Environmental Earth Sciences (2019) 78:4
https://doi.org/10.1007/s12665-018-7988-z

ORIGINAL ARTICLE

Assessment of chitin variation in seston of a temperate estuary (Bahía Blanca, Argentina)

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Received: 21 December 2017 / Accepted: 15 September 2018

Abstract
Chitin was quantified in seston samples to determine its seasonal and spatial distribution in the estuary of Bahia Blanca. Sampling was conducted at three sites: Puerto Cuatreros, Maldonado, and Canal Vieja in autumn, winter, spring, and summer (2012–2013). Chitin concentrations were 3.79 ± 0.75 and 0.61 ± 0.36 chitin mg L^{-1} in autumn and winter, respectively. The proportion of chitin in the suspended particular matter (SPM) reached a maximum of 4% in autumn. There were statistical differences in chitin between seasons but not between sampling points. The particulate organic carbon (POC) ranged from 337.25 ± 30.52 µM in winter to 54.93 ± 26.10 µM in summer. The SPM varied from a maximum of 1172.17 ± 17.53 mg L^{-1} in spring to a minimum 71.42 ± 5.59 mg L^{-1} in autumn. In both cases, statistical differences were found between seasons but not between sampling points. The results indicated that the concentration of chitin as well as SPM and POC had a strong seasonal trend. The principal component analysis and the canonical analysis on the principal coordinates of environmental variables also highlighted the seasonal variation. The micro-detritus and planktonic organisms (fungi, diatoms, and copepods) in SPM and POC represent a chitinaceous substrate with a great importance for the study of the incorporation of compounds of nitrogen and carbon to the Bahía Blanca system.

Keywords Chitin · Biopolymer · Seston · Seasonal changes · Biogeochemical cycles · Estuary

Introduction

Chitin, a carbohydrate polymer composed of β-(1, 4)-*N*-acetyl-D-glucosamine, is the most abundant organic compound in nature after cellulose (Khoushab and Yamabhai 2010). It is synthesized by a large number of marine organisms, being a significant source of nitrogen and carbon (C:N = 8:1) (Montgomery et al. 1990). It is found in zooplankton organisms as the main component of the exoskeleton of euphausiids and copepods, and in certain diatoms within the genera *Thalassiosira* and *Skeletonema* (Durkin et al. 2009; Jeuniaux and Voss-Foucart 1991; Smucker 1991). This polymer is also present in cell walls of fungi, yeast, in cyst walls of some ciliates and amoebae, and in the lorica walls of some ciliates (Gooday 1990).

Dynamic processes such as molting cuticles and senescence of planktonic organisms are in a steady rain of chitin, which is deposited in the deep ocean known as "marine snow" (Alldredge and Gotschalk 1990; Simon et al. 2002). Organic detritus, micro-organisms (bacteria, fungi, phytoplankton, flagellates, and protozoans), and clay minerals exist in the ocean as aggregates, constituting the part of the suspended matter. Furthermore, that "marine snow" is an important site for biological processes of production, decomposition, nutrient recycling, and food sources for large particle feeders including fish and zooplankton, in the water column (Alldredge and Gotschalk 1990).

In this context, aquatic bacteria, which are present in the marine environment, are responsible for a significant portion, even most, of the recycling process of chitin (Beier and Bertilsson 2013). It is well known the specific interaction of certain members of the aquatic bacterial communities with chitin particles, i.e., *Vibrio cholera* (Pruzzo et al. 2008). Without the presence of these bacteria, marine environment would be

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Published online: 19 December 2018

significantly depleted in nitrogen in a relatively short period (Yu et al. 1991). However, it has been recently reported that the production of extracellular chitinases has been detected in other bacterial species (Kharade and McBride 2014). Occasionally, fungi and diatoms have also been shown as hydrolyze chitin oligomers (Wurzbacher et al. 2010; Vrba et al. 1997). Indeed, it has been recently proposed that the micro-organism community composition might actually be a significant player in chitin degradation rates, modulated by environmental factors as temperature and abundance of zooplankton and phytoplankton (Beier and Bertilsson 2013).

One of the phenomena that characterizes the estuary of Bahia Blanca is the suspended particulate matter (SPM), in which the inorganic fraction includes high concentrations of fine sediments (silt, clay, and sand) (Federici et al. 2004). The organic fraction, the particulate organic matter (POM), contributes more than half of the total suspended matter during phytoplankton bloom in winter, and is constituted by a high proportion of detritus, and ultra-, nano-, and microplankton (Diodato and Hoffmeyer 2008; Guinder et al. 2009a). During the rest of the year, only detritus appears to play an important role as food for filter feeders (Diodato and Hoffmeyer 2008). The zooplankton community, which is well known in the BBE (Hoffmeyer 1994; Biancalana et al. 2012; Dutto et al. 2012; Lopéz Abbate et al. 2016), also contributed to the micro-detritus in the form of exuviae and fecal pellets principally during spring–summer (Biancalana et al. 2017a, b).

The largest part of chitin was found in the seston from the BBE, suggesting that nano-phytoplankton, micro-detritus (exuviae and pellets), and other cell (fungi and bacteria) play an important contributing to the chitin pool in this system (Biancalana et al. 2017a). However, the changes in the amount of chitin in seston, depending on the community composition and activities, as well as its modulation by environmental variables, remain still unclear in BBE. The aims of the present study were to (1) determine the concentration of chitin in seston samples, (2) analyze the seasonal and spatial pattern of this polymer, SPM, and POC, and (3) identify the possible sources of this polymer and the relationship among the chitin concentration, SPM, POC, and the environmental and biotic variables. This work is also a contribution to understanding not only the production and degradation processes but also the biogeochemical role of chitin in the BBE.

Materials and methods

Study area

The Bahía Blanca Estuary (38°44′–39°27′ S and 61°45′–62°30′ W) (Fig. 1) is a mesotidal, temperate, and turbid estuary, located in the southwestern Atlantic Ocean and with a total area of 2300 km^2 at high tide (Perillo et al. 2004). Winds strongly modify the tidal wave, being the wind from the North–Northwest, the one which reduces sea level and from the Southeastern the one which generates the opposite effect (Piccolo et al. 2008). Salinity varies from 17.9 to 41.3 depending on seasonal rainfall, winds, and temperature (Freije and Marcovecchio 2004). The mean surface water temperature varies from 7 °C in winter to 23 °C in summer (Freije et al. 2008). Mean dissolved oxygen is close to 7 mg L^{-1}, reaching 13 mg L^{-1} during periods of phytoplankton blooms (winter and late-summer) (Freije et al. 2008). The BBE is recognized as a nutrient-enriched system due to the significant levels of inorganic nutrients (nitrate, nitrite, phosphate, and silicate) which are maintained during most of the year (Freije et al. 2008). The major freshwater inputs come from small tributes: The Sauce Chico River and the Naposta Grande Stream, which enter along the northern shore of BBE, provide an annual mean runoff of 1.9 and 0.8 m^3 s^{-1}, respectively. These inputs are only significant during rainy periods. The largest input is the sewage discharge from Bahia Blanca, Punta Alta, and Ingeniero White cities (Piccolo et al. 2008). The inner zone of this estuary is the most exposed to anthropogenic pressure as a consequence of human settlement, commercial ports, and petrochemical and chemical industries located on its northern coast (Arias et al. 2010).

The sampling points are located in the inner area of BBE (Fig. 1): (1) Puerto Cuatreros (PC), which is an area with high nutrients concentration, high phytoplankton biomass, and sparse vegetation on the tidal flats (Popovich et al. 2008; Negrin et al. 2013; Spetter et al. 2015; Guinder et al. 2009a), is the innermost point located far away from the sources of contamination in the BBE. (2) Maldonado (M) which is an area located between two ports, Puerto Cuatreros and Puerto Galvan, receives sewage from the "Tercera Cuenca" treatment plant, which corresponds to an old landfill that is currently out of use but still receives illegal dumps. (3) Canal Vieja (CV) which is a channel that receives untreated sewage (mean flow 0.59 m^3 s^{-1}) from Bahia Blanca and Ingeniero White city (400,000 inhabitants) (Tombesi et al. 2000).

Sampling and laboratory analysis

To detect the differences in the concentration of chitin between seasons and sampling points, nine sampling campaigns were carried out during autumn (04/19, 05/21, and 06/06/2012), winter (08/02 and 09/09/2012), spring (11/06 and 12/03/2012), and summer (01/02 and 02/02/2013) at the three sampling points. Seasonality was considered because of the changes on the plankton composition and abundance, and environmental variables of the BBE, being possible to distinguish different sources of chitin. The three sampling

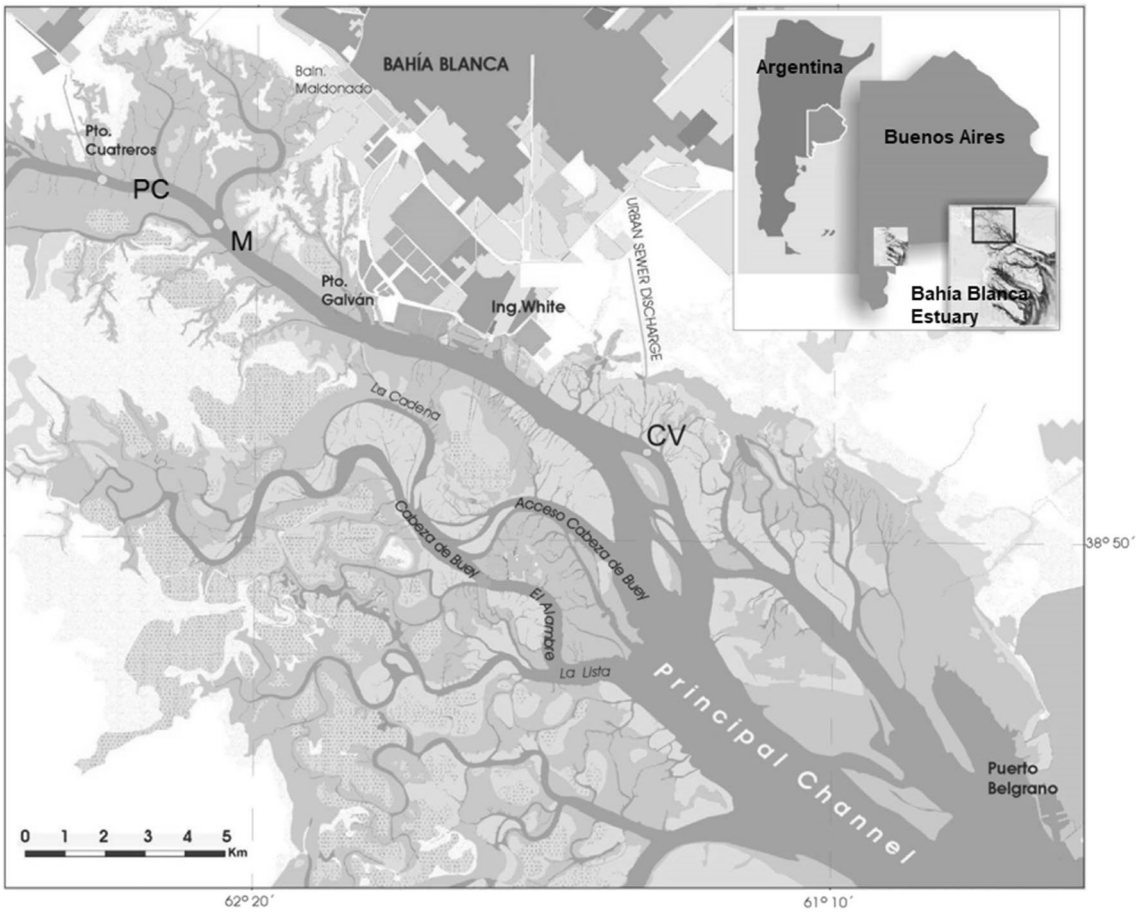

Fig. 1 Map of the study area showing the location of the sampling points: Puerto Cuatreros (PC), Maldonado (M), and Canal Vieja (CV)

points were located from the standpoint of waters quality and density of plankton.

Seston samples were collected at the surface using a 5 L Van Dorn bottle and were vacuum-filtered by glass fiber filters (GAMAFIL-GF/F − 0.7 µm pore), precombusted at 450 °C in a muffle over 4 h. After that, the filters were dried at 50 °C over 12 h. Chitin content was measured by the fluorescein succinylated wheat-germ agglutinin (FITC-WGA) method after Montgomery et al. (1990). The method was calibrated with purified crab chitin (Sigma-Aldrich, St Louis, MO, USA) as standard, quantified by fluorometric measurement of excess WGA after its reaction with fluorescein isocyathionate (FITC). Wheat germ agglutinin (WGA) after its reaction with fluorescein isocyanate (FITC). WGA is a sugar-binding protein that has a high affinity for *N*-acetyl glucosamine residues (Allen et al. 1973), which is the major component of chitin. The FICT-WGA has been shown to bind specifically to this polymer, even when samples contain high concentrations of cellulose, clay, and bacteria (Montgomery et al. 1990).

Temperature (*T*, °C), salinity (*S*), dissolved oxygen (DO), and pH were measured by HORIBA U-10 multiparameter sensor. Salinity is on the practical salinity scale PSS-78, defined as a conductivity ratio; following UNESCO (1985) recommendations, salinity on practical salinity scale is unitless.

Water samples for particulate organic carbon (POC) determination were filtered through muffled (450 °C, 1 h) glass fiber filters (GAMAFIL-GF/F − 0.7 µm pore). The POC concentration (µM) was measured following the Strickland and Parsons (1968), using a UV–Vis Jenway 6715 spectrophotometer. The SPM (mg L^{-1}) was determined gravimetrically after drying at 50 °C to a constant weight. In addition, water samples were collected to determine inorganic dissolved nutrients, chlorophyll *a* (Chl. *a*), and phaeopigments (phaeo.). Nitrate (NO_3^-), nitrite (NO_2^-), orto-phosphate (PO_4^{3-}), and silicate (SiO_2^-) concentrations were determined using an automatized and five-channel upgraded Techinicon AutoAnalyzer II according to Treguer and Le Corre (1975), Hansen and Grasshoff (1983), Eberlein and Kattner (1987), and Technicon (1973), respectively. Chl. *a* and phaeopigments were analyzed using the spectrophotometric method APHA–AWWA–WEF (1998) by extraction with acetone 90% during 24 h at − 4 °C using

a UV–Vis Jenway 6715 spectrophotometer. The chemical analyses were done by the Marine Chemistry Laboratory at IADO. Mesozooplankton samples were collected with a 200-µm mesh, 0.30-m open-mouth net by horizontal tows in the upper layer (0–2 m) during mid-ebb tide. A mechanical flowmeter was used to estimate the filtered volume of water and samples were preserved in 4% formalin. Mesozooplankton samples were qualitatively and quantitatively analyzed under a Wild M5 stereomicroscope. Total mesozooplankton abundance (TMA) was expressed as individuals per cubic meter (ind m^{-3}).

Data analysis

Non-parametric statistical procedures were applied, because the data did not meet the assumptions of normality and homoscedasticity. The Kruskal–Wallis *H* test was used to determine differences on chitin concentration, POC, SPM, environmental (*T, S*, DO, pH, Chl. *a*, phaeo., NO_3^-, NO_2^-, PO_4^{3-}, and SiO_2^-) and biotic (TMA) variables between sampling points and seasons. When statistical differences were detected in the Kruskal–Wallis *H* test, the Mann–Whitney *U* test with Bonferroni correction was applied. This correction is based on the division of the significant level ($p = 0.05$) by the number of comparisons of pairs (six seasonal comparisons and three spatial comparisons). This analysis was conducted at a significant level of 0.05, by SPSS 15.0 software. Principal component analysis (PCA) was performed to visualize the patterns of chitin concentration, POC, SPM, and environmental variables (*T, S*, DO, pH, Chl. *a*, phaeo., NO_3^-, NO_2^-, PO_4^{3-}, and SiO_2^-). Permutational Multivariate Analysis of Variance (PERMANOVA) was applied to detect the differences in the patterns of environmental data regarding the seasons and sampling points, as well as the interaction between both. Canonical analysis on the principal coordinates (CAP) was used to make an ordination of taking into account the design of the data in the PCA. Seasons were selected as a factor for groups in CAP. The Euclidean distance matrix was used in the analyses above. The Spearman's rank correlation coefficients were calculated to determine the relationship among chitin concentration, POC, SPM, environmental (*T, S*, DO, pH, Chl. *a*, phaeo., NO_3^-, NO_2^-, PO_4^{3-}, and SiO_2^-) and biotic (TMA) variables. Previously, all data were standardized. These analyses were done using the PRIMER 6 and SPSS 15.0 software, respectively.

Results

Chitin, POC, and SPM

Chitin concentration was the highest in autumn and the lowest in winter (3.79 ± 0.75 and 0.61 ± 0.36 mg chitin L^{-1}, respectively) (Fig. 2). In addition, chitin was the highest (2.16 ± 0.72 mg L^{-1} chitin) in CV and the lowest (1.88 ± 0.70 mg chitin L^{-1}) in PC (Fig. 2). There were statistical differences between seasons (the Kruskal–Wallis test, $H = 8.87$, $p = 0.03$) but not between sampling points (the Kruskal–Wallis test, $H = 0.15$, $p = 0.93$) (Table 1). Significant differences in chitin concentration were found between autumn and winter (the Mann–Whitney test $U = 5$, $p \leq 0.008$) (Table 1).

The highest POC concentration was 337.25 ± 30.52 µM in winter and the lowest was 154.93 ± 26.10 µM in summer (Fig. 2). The POC concentration varied from 256.27 ± 42.26 µM at CV to 230.49 ± 26.39 µM at M (Fig. 2). In addition, SPM concentration ranged between 172.17 ± 17.53 mg L^{-1} in spring and 71.42 ± 5.59 mg L^{-1} in autumn (Fig. 2). SPM concentration was the highest at M and the lowest at CV (129.88 ± 16.38 mg L^{-1} and 97.21 ± 12.29 mg L^{-1}, respectively) (Fig. 2). In both cases, POC and SPM presented differences between seasons (the Kruskal–Wallis test, $H = 10.74$, $p = 0.01$ and the Kruskal–Wallis test, $H = 14.21$, $p = 0.003$, respectively) but not between sampling points (POC—the Kruskal–Wallis test, $H = 0.16$, $p = 0.93$; SPM—the Kruskal–Wallis test, $H = 3.77$, $p = 0.14$) (Table 1). Differences in POC concentrations were only found between winter and summer (the Mann–Whitney test $U \leq 0$, $p \leq 0.008$). Significant differences of SPM concentration were found between winter and spring (The Mann–Whitney *U* test ≤ 0, $p \leq 0.008$), as well as between autumn and spring (The Mann–Whitney test $U \leq 0$, $p \leq 0.008$) (Table 1).

The proportion of chitin to SPM (weight/weight) ranged from 1% in spring to 3% in autumn (Fig. 3). The proportion of POC to SPM (w/w) reached a maximum of 5% in winter (Fig. 3). In spring and summer, the SPM reached the maximum percentages (98% and 97%, respectively) and the proportion of chitin and POC to SPM (w/w) did not exceed the 1% and 2%, respectively (Fig. 3).

Environmental and biotic variables

Temperature varied from 5.6 ± 2.51 °C in winter to 21.9 ± 0.55 °C in spring. The highest temperature was 16.13 ± 2.05 °C at PC (Fig. 4). The lowest salinity value was observed in winter, while the highest was in autumn (16 ± 7 to 37 ± 0.34, respectively). Salinity values varied from 29.34 ± 3.65 at CV to 35.00 ± 0.76 at PC (Fig. 4). The dissolved oxygen values ranged between 1 ± 0.13 mg L^{-1} in summer and 7 ± 0.72 mg L^{-1} in autumn. The highest DO value was 5.31 ± 1.14 mg L^{-1} at M (Fig. 4). The lowest pH value was registered in winter and the highest in summer (3.94 ± 1.76 and 9.42 ± 0.45, respectively). The highest pH value was 8.89 ± 0.43 at PC (Fig. 4). NO_2^- concentration varied from 0.92 ± 0.21 µM in winter to 2.53 ± 0.39 µM

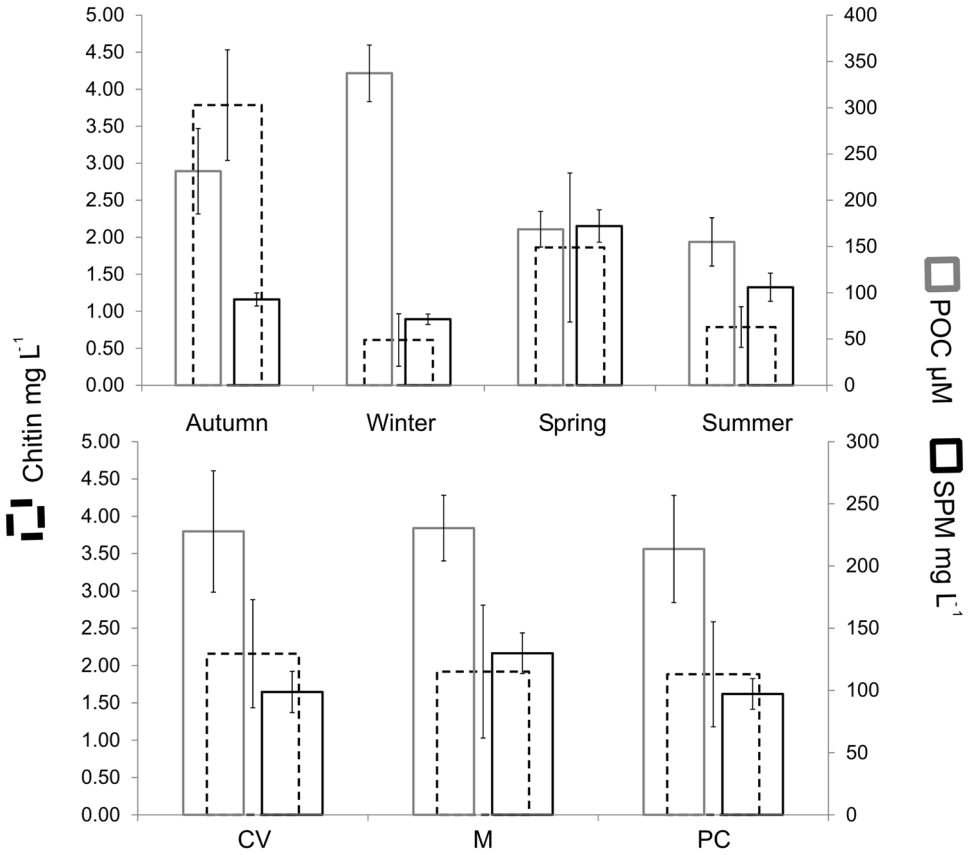

Fig. 2 Seasonal and spatial concentration of chitin, POC, and SPM (autumn, winter, summer, and spring) (CV: Canal Vieja, M: Maldonado, and PC: Puerto Cuatreros)

Table 1 Kruskal–Wallis *H* test and Mann–Whitney *U* test with Bonferroni correction

Variables	POC	Chitin	SPM
The Kruskal–Wallis *H* test (variable of agrupation: sampling points)			
H	0.16	0.15	3.87
df	2	2	2
p	0.93	0.93	0.14
The Kruskal–Wallis test (variable of agrupation: seasons)			
H	10.74	8.87	14.21
df	3	3	3
p	0.01	0.03	0.003

*$p \leq 0.05$ significant difference; **$p \leq 0.01$ high significant difference. *H* = statistic, *df* = degrees of freedom

The Mann–Whitney *U* test with Bonferroni correction	Signification
Chitin	
Autumn–winter	*
POC	
Winter–summer	*
SPM	
Winter–spring	*
Autumn–spring	*

*$p \leq 0.008$ significant difference (0.05 significant level/six pair of comparisons)

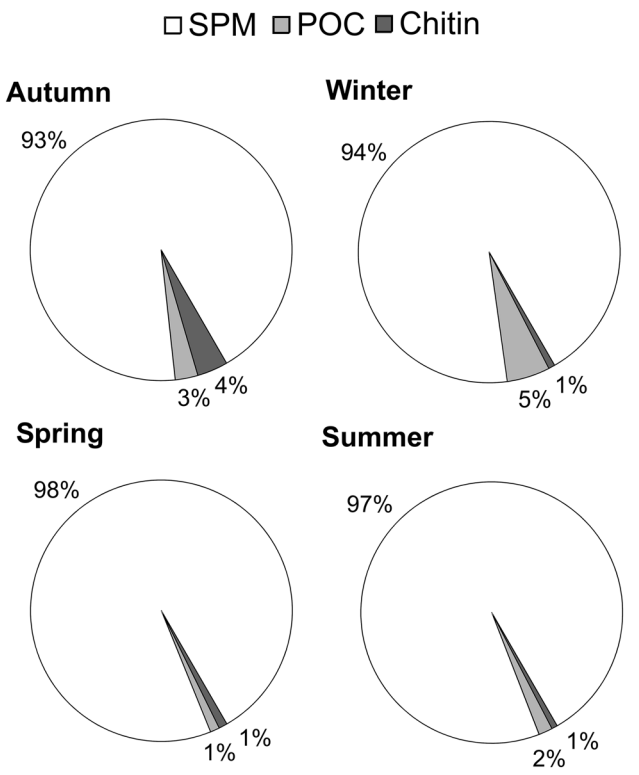

Fig. 3 Proportion of chitin and POC in SPM, and the contribution of chitin to POC in autumn, winter, summer, and spring. Data expressed as percentage (%)

in autumn, being 2.09 ± 0.39 µM the highest concentration at PC (Fig. 4). NO_3^- concentration was the lowest in winter and the highest was in summer (5.6 ± 1.01 µM and 9.56 ± 1.89 µM, respectively). The lowest NO_3^- concentration was 5.86 ± 1.86 µM at CV (Fig. 4). PO_4^{3-} concentration was the lowest (1.99 ± 0.71 µM) in winter and the highest (7.12 ± 3.38 µM) in autumn. PO_4^3 concentration varied from 1.77 ± 0.29 µM at PC to 7.48 ± 3.35 at CV (Fig. 4). SiO_2^- concentration was the lowest (40.92 ± 6.29 µM) in summer and the highest (83.63 ± 13.90 µM) in autumn. The lowest SiO_2^- concentration was 36.86 ± 6.14 at CV (Fig. 4). Chlorophyll *a* concentration ranged between 0.64 ± 0.42 µg L^{-1} in summer and 7.22 ± 3.19 µg L^{-1} in spring. The lowest Chl. *a* concentration was 2.86 ± 1.23 µg L^{-1} at PC (Fig. 4). Phaeopigment concentration reached a maximum of 6.90 ± 1.34 µg L^{-1} in summer, ranging between 3.45 ± 1.60 µg L^{-1} at CV to 5.30 ± 1.68 µg L^{-1} at M (Fig. 4). The total mezooplankton abundance was the lowest in autumn and the highest in summer (19.85 ± 12.02 and 78.31 ± 40.40 ind. m^{-3}, respectively). The highest TMA was 40.12 ± 21.31 ind. m^{-3} at CV (Fig. 4).

Statistical differences between seasons were detected in temperature (the Kruskal–Wallis test, $H = 17.31$, $p = 0.001$), salinity (the Kruskal–Wallis test, $H = 15.59$, $p = 0.001$), DO (the Kruskal–Wallis test, $H = 14.57$, $p = 0.002$), pH (the Kruskal–Wallis test, $H = 16.71$, $p = 0.001$), and NO_2^- (the Kruskal–Wallis test, $H = 11.91$, $p = 0.008$) (Table 2). Temperature, salinity, and DO presented significant differences between autumn and summer, and pH significantly differed between autumn and spring. Differences in NO_2^- concentration were detected between autumn and winter, and winter and spring (Table 2). Only difference in the PO_4^3 concentration was detected between sampling points (the Kruskal–Wallis test, $H = 0.16$, $p = 0.024$). The PO_4^3 concentration was significantly differed between CV and PC, and M (Table 2).

Multivariate analyses

To identify the major sources of variation and emphasize the differences between seasons of the environmental data, a PERMANOVA, PCA, and CAP were applied. Spearman's rank correlation matrix was used to explore relationships among chitin concentration, POC, SPM, and environmental and biotic variables.

The first two components of PCA explained the 83.80% of the total variation (Fig. 5). POC had the highest percentage contribution to the first axis (PC1 89.40%) and SiO_2^- had the highest percentage contribution to the second axis (PC2 8.70%), being both, POC and SiO_2^-, the major sources of variation (Fig. 5). POC, which was positively associated with the PC1 axis, was the variable that best explained the first axis (Fig. 5). The silicate and SPM were the variables that best explained the second axis, associated with samples that formed the group summer–spring (Fig. 5). SiO_2^- positively associated with PC2 axis and the SPM were negatively associated with PC2 axis (Fig. 5). Although the concentration of chitin was not well-represented in PCA plot, it had a negative association with the PC1 and positive with PC2, indicating the same pattern of SiO_2^-, which was positively associated with two axes, and inverse of POC.

Variables with strong seasonal variability (PERMANOVA F: 3.6, $p \leq 0.05$) formed graphically two clearly arranged groups (summer–spring vs. autumn–winter) along the CAP 1 axis (Table 3; Fig. 5).

Table 3 shows the significant correlations between the environmental and biotic variables. POC was high and negatively correlated with SPM ($r_s = -0.79$, $n = 27$, $p \leq 0.01$), temperature ($r_s = -0.76$, $n = 27$, $p \leq 0.01$), pH ($r_s = -0.61$, $n = 27$, $p \leq 0.01$), nitrite ($r_s = -0.55$, $n = 27$, $p \leq 0.01$), and salinity ($r_s = -0.55$, $n = 27$, $p \leq 0.01$). These variables were negatively associated with PC1 axis (Fig. 5). TMA was significantly and negatively correlated with silicate ($r_s = -0.40$, $n = 27$, $p \leq 0.05$). On the other hand, SPM was high and positively correlated with temperature ($r_s = 0.70$, $n = 27$, $p \leq 0.01$), nitrite ($r_s = -0.52$, $n = 27$, $p \leq 0.01$), and salinity ($r_s = -0.52$, $n = 27$, $p \leq 0.01$), and significantly correlated with pH ($r_s = -0.43$, $n = 27$, $p \leq 0.05$). Temperature presented a high and positive significant correlation

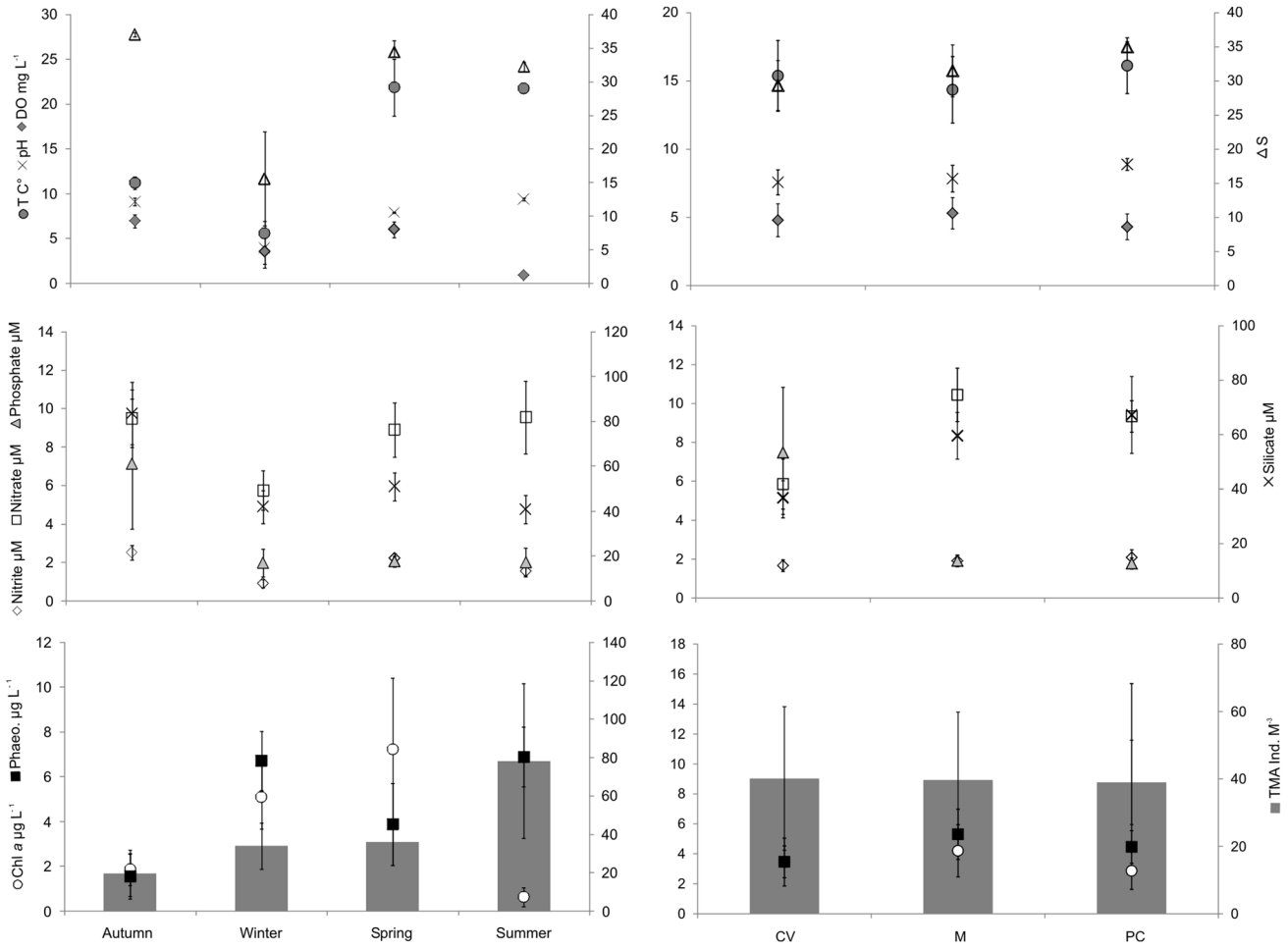

Fig. 4 Environmental variables: temperature (°C), pH, dissolved oxygen (DO), salinity, nitrite, nitrate, phosphate, silicate, chl. *a*, phaeo., and biotic variable: total mesozooplankton abundance (TMA), in Bahía Blanca estuary during the study period

with pH and salinity ($r_s = -0.65$, $n=27$, $p \leq 0.001$, and $r_s = 0.65$, $n=27$, $p \leq 0.001$, respectively). The pH and dissolved oxygen were high and significantly correlated with salinity ($r_s = 0.83$, $n=27$, $p \leq 0.01$ and $r_s = 0.54$, $n=27$, $p \leq 0.01$, respectively). Nitrite was positively correlated with temperature ($r_s = 0.56$, $n=27$, $p \leq 0.01$), pH ($r_s = 0.59$, $n=27$, $p \leq 0.01$) and salinity ($r_s = 0.57$, $n=27$, $p \leq 0.01$). Phosphate was positively correlated with DO ($r_s = 0.39$, $n=27$, $p \leq 0.05$), while silicate was high and positively correlated with nitrate ($r_s = 0.53$, $n=27$, $p \leq 0.01$) and pH ($r_s = 0.42$, $n=27$, $p \leq 0.05$). The concentration of chlorophyll *a* was negatively correlated with phaeopigments ($r_s = -0.52$, $n=27$, $p \leq 0.01$) and with pH ($r_s = -0.49$, $n=27$, $p \leq 0.05$) (Table 4).

Discussion

Seasonal and spatial changes: chitin, SPM, POC, and environmental and biotic variables

The chitin values of total seston registered in this study were comparable to those chitin values measured in different marine environments. Montgomery et al. (1990) registered values of chitin that ranged between 0.004 and 0.021 mg L^{-1} in Delaware Bay (USA), reflecting the seasonal decline in primary production, and a considerable supplied of copepods that were abundant in this environment in September.

Table 2 Kruskal–Wallis *H* test and Mann–Whitney *U* test with Bonferroni correction—environmental variables

Variables					PO_4^{3-}
The Kruskal–Wallis *H* test (variable of agrupation: sampling points)					
H					0.16
df					2
p					0.024
$^*p \leq 0.05$ significant difference; $^{**}p \leq 0.01$ high significant difference. *H* = statistic, *df* = degrees of freedom					
The Mann–Whitney *U* test with Bonferroni correction					Signification
PO_4^{3-}					
CV–M					*
CV–PC					*
$^*p \leq 0.02$ significant difference (0.05 significant level/three pair of comparisons)					
Variables	*T*	*S*	DO	pH	NO_{2-}
The Kruskal–Wallis *H* test (Variable of agrupation: seasons)					
H	17.31	15.59	14.57	16.71	11.91
df	3	3	3	3	3
p	0.001	0.001	0.002	0.001	0.008
$^*p \leq 0.05$ significant difference; $^{**}p \leq 0.01$ high significant difference. *H* = statistic, *df* = degrees of freedom					
The Mann–Whitney *U* test with Bonferroni correction					Signification
T					
Autumn–spring					*
Autumn–summer					*
S					
Autumn–summer					*
DO					
Autumn–summer					*
Spring–summer					*
pH					
Autumn–spring					*
Spring–summer					*
NO_{2-}					
Autumn–winter					*
Winter–spring					*
$^*p \leq 0.008$ significant difference (0.05 significant level/six pair of comparisons)					

Lara et al. (2011) suggested that a large amount of chitin in SPM in the Sunderbans Estuary, ranging from 1 to 2 mg L^{-1}, was concentrated in a micro-detritus degraded form either as exuviae or absorbed on particles constituting the sediment fraction at less than 20 µm, which would also have included by bacteria and fungi. Nicol and Hosie (1993) have discussed that chitin concentration of the suspended matter from the North Sea in January and October 1992 and March 1993 was composed of phyto- and zoo-planktonic components with probable some bacterial and/or terrestrial materials, as well. Values of chitin reached a maximum of 6.1 mg L^{-1} in seston fraction ≤ 20 µm in summer have already been registered in the BBE (Biancalana et al. 2017a). In the latter work, it is suggested that the concentration of chitin followed the same pattern of the dynamic of plankton, being the contributors of chitin in that fraction, fungi and some structure of bacteria, small phytoplankton, and micro-detritus. Although the determination of chitin in this study was performed on total seston, the concentration of chitin represented the same distribution trend: the maximum values during warm seasons and minor values during winter comparing with the results registered in sestonic fraction ≤ 20 µm in the BBE during 2008–2009. Therefore, taking into account the previous results, it is assumed that chitin in total seston was associated with the presence of phytoplankton in cold seasons and with the increase of micro- and mesozooplankton during warm seasons in the Bahia Blanca Estuary.

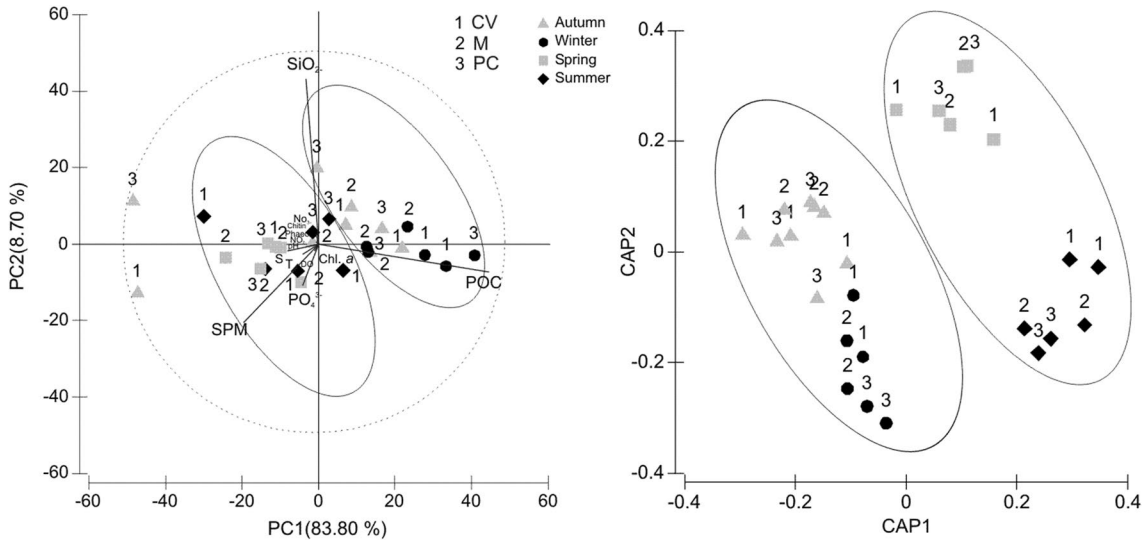

Fig. 5 PCA plot of chitin concentration, POC, SPM, and environmental and biotic variables

Table 3 PERMANOVA results

Source	*df*	SS	MS	Pseudo-*F*	*p*
Seasons	3	5803.9	1934.6	3.96	0.02
Sampling points	2	251.23	125.62	0.26	0.89
Seasons × sampling points	6	1404.60	234.10	0.48	0.88
Residual	15	7320.60	488.04		
Total	26	14,856.00			

df degree of freedom, *SS* sum square, *MS* mean square ($p \leq 0.05$)

The concentration of chitin, POC, and SPM had a strong seasonal trend, while the spatial pattern seemed to be similar in the three sampling points. A greater amount of POC and lower concentration of chitin and SPM occurred in winter. The inverse pattern was observed during spring, although the maximum value of chitin was observed in autumn. In addition, an inverse correlation between POC and temperature, salinity, and pH was observed in this study, corroborating the strong seasonal variation. The values of POC and SPM found in this study were similar to those previously reported

Table 4 Coefficients of Spearman's rank correlation (r_s)

	NO_{2-}	NO_{3-}	PO_4^{3-}	SiO_{2-}	TMA	POC	Chitin	SPM	Chl. *a*	Phaeo.	*T*	pH	DO
NO_{3-}	0.31												
PO_4^{3-}	0.15	−0.19											
SiO_{2-}	0.35	0.53**	−0.04										
TMA	−0.27	−0.27	−0.35	−0.40*									
POC	−0.55**	−0.20	−0.09	−0.07	−0.24								
Chitin	0.38	0.18	0.26	0.13	−0.33	−0.17							
SPM	0.52**	0.14	−0.03	−0.14	0.05	−0.78**	0.19						
Chl. *a*	−0.03	−0.27	−0.32	−0.34	0.14	0.34	−0.19	0.04					
Phaeo	−0.27	−0.00	−0.26	0.17	0.10	−0.16	−0.20	0.07	−0.51				
T	0.56**	0.08	0.14	−0.02	0.05	−0.76**	0.03	0.70**	−0.31	0.29			
pH	0.59	0.29	0.21	0.42*	−0.34	−0.61**	0.31	0.43*	−0.49*	0.33	0.65**		
DO	0.28	0.03	0.39*	0.06	−0.22	−0.15	0.13	0.20	−0.00	−0.25	0.08	0.24	
S	0.57**	0.31	0.20	0.35	−0.25	−0.55**	0.25	0.52**	−0.29	0.22	0.65**	0.83**	0.54**

NO_{2-} nitrite (μM) ($n=27$), NO_{3-} nitrate (μM) ($n=27$), PO_4^{3-} phosphate (μM) ($n=27$), SiO_{2-} silicate (μM) ($n=27$), TMA total mesozooplankton abundance (Ind. m^{-3}) ($n=27$), POC particulate organic carbon (μM) ($n=27$), Chitin ($mg.L^{-1}$) ($n=27$), SPM-suspended particulate matter ($mg.L^{-1}$) (n:27), T temperature (°C) ($n=27$), pH ($n=27$), DO-dissolved oxygen ($mg.L^{-1}$) ($n=27$), S salinity ($n=27$), Chl. *a* chlorophyll *a* ($mg.L^{-1}$) ($n=27$), and Phaeop. phaeopigments ($mg.L^{-1}$) ($n=27$). (−) *$p<0.05$ significant difference; (−) **$p<0.01$ high significant difference

in the same seasons in the BBE (Diodato and Hoffmeyer 2008; Federici et al. 2004; Guinder et al. 2009a). Both, SPM and POC, were influenced by the precipitation and freshwater inputs as well as strong winds and tidal currents, which induce sediment resuspension of fine particles from the tidal flats (Perillo and Piccolo 1991; Guinder et al. 2009a). In addition, detritus originates from the saltmarshes, in which the principal component was *Spartina alteniflora*, and has a crucial role as one of the main contributors of organic matter to SPM followed by plankton in the BBE (Dutto et al. 2014). A negative correlation between the concentration of POC and SPM indicated that both were controlled by common processes but produced inverse effects on them. The latter is also reflected in the PCA analysis, in which POC positively contributed to the first axis and SPM negatively associated with it.

The maximum values of POC have been closely correlated with high biomass and abundance of phytoplankton during the winter period (Guinder et al. 2009a; Spetter et al. 2015). The beginning of the phytoplankton winter bloom (July) has been characterized by high dissolved nutrient concentrations due to autumn rains (Guinder et al. 2009b; Popovich et al. 2008), an increase through light penetration in the water column resultant of less suspended sediments (Guinder et al. 2009a) and low zooplankton grazing pressure related to low water temperatures (Berasategui et al. 2009; Pettigrosso and Popovich 2009). In the present study, the previous stage of phytoplankton winter bloom (autumn) was recorded, and has represented similar conditions to those mentioned above, e.g., high concentration of inorganic nutrients. In addition, the increase of phytoplankton in the autumn coincided with the highest value of DO and an increasing pH. Furthermore, the collapse of the phytoplankton winter bloom (August–September) was likely related to NO_3^-, NO_2^-, PO_4^{3-}, and SiO_{2-} depletions, associated with phytoplankton uptake, as well as to micro- and mesozooplankton grazing. The latter was reflected in the increase of phaeopigments that showed an inverse pattern to that of chlorophyll *a*. As a consequence of the grazing and degradation activities, a decrease of DO was also observed. In addition, in this period, low salinity was registered as a consequence of the increase in rain (accumulated precipitation of this period 88.18 mm) and freshwater inputs (Vitale et al. 2012). Thus, the predominance of POC (5%) in SPM in winter was probably caused by the combination of phytoplankton winter bloom depletion and by detritus from the resuspension of sediment and tidal flats as a consequence of rain and freshwater inputs. In this context, the aggregation of detritus, senescence of microalgae, and an increase of zooplankton contributed to chitin (1%) in SPM during winter.

As mentioned above, chitin and SPM concentration increased, while POC diminished in spring–summer. In spring, chl *a* showed a second peak, reflecting a second phytoplankton bloom as had been previously mentioned by Guinder et al. (2009b). An increase in the total abundance of zooplankton was also registered in spring, reaching the maximum value during summer. In addition, the inverse correlation of total abundance of zooplankton and silicate (Turner et al. 1998), which represented minimum values in winter and summer, indicated that zooplankton grazing was taking place in the BBE. In addition, the highest concentrations of phaeopigments were observed in summer, indicating the degradation of chlorophyll *a* (Biancalana et al. 2012; Dutto et al. 2012; Spetter et al. 2015). Moreover, an elevated pH and dissolved oxygen depletion was observed during summer in this study. Thus, the inverse correlation between chlorophyll *a* and pH corroborated biological activity. Therefore, as seen in winter, the supply of chitin from zoo- and phytoplankton constituted as a degradation component of the micro-detritus in the seston from the BBE.

Alternatively, salinity, which increased during spring–summer due to evaporation processes a product of high temperatures in the inner zone of the BBE (Piccolo et al. 2008 1990), regulated the amount of chitin, SPM, and POC. Moreover, low precipitation contributed greatly to this scenario.

In this context, dissolved inorganic nutrients, especially NO_{2-}, DO, and pH, presented marked seasonal differences, depending on the development and growth of phytoplankton during winter as well as degradation processes after phytoplankton depletion and mesozooplankton increased during spring–summer in the BBE.

In addition, the microphytobenthos mostly formed by cyanobacteria and diatoms organisms could be considered. This community is responsible for the secretion of large amounts of extracellular polymeric substances (EPS) (Pan et al. 2013). In the estuary, the biovolume of this community estimated by total biomass is considerably less in the summer (Pan et al. 2013); thus, the biofilms are thinner, allowing sediments to remobilize and, therefore, increases the amounts of SPM and the chitin conglomerated with it in the water column. Greater amounts of chitin were registered in seston samples from the water column in spring and summer. The latter context could also be representative of the autumn period, in which chitin represented the highest value (3% of SPM).

In term of spatial distribution, no statistical differences were found in chitin concentration, POC, and SPM between stations in this study. In addition, there were not statistical differences in the spatial distribution among sampling point of the environmental and biotic variables, except phosphate. The three samples points were influenced by freshwater and detritus inputs from *S. alterniflora*, contributing with organic matter and sediment to SPM. In addition, both sampling points, CV and M, received sewage, containing large amounts of organic matter (Curds 1982). In addition, spatial

studies have shown that water turbidity, chlorophyll *a* concentration, and phytoplankton and zooplankton abundance changes were related to the meteorological and hydrodynamic conditions (i.e., intensive tidal advection and suspension, low river inflow, high residence time, winds, and precipitation) (Popovich and Marcovecchio 2008; Biancalana et al. 2012; Dutto et al. 2012; Menendéz et al. 2016). Winds and tides have been identified as the main inputs of energy, especially in the inner zone of the BBE (Piccolo et al. 2008). Thus, the water column in the inner area of the BBE is partially mixed with a strong tendency to be vertically homogeneous under ressupension and mixing/advection processes as well as low freshwater discharge. Due to the above-mentioned chitin concentration, POC, SPM, as well as water quality presented similar spatial distribution without finding significant statistical differences among them.

Although, not many differences were found between sampling point consideration to other points which is necessary. The PO_4^3 concentration was the only dissolved nutrient that showed significant differences between CV and the other sampling points (PC and M). As it is known, disposal of sewage in the sea is considered the major sources of addition of compounds of nitrogen and phosphorus to the environment (Curds 1982). In our study, PO_4^3 concentration was the variable with the clearest association with the sewage contribution, especially at CV where the maximum value of phosphate was observed. In particular, CV reflected the small-scale local changes produced by sewage.

Chitin sources: preliminary implications in production and degradation process

Phytoplankton, in which diatoms' bloom was the most important event on primary biomass production during winter and early spring in the BBE (Popovich et al. 2008), could be a relevant source of chitin in the seston of the BBE. It is well known that several common genera of diatoms, such as *Thalassiosira, Skeletonema*, and *Cyclotella*, which were found in the phytoplankton community of the BBE, produced chitin as an important portion of their biomass (Durkin et al. 2009; Smucker 1991). The same pattern of chitin and SiO_2_ in the PCA corroborated the assumption of the phytoplankton as a source of chitin in the BBE. Moreover, the maximum POM concentration in the BBE during winter 2003 was correlated with the higher total abundance of diatoms, especially *T. curviseriata* followed by *Cylotella* sp. (Spetter et al. 2015). In addition, the higher levels of fatty acid marker of diatoms were found in winter 2009–2010, asseverating the important contribution of diatoms to the estuarine seston of the BBE (Dutto et al. 2014).

Alternatively, diatoms are part of the microphytobentonic community integrating biofilms and microbial mats (Parodi and Barria de Cao 2003; Pan et al. 2013). As previously mentioned, EPS from diatoms, bacteria, and other microorganisms are the main binding agents of sediment but also of fecal pellets (Wotton 2011). The fecal pellets of planktonic crustaceans, principally copepods, are wrapped in a peritrophic matrix after ingestion. This matrix was formed of chitin (Yoshikoshi and Ko 1988). In addition, EPS were captured and digested by organisms such as amphipods and harpacticoid copepods, which were part of the meiobentonic community of the BBE. As discussed above, the environmental conditions during spring–summer facilitated the resuspension of SPM, and therefore, the organisms of those communities which also contributed to the chitin source.

Dynamic processes such as molting cuticles (exuviae) and senescence of planktonic organisms result in a continuous supply of chitin in the marine seston (Alldredge and Gotschalk 1990). Consequently, fecal pellets production for zooplankton was an important source of chitin (Kirchner 1995). In winter, the lowest water temperatures provoked the decrease in total zooplankton abundance in BBE (Biancalana et al. 2012; Dutto et al. 2012). Thus, senescence of zooplankton also contributed to chitin pool. In spite of this general decrease of zooplankton during winter, some species of meso and micro-zooplankton had their peaks of abundance (Barria de Cao 1992; Berasategui et al. 2009, 2012) and continued to contribute to the chitin pool. Moreover, chitin is found as a structural material for cyst and lorica walls of some ciliates (Gooday 1990; Tuner 2002) which also are part of the microplankton of the BBE. The zooplankton abundance increased in summer, being the chitin supply generated by molting cuticles and egg production of different genera (Berasategui et al. 2012). Moreover, *Acartia tonsa* and *Eurytemora americana*, the two principal species of the BBE had their resting benthic egg banks in the inner zone of this estuary (Berasategui et al. 2009, 2012). This provided them temporary refuge under adverse environmental conditions (i.e. warm season for *E. americana* and cold season for *A. tonsa*) (Berasategui et al. 2009, 2012), thereby contributing to the chitin source in different seasons.

Fungi and certain bacterial structures (Gooday 1990; Lara et al. 2011) could be an important source of chitin in the seston of the BBE. It is known that certain marine fungi have chitin in their walls (Wursbacher and Grossart 2012). Particularly, in the BBE, information on these important components and its relation with chitin is scarce. Currently, studies on this association between fungi and bacteria with chitin have been carried out in the BBE. Moreover, chitin was observed on the structures of fungi in seston samples from the BBE (Biancalana et al. 2017b). This allows us to think that fungi could be another source of chitin in the estuary.

Biodegradation processes of chitin were performed by bacteria (e.g., *Vibrio* and genus containing chitinase) and other organisms such as fungi and diatoms (Beier and

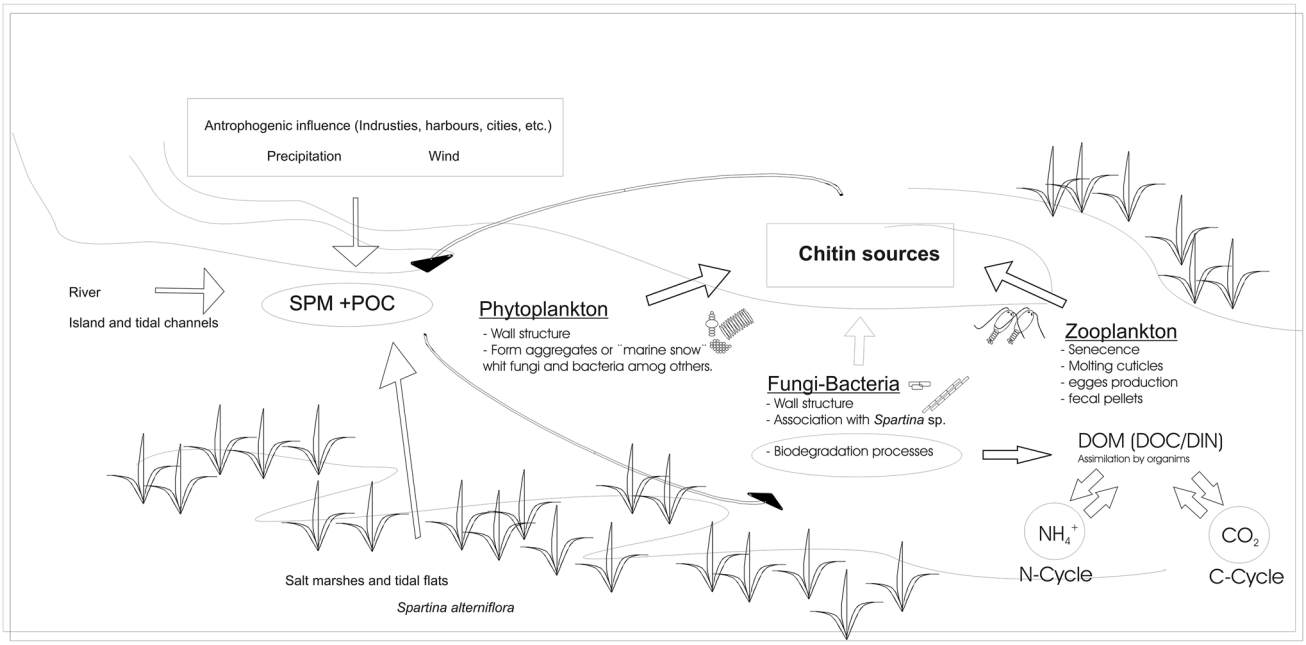

Fig. 6 General representation of possible sources of chitin and processes that affecting SPM, POC (extracting from Guinder et al. 2009), and sources of chitin, and its possible function in biogeochemical cycles in the BBE

Bertilsson 2013; Wurzbacher et al. 2010; Vrba et al. 1997). Fungi, as well as bacteria, were also involved in *Spartina* and aggregates or "marine snow", which formed during the decaying stage of a phytoplankton bloom, particularly diatoms are capable of secreting large amount of mucus that form a conspicuous aggregate in the water column, decomposition (Lee et al. 1980; Menendez and Sanmarti 2007). These kinds of aggregates in which diatoms and fungi were involved have been observed in seston samples from the BBE (Biancalana et al. 2017b). The latter confirmed us the potentiality of both, especially fungi, to contribute as a source of chitin, being also degrader components of this polymer in seston of the BBE. Moreover, the degradation of this polymer by bacteria and fungi produced the incorporation of nitrogen and carbon (Wurzbacher et al. 2010; Kirchman and White 1999), and depended on temperature and the abundance of the phyto and zooplankton communities as a chitin supply to the system (Beier and bertilsson 2013). In this study the highest deflection of dissolved oxygen with high temperatures, and the negative correlation between phaeopigments and Chlorophyll *a*, suggested us that a process of degradation was taking place in the BBE, especially during the summer. Therefore, the degradation of chitin might be considered as a factor that produced the low concentration of this polymer during summer in this study.

To summarize, Fig. 6 represents a general diagram of chitin sources and their contribution from components of seston in the BBE.

Conclusion

The marked seasonality could be due to (1) the contribution by phytoplankton organisms such as diatoms, which are one of the most abundant groups in winter in the BBE (2) as well as small particles from processes such as the change of the cuticles (exuvias), fecal pellets, and production senescence of planktonic organisms, which are a continuing contribution of chitin seston in the BBE, and (3) the potential contribution of chitin by fungi and certain bacterial structures that develop component biodegradation processes during some periods of the year.

Accordingly, the seasonal marked differences of the concentration of chitin, POC and SPM were highly related to biological community activity, but the similar spatial distribution of these variables apparently depended on hydrodynamic conditions of the BBE. It could be pointed out some distinctions on the spatial distribution of nutrients, especially phosphate, as a consequence of sewage in particularly in CV.

From a biogeochemical standpoint, chitin played an important role as a source of carbon and nitrogen in marine systems. Therefore, the identification of its sources and its dynamics (processes of production and degradation) is relevant to clarify its function in biogeochemical cycles in the BBE.

Acknowledgements This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 467 2011-2014 and PICT

910 2011-2013). We are grateful to IADO boat staff Enio Redondo and Alberto Conte for their technical support and help during sampling.

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