Estuaries and Coasts

Phenological changes of blooming diatoms promoted by compound bottom-up and top-down controls
--Manuscript Draft--

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Abstract:	Understanding phytoplankton species-specific responses to multiple biotic and abiotic stressors is fundamental to assess phenological and structural shifts at the community level. Here we present the case of Thalassiosira curviseriata, a winter blooming diatom in the Bahía Blanca Estuary, Argentina, which displayed a noticeable decrease in the past decade along with conspicuous changes in phenology. We compiled interannual field data to assess compound effects of environmental variations and grazing by the invasive copepod Eurytemora americana. The two species displayed opposite trends over the period examined. The diatom decreased towards the last years, mainly during the winters, and remained relatively constant over the other seasons, while the copepod increased towards the last years, with an occurrence restricted to winter and early spring. A quantitative assessment by structural equation modeling unveiled that the observed long term trend of T. curviseriata resulted from synergistic effects of environmental changes driven by water temperature, salinity and grazing. These results suggest that the shift in the abundance distribution of T. curviseriata towards higher annual ranges of temperature and salinity -as displayed by habitat association curves- constitutes a functional response to avoid seasonal overlapping with its predator in late winters. The observed changes in timing and abundance of the blooming species resulted in conspicuous shifts in primary production pulses. Our results provide insights on mechanistic processes shaping the phenology and structure of phytoplankton blooms.

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23 June 2016

Deana Erdner
Associate Editor
Estuaries and Coasts

Dear Deana Erdner,

Please find enclosed the amended version of our manuscript "Phenological changes of blooming diatoms promoted by compound bottom-up and top-down controls". We would like to thank for the constructive comments to improve the manuscript and for considering our work for its publication in Estuaries and Coasts. We have addressed and corrected all the issues raised by you and the reviewers. The answers to the reviewers are below this letter. We believe the manuscript has gained additional clarity on the presentation of results and discussion. We have taken into consideration all suggestions to prepare the new version of the manuscript.

We hope that you find our revised manuscript and response to the reviewers' comments satisfactory. Thank you again for this opportunity and we would be more than happy to discuss any further concerns.

Yours faithfully,

Valeria Ana Guinder
on behalf of coauthors

Associate Editor: This submission has been reviewed by two experts in the field. Both reviewers agreed that the manuscript was acceptable for publication, after some revision.

Both reviewers provided detailed comments, which should help the authors to improve the manuscript, and raised larger issues as well. Specifically, one of the reviewers requests much more detail for the statistical analyses, as they form the basis for all of the results and conclusions in the study. The other reviewer requested better explanation and justification of the choice of time periods. This is important because the time periods for the two main species of question, Thalassiosira and Eurytemora, do not overlap, and this choice should be well justified.

Authors: we have modified the original manuscript according to the reviewer's comments. On the one hand, new figures (1b and Fig. 5) have been incorporated as proposed by reviewer 1, figure 2 has been modified and we have provided further explanation of the choice of the time periods. On the other hand, the statistical analyses have been explained more in detail and we have justified why they were chosen for this work, according to the suggestions of reviewer 2. Overall, all the general and specific comments of the reviewers have been taken under consideration to prepare the revised version of the manuscript.

Reviewer #1: The objective of this study was to examine the factors influencing the timing and magnitude of blooms of the winter blooming diatom, Thalassiosira curviseriata. Data collected over an 18-year period (although not continuous) was examined and structural equation modeling was employed to explain observed trends in abundance. Results identified the synergistic effects of changes in water temperature, salinity and grazing to explain the shifts in bloom timing and magnitude.
The results of this study show how environmental modifications and an introduced predator in the Bahía Blanca Estuary produced dramatic shifts in the plankton community structure.

I would recommend publication after some revision:
In the introduction, the authors should explain why the 3 periods were picked? Why differentiate 2002-2005 (which is a bit misleading since there are only 2 yrs of data for this period for the diatom and copepod—and these years do NOT coincide. And only 2 years of data for 2006-2008—and again these data do not overlap. So, this is a very limited time series and because data do not overlap, it would seem more realistic to compare the 1990s vs. 2002-2008?

Authors: We acknowledge that the time series has limitations; however the years where the species counting was performed provide valuable information of different environmental periods. The available data of the diatom and the copepod were grouped in 3 periods because previous studies in the Bahía Blanca Estuary (e.g. Guinder et al. 2010 and 2013; Winder and Cloern 2010; López Abbate et al. 20015) have detected different environmental conditions in these three periods governed by changes of salinity, temperature and precipitation. We believe that such contrasting conditions provide a template to assess phytoplankton responses to varying scenarios.

According to the reviewer suggestion, we have specified in the Introduction (Lines 111-118) that continuous data of chlorophyll *a* and environmental variables (e.g. nutrients, temperature, salinity) were used covering the period 1990-2008, whereas discrete, irregular data correspond to planktonic species abundance.

In Materials and Methods and in figure 3a, the available years with species abundance are indicated (lines 141-147), i.e. *Thalassiosiosira curviseriata*: June 1991-February 1995, May-August 2002, April - November 2003 and February 2006-February 2008; *Eurytemora americana*: 1990, 2002, 2005, 2007 and 2008.

Specific suggestions (although more thorough editing for English usage is needed):
Line 36: From these results, we hypothesized that….

Authors: we have changed this expression according to the reviewer's remark.

Line 110: "multi-annual" is more common usage than pluriannual.

Authors: this word has been changed.

Line 112-3: revise an incomplete and ? sentence: We aimed to track population variability in relation to environmental changes.

Authors: we have modified this sentence by adding the information mentioned before, according to the reviewer suggestions.

Line 114: quantified (here and elsewhere, verbs should be past tense?)

Authors: we have changed the verbs into past tense.

Line 155: replace "quantification of methods" with "detection"

Authors: done.

Line 157:
Authors need to explain why 2 types of sample preservation were used. I assume lugol's for counting and formalin-preserved for finer taxon id (confirming).

Authors: we have clarified this in the revised manuscript according to the reviewer's suggestion.

Line 180; "species" is correct; remove "specie"

Authors: done.

Line 283: autochtonous?

Authors: The sentence was modified. We have replaced autochthonous by native. The sentence in the new version is: "the native copepod Acartia tonsa…"

Line 300 replace "," with period. Begin new sentence, delete "which".

Figures:
My version had very low resolution figures; hopefully final version is high res.
Fig. 1. add 3rd set of figures plotting deviation from long-term mean (anomaly from the average for the entire 20 year time period)— for the total cells and diatom abundance— it is very difficult to see a difference in the log plots.

Authors: we have uploaded the figures with high resolution in the revised version of the manuscript. In addition, we have added a new figure suggested by the reviewer (Fig. 1b) with the anomaly of the abundance of *T. curviseriata* and of total diatoms from the average of the period 1990-2008.

Fig. 2. An additional bar-graph for the mean of each period (see general comment above) with statistical significance test between the 2 periods might appear more convincing?

Authors: we have changed this figure in the new version of the manuscript. GAM analysis was deleted. Here we show the data of winter (June-August) temperature, salinity and chlorophyll together with their temporal trend depicted from filtered values with low-pass Loess filter to eliminate short-scale noise.

Reviewer #2: --
ESCO review by Guinder et al. Phenological changes of blooming diatoms promoted by compound bottom-up and top-down controls

This is a study of phytoplankton in estuarine waters; particularly focusing on the potential shifts of the dominant blooming taxa, and the exploration of the involved abiotic forcing and biotic interactions. One of the real major challenges in environmental studies is to evaluate the relative importance of local and global forcing in ecological trajectories such as the relative importance of regulatory factors 'nutrients' and 'temperature' in the dynamics of taxa. From this point of view the approach adopted by the authors in the exploration of this data set is attractive and I would fully agree with them that it provides 'insights' and ideas into 'tracking distributional shifts in the plankton'. Although, the data set of phytoplankton counting has important gaps and the one of zooplankton is based on only five-years sampling, I believe that the authors succeeded in putting together this information into a balanced and synthetic scenario. I greatly appreciated the 'natural history' part , the effort to exploit the possibility of increased grazing by an invasive species and most of all the effort to put together all this information into a conceptual model-figure (Fig. 5). In this -otherwise very well written- paper, a lot more explanations on the methods used for statistical analysis should be given, the basic steps of each analysis, what was expected of each of them (i.e. justify why each analysis was chosen), and all the related references. I hope that the authors can easily provide the clarifications needed, and listed below, in a way that a reader can follow and eventually 'repeat' what was done here. I believe that the paper deserves to be published provided that the following clarifications are given:

Specific comments

- The Structural Equation Modeling -which I suppose is what we see on Fig. 5- (check the legend) is mentioned in the introduction (L114) and again at the end of the results (L248). The choice of the model, the principle of its calculation, and the related references should be explicitly given in the M+M section.

Authors: we thank the reviewer's remark and have accordingly changed the text in the revised version of the manuscript.

We used structural equation modeling (SEM), which is a generalized multiequation framework whereby complex hypotheses, particularly those involving networks of path relations, are evaluated against multivariate data (Bollen 1989, Grace 2006). This approach has gained attention in ecological research as it is suited to test for the nature and magnitude of direct and indirect effects of multiple interacting factors.

- Moreover, what does the BIC show exactly here? What does the chi2 calculated here indicate? Is it for the whole model? Why chi2? is the calculation based on % or proportions? If yes why, and which ones exactly? Are the abiotic parameters normalized? What is the criterion for the positive or negative significant relationships? Correlation? Other? What exactly is, and how does this fit in, a chi2 model? Please explain, clarify, and complete the legends.

Authors: data were analyzed by comparing the model with the observed covariance matrix, using maximum likelihood and Chi-square as goodness-of-fit measures. Chi-square is the original fit index for

structural models. We also used BIC as it favors parsimonious models with few parameters. Significance levels for individual paths between variables were set at α = 0.05. At each significant path the standardized coefficients are represented and interpreted as follows: if, for example, temperature goes up by 1 SD, the abundance of *Thalassiosira curviseriata* goes down by 0.44 SD.

- Same goes for the GAM model. Page 8 should be rewritten and expanded. I am willing to believe the authors, but they have to convince me that what I see on figure 2 are not just random points, most of which are outside the shaded interval (Is this the confidence interval?). (Fig2 and L219-224)

Authors: The GAM analysis has been removed from the revised version of the manuscript and this figure has been replaced by interanual variability of chlorophyll, temperature and salinity in winter (June-August) and their long-term trend depicted by the filtered data with low-pass filter.

-I am very intrigued also with figure 3d. Is the regression based on only five points for zooplankton meaningful? Given the huge seasonal variability (Fig3c), I think 'means' are meaningless, and integrated values rather than means should be used here. Please also note that this fig. is incorrectly cited as 3c, while figure 3c is not cited in the paper.

Authors: we have deleted the figure of the regressions from the revised version of the manuscript.

The indication of the figures have been corrected.

We have added a new figure (Fig. 5 in this new version) where the abundance of *T.curviseriata* has been plotted against temperature for the periods 1991- 1994 (Fig. 5a) and 2002-2008 (Fig. 5b). In addition, the annual means of the abundance of *T. curviseriata* and *E. americana* with their standard deviations (Fig. 5c) were plotted together to see their interanual variability.

- Same for the 'path model', the 'HA', and 'CFD'. Why were they chosen among others, what do they exactly show, and what is the basic principle of their calculation, etc, etc ...; as above

Authors: we have rewritten the corresponding part in the Data analyses section. In the new version the paragraph states:

Habitat association curves were assessed by means of a nonparametric test using the maximum absolute difference between the cumulative frequency distribution (CFD) of the environmental factor (temperature, salinity) and the abundance-weighted CFD of that variable. Briefly it was performed as follows: the relative cumulative frequency distribution (CFD) was calculated for each of the environmental variables (temperature and salinity). Subsequently we weighted the CFD for each environmental variable by the abundance of each species (*T. curviseriata* and *E. americana*). The maximum absolute difference between the two CFD curves (unweighted and weighted) indicates the association of the population with that environmental variable. If the density of the population is randomly distributed in relation to the environmental variable, the cumulative pattern will increase similarly in the two curves and they will not be significantly different. By contrast, if the population is associated with a particular range in the environmental variable, the slope of the weighted CFD should be steeper than that of the unweighted environmental variable. The degree of association between the species abundance and environmental factors was tested by a Monte Carlo randomization procedure

(1000 randomnizations). A similar approach has been used to depict the habitat association of fish (Perry and Smith 1994), copepods (Molinero et al. 2009) and jellyfish (Marques et al. 2015).

-Important - I think that the nutrient data should also be presented in the paper along with chl (Fig.), since they directly influence phytoplankton (see significative relations of figure 5).

Authors: We agree with the reviewer that nutrients shape phytoplankton dynamics. In the particular case of the Bahía Blanca Estuary, dissolved nutrient concentrations have been presented in several studies (Freije et al. 2008; Popovich and Marcovecchio 2008), and already discussed in relation with phytoplankton variability (e.g. Gayoso 1998; 1999; Popovich et al. 2008; Guinder et al. 2010; 2012; 2013; López Abbate et al. 2015; Spetter et al. 2015). Over the last three decades, nutrients have been in high concentrations in the inner zone of the estuary and thus this area has been characterized as hypereutrophic with no limiting levels for phytoplankton development.

Phenological changes of blooming diatoms promoted by compound bottom-up and top-down controls

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Author Contributions: VAG and JCM originally formulated the idea, and VAG, JCM and CLA analyzed the data. VAG, AAB, CAP, CVS, JEM and RHF conducted field- and laboratory work. VAG and JCM wrote the manuscript. All the authors fully discuss the results and provided editorial advice.

24   **Abstract**

25   Understanding phytoplankton species-specific responses to multiple biotic and abiotic

26 stressors is fundamental to assess phenological and structural shifts at the community level. Here

27 we present the case of *Thalassiosira curviseriata*, a winter blooming diatom in the Bahía Blanca

28 Estuary, Argentina, which displayed a noticeable decrease in the past decade along with

29 conspicuous changes in phenology. We compiled interannual field data to assess compound

30 effects of environmental variations and grazing by the invasive copepod *Eurytemora americana*.

31 The two species displayed opposite trends over the period examined. The diatom decreased

32 towards the last years, mainly during the winters, and remained relatively constant over the other

33 seasons, while the copepod increased towards the last years, with an occurrence restricted to

34 winter and early spring. A quantitative assessment by structural equation modeling unveiled that

35 the observed long term trend of *T. curviseriata* resulted from synergistic effects of environmental

36 changes driven by water temperature, salinity and grazing. These results suggest that the shift in

37 the abundance distribution of *T. curviseriata* towards higher annual ranges of temperature and

38 salinity –as displayed by habitat association curves- constitutes a functional response to avoid

39 seasonal overlapping with its predator in late winters. The observed changes in timing and

40 abundance of the blooming species resulted in conspicuous shifts in primary production pulses.

41 Our results provide insights on mechanistic processes shaping the phenology and structure of

42 phytoplankton blooms.

43

44   **Key words:** plankton, grazing selectivity, phenology, structural equation modeling,

45 estuaries

46

47

Introduction

Research efforts concerning phytoplankton responses to environmental forcing have mainly focused on bulk biomass or chlorophyll changes (e.g. Winder and Cloern 2010; Demarcq et al. 2012; Racault et al. 2012). However, shifts at the community level are emergent properties of the multiple processes taking place at the population level (Litchman et al. 2012; Walters et al. 2013). Hence, overlooking proximal effects on species life history and inter-specific interactions (Schlüter et al. 2012) yields a limited understanding of mechanisms driving phenological patterns at the base of the pelagic food web. In temperate coastal systems, seasonal succession in phytoplankton species is a natural phenomenon driven by seasonal changes in resource availability -light and nutrients-, physical properties of the water column and grazing pressure (Sommer et al. 2012). Whilst this process arises from physiological responses to environmental changes, phytoplankton bloom events are triggered by species' life history and constitute recurrent annual events in ecosystems with marked seasonality, commonly composed by the same cluster of species (Smayda 1998). Such interannual recurrence confers these events reliability as indicators of the ecosystem state. For instance, structural changes in species composition may point out alterations in habitat conditions (Bosak et al. 2012). Thus, tracking species' range boundaries in relation to water chemistry and ecological interactions, e.g. competition and grazing (Litchman et al. 2012; Ratti et al. 2013), help understanding shifts in the seasonal timing of phytoplankton (Walters et al. 2013).

In the Bahía Blanca Estuary, Argentina, the phytoplankton variability has been surveyed over the last three decades. Surface chlorophyll a has been measured on a continuous fortnightly basis, while phytoplankton cells enumeration and species identification have been performed during some years of the mentioned period, also in a fortnightly frequency. A recurrent winter-early spring diatom bloom portrays the main biomass event in the annual cycle. Species of the

genera *Thalassiosira* followed by *Chaetoceros* have characterized the bloom succession, with *T.*
curviseriata as the dominant species from 1981 to 2002 (Gayoso 1999; Popovich and Gayoso
1999; Popovich and Marcovecchio 2008). In the last years, changes in the phytoplankton
phenology and structure have been ascribed to modifications in regional hydro-climate
conditions, i.e. warmer and dryer conditions (Guinder et al. 2010, 2013). Phytoplankton changes
embraced a decline in bloom magnitude concurrently with an earlier peak of the winter bloom *ca.*
one month. Further changes included modifications in the community composition and size
structure, with a decline in the population of the diatom *T. curviseriata*. Although climate
variations have promoted shifts in the governing environmental conditions, further factors behind
the observed changes in phytoplankton structure and seasonality are not discarded. In particular,
the grazing pressure promoted by the invasive copepod *Eurytemora americana*, the abundance of
which markedly increased since its introduction in the Bahía Blanca Estuary (Hoffmeyer 2004).
However, the lack of information on the potential effect of grazing precludes quantifying the real
effect of predation (Guinder et al. 2010).

Natural History

Thalassisira curviseriata Takano 1981 is a centric marine diatom (6-21 μm in diameter) arranged
in curved chains of several cells, commonly found embedded in mucilage. It has been firstly
registered in the Bay of Tokyo, Japan (Takano 1981), and afterwards in coastal waters of
Australia (Hallegraeff 1984), in the Skagerrak, North Atlantic (Lange et al. 1992), in the North
Sea (Hoppenrath et al. 2009) and in the eutrophic Bahía Blanca Estuary, Argentina (Gayoso
1981; 1989). Empirical research with *T. curviseriata* isolated from the Bahía Blanca Estuary
showed that this species is eurythermal (5-20 °C) and euryhaline (20-40), and well adapted to
growth at relatively low light intensity (growth light saturated between 70-80 μmol m^{-2}s^{-1},

96   inhibited at ~150 µmol $m^{-2}s^{-1}$) (Popovich and Gayoso 1999). *T. curviseriata* has been a dominant

97   diatom during the blooming season, i.e. winter-early spring (e.g. Gayoso 1998, 1999; Popovich

98   and Gayoso 1999; Popovich et al. 2008) and therefore appears as a suitable species to track

99   changes in the phytoplankton dynamics.

100   *Eurytemora americana* Williams 1906 is an estuarine copepod native to the Northern

101   Hemisphere (Kos 1977) and generally observed in relatively low abundance in comparison to

102   other estuarine copepods such as *E. affinis*, *E. hirundoides*, *Acartia tonsa* and *A. clausi*. This

103   species is adapted to temperatures between <1 and 15 °C and salinity between 10 and 33 (Avent

104   1998). In the Southern Hemisphere, *E. americana* has been reported as an invader species in the

105   Bahía Blanca Estuary (Hoffmeyer 2004). Since its introduction around the late 1980s early

106   1990s, it has increased progressively (Hoffmeyer 2004; Berasategui et al. 2009; Fernández

107   Severini et al. 2011) and developed a recurrent abundance peak in winter-early spring, beginning

108   in June and lasting until October. During the rest of the year, the species experiences a

109   developmental arrest (resting eggs), while remains in bottom sediments (Hoffmeyer 2004;

110   Berasategui et al. 2009).

111   Here we assessed multi-annual patterns of these two prominent species in the plankton of

112   the Bahía Blanca Estuary; the diatom *Thalassiosira curviseriata* and the copepod *Eurytemora*

113   *americana* both dominant during the winter early-spring phytoplankton bloom. We aim tracking

114   their population variability in relation with environmental changes over the period 1990-2008,

115   analyzing monthly data of chlorophyll *a* and dissolved nutrient concentrations, water temperature

116   and salinity, and records of the species abundances gathered during the years: 1991- 1995, 2002,

117   2003 and 2006-2008 for *Thalassiosiosira curviseriata*, and 1990, 2002, 2005, 2007 and 2008 for

118   *Eurytemora americana*. We tackle this question by means of structural equation modeling

119   (SEM), a generalized multiequation framework whereby ecological hypotheses, particularly those

120   involving networks of path relations, are evaluated against multivariate data (Grace 2006). In

121   recent years, this approach has gained attention in ecological research as SEM test for the nature

122   and magnitude of direct and indirect effects of multiple interacting factors shaping ecological

123   phenomena. In this study, we quantified both direct and indirect effects of biological and

124   physico-chemical factors to understand the temporal pattern of *T. curviseriata* seasonal timing.

125

126   **Materials and Methods**

127   *Biological data*

128   The sampling of physicochemical and biological variables was carried out in the inner zone of the

129   Bahía Blanca Estuary, at Puerto Cuatreros station (38°50´ S; 62°20´ W), during midday and high

130   tide. While sampling started in 1978, the data series was not complete in terms of physico-

131   chemical and biological parameters. Thus, here we cover the years 1990 to 2008, period during

132   which chlorophyll *a*, dissolved nutrients and *in situ* water temperature and salinity have been

133   recorded on a fortnightly basis. Likewise, surface (approx. 0.5 m depth) water temperature and

134   salinity were measured on a fortnightly basis using a digital multisensor Horiba U-10. Surface

135   water samples for chlorophyll *a*, dissolved nutrients and phytoplankton quantitative analyses

136   were collected with a van Dorn bottle (2.5 l). Phytoplankton samples were preserved with acid

137   Lugol's solution to stain the cells for counting, and the chlorophyll *a* and nutrient samples were

138   stored in a cooler and transported to the laboratory. For taxonomic identification, water samples

139   were collected using a Nansen net (30 μm mesh) and preserved with formalin (final concentration

140   4 % v/v). Phytoplankton cell counting was performed biweekly only during the periods: June

141   1991- February 1995, May - August 2002, April - November 2003 and February 2006 - February

142   2008. Mesozooplankton was sampled monthly by means of subsurface horizontal tows (0.5 m

143   depth) using a 200-μm mesh size plankton net. Samples were preserved in 4% buffered formalin.

144 *E. americana* was not collected during the whole period, but during the years 1990, 2002, 2005,

145 2007 and 2008. Records of the species abundance have shown a noticeable abundance increase

146 since its introduction in the estuary (Fernández Severini et al. 2011). Abundance was calculated

147 taking into account the corresponding sample volume and expressed in number per cubic meter

148 (Boltovskoy 1981).

149 *Meteorological data*

150 Regional records of monthly precipitation over the period 1990-2008 were obtained from the

151 Argentine National Meteorological Service (SMN). In addition, monthly air temperature during

152 the mentioned period was taken from the dataset of the National Center for Environmental

153 Prediction–National Center for Atmospheric Research (NCEP–NCAR) gridded reanalysis

154 (Kalnay et al. 1996).

155

156 *Laboratory determinations*

157 Chlorophyll *a* (in μg l^{-1}) was measured spectrophotemetrically according to Lorenzen and Jeffrey

158 (1980). Water samples (250 ml) were filtered through Whatman GF/C filters, and immediately

159 stored at -20 °C. Pigment extraction was done in 90% acetone (24 h, 4 °C).

160   For dissolved nutrient determinations, water samples were filtered through Whatman

161 GF/F filters and frozen in plastic bottles until analysis. Dissolved nitrate (NO_3^-), nitrite (NO_2^-),

162 phosphate (PO_4^{3-}) and silicates concentrations were determined by Treguer and Le Corre (1975),

163 Grasshoff et al. (1983), Eberlein and Kattner (1987) and Technicon (1973), respectively, using

164 an automatized Technicon AutoAnalyzer II. The limit of detection is 0.10 μM for nitrate, 0.02

165 μM for nitrite, 0.01 μM for phosphate and 1.00 μM for silicates.

166   Due to the amount of suspended solids in the inner zone of the Bahía Blanca Estuary,

167 phytoplankton >3 μm was counted with a Sedgwick–Rafter chamber (1 ml) from the samples

168 fixed with Lugol. The entire chamber was examined at x200 and each algal cell was counted as a

169 unit (McAlice 1971). Phytoplankton species identification was done from the formalin fixed

170 samples, using a Zeiss Standard R microscope and a Nikon Eclipse microscope with x1000

171 magnification and phase contrast. The quantification of *E. americana* was done in counting

172 chambers using a Wild M5 stereoscopic microscope.

173

174 *Data analyses*

175 Chlorophyll *a* concentration and the abundance of total diatoms and *Thalassiosira curviseriata*

176 were examined to evaluate the interannual variability of phytoplankton seasonal signals over the

177 period 1990-2008. In addition, interannual trends of winter (June-August) temperature, salinity

178 and chlorophyll over the period 1990-2009 were depicted by filtration of the data with low-pass

179 Loess filter to eliminate short-scale noise. Interannual and seasonal changes in *T. curviseriata* and

180 *E. americana* abundances were assessed by monthly means of the log-transformed data and

181 thereafter displayed as heat maps, where a grey-color scale was assigned to the abundance range,

182 from low (light grey) to high (dark grey) abundance. Further, the annual cycles of the species

183 were calculated for the periods: 1990-1994, 2002-2005 and 2006-2008 defined by the first and

184 the last year with data either of *T. curviseriata* and/or *E. americana*. The years 2002-2005 were

185 analyzed separately from 2006-2008 in accordance with the environmental shift registered in the

186 estuary at the beginning of the 2000s (Guinder et al. 2010, 2013; Winder and Cloern 2010) and

187 with the changes in chlorophyll shown in the present work.

188 Potential changes in the habitat preferences of *T. curviseriata* and *E. americana* were

189 assessed using habitat association curves (HA), which were computed by means of a

190 nonparametric test using the maximum absolute difference between the cumulative frequency

191 distribution (CFD) of the environmental factor (temperature, salinity) and the abundance-

weighted CFD of that variable. Briefly it was performed as follows: the relative cumulative

frequency distribution (CFD) was calculated for each of the environmental variables (temperature

and salinity). Subsequently we weighted the CFD for each environmental variable by the

abundance of each species (*T. curviseriata* and *E. americana*). The maximum absolute difference

between the two CFD curves (unweighted and weighted) indicates the association of the

population with that environmental variable. If the density of the population is randomly

distributed in relation to the environmental variable, the cumulative pattern will increase similarly

in the two curves and they will not be significantly different. By contrast, if the population is

associated with a particular range in the environmental variable, the slope of the weighted CFD

should be steeper than that of the unweighted environmental variable. The degree of association

between the species abundance and environmental factors was tested by a Monte Carlo

randomization procedure (1000 randomnizations). A similar approach has been used to depict the

habitat association of fish (Perry and Smith 1994), copepods (Molinero et al. 2009) and jellyfish

(Marques et al. 2015). HA were estimated for those years when the phytoplankton and the

mesozooplankton were counted all year-round, *i.e.* 1992-1994 and 2006-2007 for *T. curviseriata,*

and 1990, and 2005, 2007 and 2008 for *E. americana*. In addition, the abundance of *T.*

curviseriata sampled fortnightly over the periods 1991-1994 and 2002-2008 was plotted against

water temperature to assess the diatom distribution in relation with seasonal temperature during

the two contrasting periods.

Finally, to gain further understanding of the multiple environmental stressors that shape

the population of *Thalassiosira curviseriata* in the Bahía Blanca Estuary, we integrated biotic and

abiotic variables in a path model, and quantified the strength of links in the planktonic network,

which encompassed the following data: abundance of *T. curviseriata* and *Eurytemora americana*,

dissolved nutrient concentrations, air temperature, precipitation, water temperature and salinity.

216   Specific effects of the analyzed factors and their co-variations on *T. curviseriata* were assessed

217   using variance partitioning and explored through SEM (Peres- Neto et al. 2006). The strength of

218   the links and the quantification of the overall model were determined by simple and partial

219   multivariate regression and Monte Carlo permutation tests (1000 replicates), while the Bayesian

220   Information Criterion (BIC) and Chi-square values were used as goodness-of-fit measures

221   (Alsterberg et al. 2013). Significance levels for individual paths between variables were set at $\alpha =$

222   0.05. Path coefficients indicate the strength of the relation between causal and response variables.

223   Path analysis was performed using the R library sem.

224

225   **Results**

226   *Chlorophyll a seasonality and diatom bloom*

227   Surface chlorophyll variability over the 1990s showed a major recurrent annual event

228   characterized by high winter abundance, as depicted by the long-term changes of the winter

229   bloom (Fig. 1a), and smaller peaks in summer. This pattern changed after 2000, when the winter

230   signal decreased yielding to similar magnitudes in both winter and summer phytoplankton bloom

231   events. The decline of the winter bloom dropped the mean annual levels of chlorophyll *a* (Fig.

232   1a), particularly in the last years (*ca.* 2005-2008). Such change in the magnitude of the winter

233   diatom bloom in recent years is shown in Figure 1b, where the deviation from the mean of the

234   period 1990-2008 shifted to negative values (i.e. values lower than the global chlorophyll

235   average) in winter for total diatoms, particularly for *Thalassiosira curviseriata*. In terms of

236   phytoplankton abundance (cells l^{-1}), during the period June 1991 to February 1995 the total

237   diatom densities reached maximal values over the winter-early spring bloom (June-September,

238   Fig. 1c). In these years, *Thalassiosira curviseriata* was the dominant species during the blooming

239   season (more than 85 % of the total diatom abundance), reaching up to 12.7 x 10^6 cells l^{-1} (97 %)

240 in winter 1991 (Fig. 1c). Records of the winter 2002 showed a similar pattern with *T. curviseriata*

241 being the dominant diatom during the bloom (up to 90 %), although the maximal population

242 density was lesser than previous years, 1.9 x10^6 cells l^{-1}. In the following years, 2003, 2006,

243 2007, the observed *T. curviseriata* abundance dropped to 0.8 x 10^6 and 0.5 x 10^6 cells l^{-1}, in

244 October 2003 and August 2007, respectively, while the species was no longer the dominant

245 winter blooming diatom.

246   Interannual variability of mean water temperature (Fig. 2a) and salinity (Fig. 2b), and

247 chlorophyll *a* (Fig. 2c) during winter, showed opposite long-term trends. Temperature displayed

248 large variations while the overall trend showed a slight increase (Fig. 2a). Salinity variations

249 showed no clear trend prior 2000, however a conspicuous increase was observed after 2000 (Fig.

250 2b). In turn, chlorophyll *a* showed an overall decline superposed to peaks of maximum

251 concentration in the early 1990s and 2000 (Fig. 2c).

252

253 *Temporal patterns of Thalassiosira curviseriata and Eurytemora americana*

254 The heat maps of *T. curviseriata* and *E. americana* (Fig. 3a) displayed interannual and seasonal

255 patterns. On the interannual scale, *T. curviseriata* decreased in the last years while the abundance

256 of *E. americana* increased, whereas on the seasonal scale, the annual cycles (Fig. 3b) of *E.*

257 *americana* showed an occurrence restricted to winter and early spring in the three analyzed

258 periods (1990-1994, 2002-2005 and 2006-2008), while the diatom displayed a decrease in the last

259 winters and a relatively constant abundance over the other seasons.

260

261 *Habitat associations and pathways linking T. curviseriata with external forces*

262 Habitat association curves showed changes in the species-specific environmental windows of *T.*

263 *curviseriata* and *E. americana* populations when contrasting the periods 1992-1994 and 2006-

264 2007, and 1990-1998 and 2005-2008, respectively (Fig. 4). While during the early periods the

265 main occurrence of *T. curviseriata* was associated with temperature and salinity of 11.2 ºC and

266 30.6, respectively, in the later years, the occurrence of the species was associated with higher

267 water temperature and salinity, 15.1 ºC and 34.5, respectively, although the species occurred

268 within a large range of these variables (Fig. 4a, c). By contrast, *E. americana* displayed a

269 narrowed distribution over the annual gradient of water temperature and salinity. The observed

270 mode of the frequency distribution shifted from 15.1 ºC in the former period to 11.4 ºC in the

271 latter. Similarly, the association with salinity showed a displacement towards lower salinities in

272 recent years, with a frequency distribution moving from 35.0 to 30.9 (Fig. 4b, d).

273   The assessment of the relationship between *Thalassiosira curviseriata* and temperature

274 displayed a conspicuous shift from a strong negative link over the period 1991-1994 to a non-

275 significant relationship during the period 2002-2008 (Fig. 5a and b). It is worth noticing that the

276 latter period is concomitant with the rise in the population of *Eurytemora americana* in the cold

277 season (Fig. 5c).

278   SEM results (Fig. 6) revealed significant links between atmospheric variability and

279 hydrological conditions in the Bahía Blanca Estuary. Results pointed out the expected close

280 relationships between air and water temperature, as well as precipitation and salinity. Water

281 temperature was tightly linked with air temperature (path coefficient = 0.58), whereas the

282 precipitation had a negative relationship with the salinity (path coefficient = -0.42). In turn, both

283 water temperature and salinity showed negative effects on the abundance of *T. curviseriata* (path

284 coefficients: -0.44 and -0.43, respectively). Likewise, negative effects on the diatom were

285 displayed by nitrates, phosphate and silicates (path coefficients: -0.42, -0.47 and -0.42,

286 respectively). In addition to the bottom-up effect promoted by environmental changes, the model

287 output further emphasized the potential top-down control promoted by *E. americana* as shown by

288 the significant negative link of the path coefficient (-0.62).

289

290 **Discussion**

291 *Environmental variables and grazing control on Thalassiosira curviseriata*

292 The species-specific approach developed here showed that the marked reduction in the

293 chlorophyll *a* levels was promoted by a conspicuous biomass drop of the phytoplankton bloom in

294 the last years driven by the abundance decline of the formerly winter dominant *Thalassiosira*

295 *curviseriata*. The modeling approach disclosed that the rising temperature and drier conditions in

296 the Bahía Blanca Estuary over the period 1990-2008 (Guinder et al. 2010) permeated the pelagic

297 environment affecting plankton communities through alterations of the water chemistry and

298 predator-prey interactions. These changes seemed to accumulate and produce a progressive

299 structural change in the bloom dynamics of primary producers, including conspicuous

300 phenological changes.

301   *T. curviseriata* was present all-year round over the studied period 1991-2008, although the

302 warmer and saltier conditions of recent winters have negatively affected its growth, probably

303 allowing other species to compete more efficiently for resources during the blooming season

304 (May-September) (Litchman et al. 2012). In addition, the model suggests a significant role of *E.*

305 *americana* in the modulation of the phytoplankton bloom, likely through exerting higher grazing

306 pressure on this small diatom (Hoffmeyer and Prado Figueroa 1997). Hence, the shift in the

307 abundance distribution of *T. curviseriata* towards higher annual ranges of temperature and

308 salinity emerge likely as a functional response to enhanced grazing pressure by the invasive

309 copepod in late winters.

310   *Eurytemora americana* was introduced in the estuary via ballast waters in the late 80´s

311 (Hoffmeyer 2004). Since its introduction this species has increased and established as the

312 dominant mesozooplankton species during winter (Hoffmeyer 2004; Berasategui et al. 2009),

313 displacing the native copepod *Acartia tonsa* towards summer-autumn periods (Hoffmeyer et al.

314 2009). The partial exclusion of *A. tonsa* in the co-occurring period may be promoted by the

315 higher efficiency of *E. americana* to assimilate the phytoplankton bloom in comparison with *A.*

316 *tonsa* (Hoffmeyer and Prado-Figueroa 1997; Hoffmeyer et al. 2009), as it shows grazing

317 preferences on small diatoms like *T. curviseriata* (Hoffmeyer and Prado Figueroa 1997). Recent

318 research on the ecology of this species in the same estuary has shown positive relationships

319 between the abundance of its developmental stages and chlorophyll *a* and salinity, whereas it

320 appeared negatively related with temperature and photoperiod (Hoffmeyer et al. 2009). For

321 instance, incubation experiments have shown larger egg hatching under low temperature ($\leq$ 6

322 °C), high salinity (32.7-36.6) and high phytoplankton density (chlorophyll *a* $\geq$ 8 µg l^{-1})

323 (Berasategui et al. 2009; 2012; 2013).

324   The above results show a close connection between the enhanced population size of the

325 invasive copepod and the decline of *T. curviseriata*. Other factors, however, should not be

326 discarded; for instance, the potential control microzooplankton might have on phytoplankton

327 abundance. Recent studies documented low grazing impact during winter (López Abbate et al.

328 2015b), although it increases along with the rising seasonal temperatures (Barría de Cao et al.

329 2005; 2011; Pettigrosso and Popovich 2009). Such trophic interaction has been recently

330 quantified (2008-2010) using dilution experiments (López Abbate et al. 2015). These

331 experiments showed that the percentage of primary production grazed by microzooplankton can

332 reach 87% in spring and fall although the relationship also displays a high variability on the

333 seasonal scale. The strongest grazing pressure appears concurrent with the highest concentration

of phytoflagellates, while the lowest consumer control was found in winter and summer, likely associated to high concentration of chain-forming diatoms, such as *Thalassiosira curviseriata* and *T. minima*. Overall, our study supports that changes at the community level of primary producers are the result of aggregated taxon phenological shifts, and highlights the importance of addressing the proximal ecology of the dominant species to gain a thorough understanding of changes in plankton ecology.

Dissolved inorganic nutrients

Former studies on the interannual and seasonal variability of nutrients have revealed that the Bahía Blanca estuary is highly eutrophic with no nutrient limitation for phytoplankton growth (*e.g.* Freije et al. 2008; Popovich and Marcovecchio 2008; Guinder et al. 2010, López Abbate et al. 2015a). It is well known that alterations in nutrient ratios have important implications for the structure of the phytoplankton community through species-specific uptake and that there is no linear response between nutrient levels and species behavior (Litchman et al. 2012). For instance, experimental studies with natural plankton under high anthropogenic pressure in the inner zone of the Bahía Blanca Estuary indicated stoichiometric deviation of nutrients from the Redfield ratio (López Abbate et al. 2015b). Dissolved inorganic nitrogen (*i.e.* ammonium, nitrate and nitrite) and phosphate were in excess with respect to silicate, which could partly explain the shift of the phytoplankton community from dominance of diatoms towards phytoflagellates in highly human-impacted areas (López Abbate et al. 2015a, 2015b, Spetter et al. 2015). In our model, SEM results showed negative effects of the dissolved nutrients –nitrate, phosphate and silicate- on *T. curviseriata*, which might be the result of contrasting long-term trends: the significant decrease on *T. curviseriata,* and the slightly rise in some nutrients over the years with the eventual unbalanced radios. In addition, precipitation and freshwater runoff have been the main

15

358   modulators of the budget of dissolved inorganic nutrients in the inner zone of the estuary, as the

359   highest annual levels of nutrients have been commonly registered during the rainy season, late

360   summer and fall (e.g. Freije et al. 2008, Popovich and Marcovecchio 2008). Accordingly, SEM

361   results pointed out the positive relationship between precipitation and nutrients concentration.

362

363   *Potential cascading effects of the restructuration of phytoplankton community*

364   In the Bahía Blanca Estuary, structural and taxonomical changes in the phytoplankton

365   community have been observed in the last years (Guinder et al. 2010, 2013, López Abbate et al.

366   2015a, 2015b, Spetter et al. 2015). The magnitude of the winter bloom has notably decreased and

367   exhibited co-occurrence of diatoms such as *Thalassiosira pacifica*, *T. eccentrica*, *T. rotula*, *T.*

368   *anguste-lineata, Chaetoceros* spp., with no conspicuous dominance of *T. curviseriata* as in

369   previous decades (Gayoso 1989, 1998, 1999, Popovich and Gayoso 1999, Popovich et al. 2008).

370   These changes are in line with alterations of the life history of a dominant taxon in plankton that

371   may translate into temporal niche differentiation and species turnover (Litchman et al. 2012).

372   Complex interactions among multiple environmental stressors affect the proximal autoecological

373   properties of phytoplankton, such as nutrients uptake, overwintering and vulnerability to

374   predation, causing new interespecific interactions and changes in the community structure (*e.g.*

375   size, stoichiometry), and in the timing of blooms as well (Sommer et al. 2012). Indeed, the size-

376   structure and phenology of the phytoplankton community in the estuary has changed in recent

377   years; with the winter bloom displaying an earlier occurrence and higher abundance of

378   phytoflagellates (Guinder et al. 2010, 2013, Spetter et al. 2015, López Abbate et al. 2015b), while

379   the summer bloom showing an increase in the abundance of the small diatom *Thalassiosira*

380   *minima* (Guinder et al. 2013). As shown here, these changes are related to compound effects of

381   hydroclimate modifications (warmer and dryer conditions), changes in nutrient ratios (López

16

382 Abbate et al. 2015, Spetter et al. 2015) and top-down control exerted by the invader copepod *E.*

383 *americana* (Hoffmeyer 2004, Berasategui et al. 2009, Berasategui et al. 2013). Moreover, the

384 introduction of this competitive species in the estuary is associated with a gradual restructuration

385 of the plankton community, as it displays a selective feeding on blooming diatoms, in particular

386 on *T. curviseriata* (Hoffmeyer and Prado Figueroa 1997). Furthermore, shifts in the species

387 composition of primary producers have wide implications upward in the food web causing

388 ultimately modifications in the structure of pelagic ecosystems and in the biogeochemical cycle

389 of elements (Finkel et al. 2010, Dutto et al. 2012). The multiple interactions between biotic and

390 abiotic variables documented here, highlight complex responses of plankton to environmental

391 modifications and consequences on predator-prey interactions (Reygondeau et al. 2015). The

392 particular case of the Bahía Blanca Estuary provides insights to track distributional shifts in the

393 plankton by the documentation of dominant species' range boundaries in accordance to

394 environmental gradients.

395

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Figure legends

571 **Figure 1:** Phytoplankton variability in the Bahía Blanca Estuary. a) Standardized and non-

572 dimensional form of surface chlorophyll *a* and b) total diatoms and *Thalassiosira curviseriata*

573 abundance measured on a fortnightly basis over the period 1990-2008. c) Seasonal and

574 interannual variability of *Thalassiosira curviseriata* (vertical bars) and total diatom abundance

575 (black dots) in cells x 10^3 l^{-1}, and sea surface temperature (red line) during the years when the

576 phytoplankton abundance and species composition were estimated (June 1991-February 1995;

577 May-August 2002-April-November 2003 and February 2006-February 2008). The gray areas

578 indicate the periods presented in figure b.

579

580 **Figure 2:** Interannual variability of winter (June-August) a) temperature, b) salinity and c)

581 chlorophyll concentration expressed by the mean values (dashed line) and the trend of the

582 filtrated data (continuous line).

583

584 **Figure 3:** a) Traffic-light diagrams illustrate the seasonal and interannual variability of the

585 abundance (log-transformed) of *Thalassiosira curviseriata* and *Eurytemora americana*. Empty

586 space denotes no data. b) Annual cycles of *T. curviseriata* and *E. americana* populations over the

587 sampling years.

588

589 **Figure 4:** Temperature and salinity windows exploited by *Thalassiosira curviseriata* (upper

590 panels) and *Eurytemora americana* (bottom panels). Curves of *T. curviseriata denote* the years

591 1992-1994 (continuous line) and 2006-2007 (dashed line), whereas in *E. americana* curves

denote the years 1990 (continuous line) and 2005, 2007 and 2008 (dashed line). The probability associated with the gradient of environmental variable is indicated.

Figure 5: Relationship between *Thalassiosira curviseriata* abundance and water temperature over the periods a) 1991-1994 and b) 2002-2008. c) Interanual variability of the diatom *T. curviseriata* and the copepod *Eurytemora americana*.

Figure 6: Path diagram showing the effects of hydroclimate-related factors, *i.e.* temperature, precipitation and salinity, nutrient concentration and *Eurytemora americana* abundance on *Thalassiosira curviseriata*. Solid paths are statistically significant, whereas dashed lines are not. Significance levels for individual paths between variables were set at $\alpha = 0.05$. At each significant path the standardized coefficients are represented and interpreted as follows: If temperature goes up by 1 SD, the abundance of *Thalassiosira curviseriata* goes down by 0.44 SD.