showed maximal abundance and diversity during late spring–summer period (Barria de Cao *et al.*, 2005; Pettigrosso and Popovich, 2009). The path of carbon flow between primary producers and mesozooplankton may become longer through heterotrophic flagellates and ciliates, which can reduce productivity of higher predators.

Both phytoplankton and zooplankton composition have changed over the last decades in the Bahía Blanca Estuary driven by human activities (e.g. dredging, species introduction) and modifications in water conditions (e.g. water temperature, turbidity). A shift in the size–structure of the phytoplankton community was previously described in the estuary during the winter bloom, with the dominance of smaller diatoms. These changes were linked to long-term climatic trends towards warmer and drier years in the Bahía Blanca region (Guinder *et al.*, 2010). The close relationship found between *T. minima* and temperature is consistent with modifications the plankton has experienced in several ecosystems worldwide, where a growing dominance of smaller species appears as a common ecological response to warming waters (Daufresne *et al.*, 2009). Whether the environmental conditions of recent summers and the potential dominance of *T. minima* in summer blooms will impact the food web structure through cascading effects associated with cell size changes remains an open question that should be addressed in future studies.

ACKNOWLEDGEMENTS

V.A.G. acknowledges the assistance of Dr A.K.S.K. Prasad in the taxonomic work on *T. minima*.

FUNDING

This work was funded by a CONICET fellowship and a DAAD fellowship to V.A.G. (A/08/73101) and the DAAD-MINCYT bilateral project “The effect of the global warming on marine plankton” (ID 50467312).

REFERENCES

- Barria de Cao, M. S., Beigt, D. and Piccolo, C. (2005) Temporal variability of diversity and biomass of tintinnids (Ciliophora) in a southwestern Adriatic temperate estuary. *J. Plankton Res.*, **27**, 1103–1111.

- Coelho, J. P., Flindt, M. R., Jensen, H. S. *et al.* (2004) Phosphorus speciation and availability in intertidal sediments of a temperate estuary: relation to eutrophication and annual P fluxes. *Estuar. Coast. Shelf Sci.*, **61**, 583–590.
- Daufresne, M., Lengfellner, K. and Sommer, U. (2009) Global warming benefits the small in aquatic ecosystems. *PNAS*, **106**, 12788–12793.
- Diodato, S. L. and Hoffmeyer, M. S. (2008) Contribution of planktonic and detritic fractions to the natural diet of mesozooplankton in Bahía Blanca Estuary. *Hydrobiol.*, **614**, 83–90.
- Eberlein, K. and Kattner, G. (1987) Automatic method for the determination of ortho-phosphate and total dissolved phosphorus in the marine environment. *Fresenius Zeitschrift für Analytische Chemie*, **326**, 354–357.
- Eilertsen, H. C., Sandberg, S. and Tellefsen, H. (1995) Photoperiodic control of diatom spore growth: a theory to explain the onset of phytoplankton blooms. *Mar. Ecol. Prog. Ser.*, **116**, 303–307.
- Finkel, Z. V., Beardall, J., Flynn, K. J. *et al.* (2010) Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res.*, **32**, 119–137.
- Gameiro, C., Cartaxana, P., Cabrita, M. T. *et al.* (2004) Variability in chlorophyll and phytoplankton composition in an estuarine system. *Hydrobiol.*, **525**, 113–124.
- Gao, Y. H., Chen, C. P. and Li, Y. (2003) Marine nanoplanktonic diatoms from the coastal waters of Hong Kong. In Morton, B. (ed.), *Perspectives on Marine Environmental Change in Hong Kong, 1977–2001*. Hong-Kong University Press, Hong-Kong, pp. 93–107.
- Gayoso, A. M. (1989) Species of the diatom genus *Thalassiosira* from the coastal zone of the South Atlantic (Argentina). *Bot. Mar.*, **32**, 331–337.
- Gayoso, A. M. (1998) Long-term phytoplankton studies in the Bahía Blanca Estuary, Argentina. *ICES J. Mar. Sci.*, **55**, 655–660.
- Gayoso, A. M. (1999) Seasonal succession patterns of phytoplankton in the Bahía Blanca Estuary (Argentina). *Bot. Mar.*, **42**, 367–375.
- Grossart, H. P., Kiorboe, T., Tang, K.W. *et al.* (2006) Interactions between marine snow and heterotrophic bacteria: aggregate formation and microbial dynamics. *Mar. Ecol. Prog. Ser.*, **42**, 19–26.
- Guinder, V. A., Popovich, C. A., Molinero, J. C. *et al.* (2010) Long-term changes in the composition, occurrence, timing and magnitude of phytoplankton blooms in the Bahía Blanca Estuary, Argentina. *Mar. Biol.*, **157**, 2703–2716.
- Guinder, V. A., Popovich, C. A. and Perillo, G. M. E. (2009) Particulate suspended matter concentrations in the Bahía Blanca Estuary, Argentina: Implication for the development of phytoplankton blooms. *Estuar. Coast. Shelf Sci.*, **85**, 157–165.
- Hasle, G. R. and Fryxell, G. R. (1970) Diatoms: cleaning and mounting for light and electron microscopy. *T. Am. Micros. Soc.*, **89**, 469–474.
- Hoffmeyer, M. S. (2004) Decadal change in zooplankton seasonal succession in the Bahía Blanca Estuary, Argentina, following introduction of two zooplankton species. *J. Plankton Res.*, **26**, 181–189.
- Hoffmeyer, M. S., Berasategui, A. A., Beigt, D. *et al.* (2009) Environmental regulation of the estuarine copepods *Acartia tonsa* and *Eurytemora americana* during coexistence period. *J. Mar. Biol. Assoc. UK*, **89**, 355–361.
- Hoppenrath, M., Beszteri, B., Drebes, G. *et al.* (2007) *Thalassiosira* species (Bacillariophyceae, Thalassiosirales) in the North Sea at Helgoland (German Bight) and Sylt (North Frisian Wadden Sea)—a first approach to assessing diversity. *Eur. J. Phycol.*, **42**, 271–288.
- Litchman, E. and Klausmeier, C. A. (2008) Trait-based community ecology of phytoplankton. *Annu. Rev. Ecol. Evol. Syst.*, **39**, 615–639.
- McQuoid, M. (2005) Influence of salinity on seasonal germination of resting stages and composition of microplankton on the Swedish west coast. *Mar. Ecol. Prog. Ser.*, **289**, 151–163.
- Molinero, J. C., Vukanic, V., Lučić, D. *et al.* (2009) Mediterranean marine copepods. Basin scale trends of the calanoid *Centropages typicus*. *Hydrobiol.*, **617**, 41–53.
- Muylaert, K. and Sabbe, K. (1996) The diatom genus *Thalassiosira* (Bacillariophyta) in the Estuaries of the Schelde (Belgium/The Netherlands) and the Elbe (Germany). *Bot. Mar.*, **39**, 103–116.
- Paerl, H. W., Valdes-Weaver, L. M., Joyner, A. R. *et al.* (2007) Phytoplankton indicators of ecological change in the eutrophying Pamlico Sound system, North Carolina. *Ecol. Appl.*, **17**, S88–S101.
- Perillo, G. M. E., Pierini, J. O., Pérez, D. E. *et al.* (2001) Suspended sediment circulation in semienclosed docks, Puerto Galván, Argentina. *Terra et Aqua*, **83**, 1–8.
- Pettigrosso, R. E. and Popovich, C. A. (2009) Phytoplankton-aloricate ciliate community in the Bahía Blanca Estuary (Argentina): seasonal patterns and trophic groups. *Braz. J. Oceanogr.*, **57**, 215–227.
- Popovich, C. A. (1997) Autoecología de *Thalassiosira curviseriata* Takano (Bacillariophyceae) y su importancia en el entendimiento de la floración anual de diatomeas en el estuario de Bahía Blanca (Pcia. Bs. As., Argentina). PhD Dissertation, Universidad Nacional del Sur.
- Popovich, C. A. and Marcovecchio, J. E. (2008) Spatial and temporal variability of phytoplankton and environmental factors in a temperate estuary of South America (Atlantic coast, Argentina). *Cont. Shelf Res.*, **28**, 236–244.
- Shikata, T., Nagasoe, S., Matsubara, T. *et al.* (2008) Factors influencing the initiation of blooms of the raphidophyte *Heterosigma akashiwo* and the diatom *Skeletonema costatum* in a port in Japan. *Limnol. Oceanogr.*, **53**, 2503–2518.
- Technicon Autoanalyzer II. (1973) Silicates in water and seawater. Industrial Method No. 186-72W/B. Technical Industrial Systems. Tarrytown, New York, 10591.
- Treguer, P. and Le Corre, P. (1975) *Analyse des sels nutritifs sur Autoanalyzer II: nitrates + nitrites. Manuel D'Analyse des Sels Nutritifs dans l'Eau de Mer*. Université du Bretagne Occidentale, pp. 11–22.
- Waring, J., Baker, N. R. and Underwood, G. J. C. (2007) Responses of estuarine intertidal microphytobenthic algal assemblages to enhanced ultraviolet B radiation. *Global Change Biol.*, **13**, 1398–1413.