Spatial variation in life-history traits of *Oithona* spp. in a shallow temperate estuarine system (Río de la Plata, south-west Atlantic) during spring

G. D. Cepeda[A,B,D], *R. P. Di Mauro*[C], *M. C. Hozbor*[B], *D. Cucchi Colleoni*[B], *D. Hernández*[B] *and M. D. Viñas*[A,B]

[A]Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Funes 3350, B7602AYL Mar del Plata, Argentina.

[B]Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo No. 1, B7602HSA Mar del Plata, Argentina.

[C]Department of Oceanography, School of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70808, USA.

[D]Corresponding autor. Email: gcepeda@inidep.edu.ar

Abstract. Oithonid species are key components of estuarine zooplankton communities. The spatial distribution of *Oithona nana* and *O. helgolandica* (syn. *O. similis*) and their population dynamics were studied for the first time in a shallow temperate estuarine system in the south-west Atlantic Ocean. Here we estimated the influence of physical (salinity and temperature) and trophic (chlorophyll-*a* and bacterioplankton concentrations) factors on the life-history traits of *O. nana* and *O. helgolandica*. The abundance and biomass of *O. nana* were higher at the surface salinity front but were not correlated with higher egg production rates, which suggests the presence of some retention mechanism that favours population aggregation. In a spatial context, the high reproductive activity near the La Plata River runoff in comparison with the coastal system nearby, suggests that this system might favour the proliferation of *O. nana* populations. However, smaller females with fairly few and smaller eggs per sac occurred under estuarine influence, which implies that there is a potential ecological advantage. The extremely low abundance and reproductive activity of *O. helgolandica* at the Río de la Plata system indicates that this system might not constitute the best habitat for this cyclopoid.

Additional keywords: frontal system, *Oithona helgolandica*, *Oithona nana*, *Oithona similis*, small copepods.

Introduction

The key role of copepods in the marine pelagic ecosystem is well known (Mauchline 1998; Uye *et al.* 2000). Nevertheless, interest in the small species (less than 1 mm) has been evident only recently in view of their role in the marine food webs and their important contribution to zooplankton biomass and production (Turner 2004, and references therein). Among small copepods, cyclopoids such as species of the genus *Oithona* are key components in zooplankton communities, where they act as a link between bacterioplankton and larger zooplankton and fish larvae (Sabatini and Kiørboe 1994; Turner 2004). It is therefore critical to understand the response of *Oithona* spp. to environmental fluctuations, because variability in its reproductive traits could act as a trigger for bottom-up interactions. An example of such is that modifications in their secondary production would affect the availability of planktonic food for fish larvae (Nielsen and Sabatini 1996), subsequently determining the recruitment success of important fish populations (Cushing 1990).

Oithonid populations are typically dominant in temperate and tropical estuarine environments (Hopcroft *et al.* 1998; Vieira *et al.* 2003; Williams and Muxagata 2006; Miyashita *et al.* 2009) with life-history traits and secondary production depending on physical and/or nutritional conditions (e.g. Williams and Muxagata 2006; Miyashita *et al.* 2009; Santhanam and Perumal 2012).

The La Plata River (south-west Atlantic Ocean, 35°S, 56°W) forms one of the most important estuarine areas in the world, sustaining valuable artisanal and coastal fisheries of two countries, Argentina and Uruguay (Acha *et al.* 2008, and references therein). Although copepods historically constitute the main zooplankton group inhabiting this environment (Ramírez 1970; Mianzan *et al.* 2001), the numerical dominance of *Oithona* spp. has been highlighted only recently (Di Mauro *et al.* 2009; Cepeda *et al.* 2012). In addition, *Oithona* spp. provide a significant contribution to the diet of larvae and adults of *Engraulis anchoita* (Padovani *et al.* 2011; Sato *et al.* 2011). Moreover, a faster growth rate of first feeding larvae of *E. anchoita* during

spring (Leonarduzzi *et al.* 2010; Viñas *et al.* 2013) has been associated with a higher proportion of cyclopoids in the zooplankton community, which suggests that *Oithona* spp. may be important in the functioning of this ecosystem. Nevertheless, knowledge of small copepods in the La Plata estuary is still scarce. In fact, neither the spatial distribution nor the demographic and reproductive traits have been studied along the estuarine salinity gradient. In order to contribute to an assessment of the ecological role of *Oithona* spp. during the massive fish spawning season, we analysed the spatial distribution of the biomass, reproductive traits and production along the La Plata estuary. To establish comparisons between sites the same analysis was performed in a non-estuarine zone.

Materials and methods

Study area

The study site covered the inner region (<50 m) of the Northern Argentine Continental Shelf, between 34 and 39°S (Fig. 1). The major hydrographic feature is the presence of the La Plata River, which constitutes the second largest watershed in South America (average discharge: $22\,000\ \mathrm{m^3\,s^{-1}}$: Framiñan and Brown 1996). This river generates a large area of Low Salinity Coastal Waters (LSCW, 0–33.3) giving rise to a broad eutrophic estuarine environment, which undergoes large seasonal fluctuations, depending on the prevailing winds (Simionato *et al.* 2004; Piola *et al.* 2005). Mid-continental Shelf Waters (MSW, 33.4–33.7) extend onto the Northern Argentine Continental Shelf from the south-west and occupies the central portion of the shelf until, at its northernmost limit, the surface mixes with the estuarine waters defining a surface salinity front (Acha *et al.* 2008).

The La Plata estuarine system is characterised by a high primary productivity (reaching up to $15.5\ \mathrm{mg\ m^{-3}}$), driven mainly by nutrient input from the river and by the high vertical stability of the water column, thereby creating a nursery for several species of coastal fishes (Acha *et al.* 2008; Carreto *et al.*

Fig. 1. Map of the study area, with the location of the oceanographic (black circles) and the zooplankton sampling stations (numbered black circles) during spring of 2002.

2008). In contrast, in the coastal system just outside the estuarine influence (S3, Fig. 1), nutrient concentrations, phytoplankton density and chlorophyll-*a* concentrations are low (<2 mg m^{-3}) throughout the year (Carreto *et al.* 1995).

Field sampling

The cruise was carried out during the spring (October–November) of 2002 as part of the Small Pelagic Fisheries Project of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Temperature and salinity profiles were obtained at ~60 stations with a conductivity–temperature–depth system (CTD) with a SBE 19 Seacat CTD profiler (Sea-Bird Electronics Inc., Bellevue, WA, USA) (Fig. 1).

Biological samples (13) were obtained along three sections (S1–S3) distributed from 34 to 39°S (Fig. 1). These sections were established according to the hydrographical conditions. Thus, S1 and S2 were located within the La Plata River plume; whereas S3 was located in an area influenced mainly by Middle Shelf Waters (MSW; 33.4–33.6) with a sporadic influence of the La Plata River waters (Lucas *et al.* 2005; Martos *et al.* 2005).

Chlorophyll-*a* and bacterioplankton estimations

Samples for chlorophyll-*a* (Chl-*a*) and bacterioplankton (Bt) analysis were collected using Niskin bottles. For Chl-*a* analyses, we used the fluorometric method according to Lutz *et al.* (2010). Immediately after collection, seawater samples were filtered onto Millipore APFF (Biopore S.R.L. Buenos Aires, Argentina) glass fibre filters. These filters were kept in liquid nitrogen (−196°C) until analysis.

For estimating Bt abundance, water samples (50 mL) were fixed with 2% formaldehyde at 4°C in the dark and processed on board within 3 h after sampling. Subsamples (2 mL) were dual stained with a combination of DAPI (4′,6-diamidino-2-phenyl-indole, Sigma–Aldrich S.A., Buenos Aires, Argentina) to a final concentration of 5 µg mL^{-1} and acridine orange (Sigma–Aldrich S.A.) to a final concentration of 1 mg mL^{-1} for counter staining (Kuwae and Hosokama 1999). Stained samples were filtered through polycarbonate black filters (0.22-µm pore size, Millipore, Biopore S.R.L.). Bacteria that were retained on the filters were examined under an Olympus IX 70 epifluorescence microscope (UV excitation) (Olympus America Inc., Bio Ana-lítica Argentina S.A., Buenos Aires, Argentina). On each filter, no less than 200 clear-edged cells in 20 microscopic fields were counted and the total concentration was expressed as number of cells per cubic metre (cells m^{-3}).

Zooplankton estimations

Samples were obtained by oblique tows (towing time: 2 min; towing rate: 20 m min^{-1}) using a small Bongo net (18-cm mouth; 67-µm mesh size) equipped with a digital flowmeter at its mouth. The samples were preserved in 4% formaldehyde–seawater solution.

Oithona taxonomic composition was determined under a compound microscope (LEICA DMIL, Leitz Wetzler Germany, Bio Optics S.R.L., Buenos Aires, Argentina). From 900 mL of each sample, a subsample of 10 mL was obtained. The number of subsamples analysed was adjusted in order to include at least 140 individuals of each species. Individuals were identified to
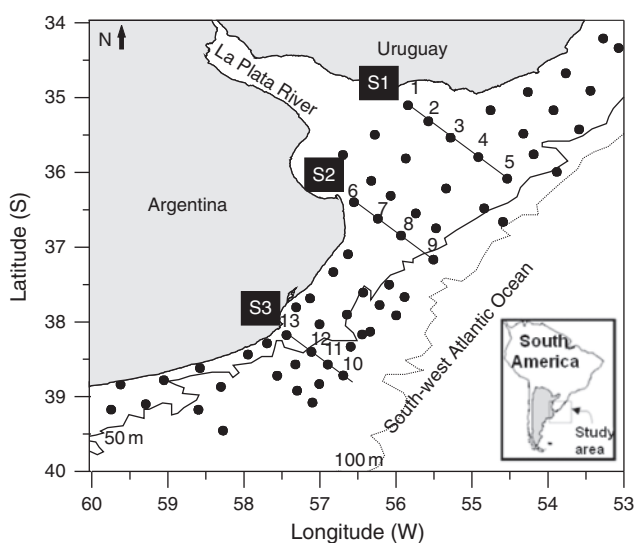
copepodite stages (C1–C5), adults (females and males) and eggs sacs. Nauplii were not considered due to the difficulty of identifying them to species. The abundance of all stages (individuals m^{-3}) was estimated from the small Bongo zooplankton counts and the filtered volumes.

From each sample, prosome length (PL) of 30 individuals of each stage (C1–C5, females and males) was measured under the compound microscope at $20\times$ and $40\times$ magnifications. For *O. nana*, the individual weight was expressed as ash-free dry-weight of each stage using the length–weight regression established by Hopcroft *et al.* (1998). Ash-free dry-weight was then converted to dry weight (DW), assuming an ash content of 6.4% (Chisholm and Roff 1990) and DW to carbon weight (C), assuming $C = 0.4$ DW (Postel *et al.* 2000). For *O. helgolandica* (syn. *O. similis*), C was estimated from the length–weight relationship suggested by Sabatini and Kiørboe (1994). In both cases, C was then multiplied by the abundance to obtain biomass.

For both species, 30 egg sacs were dissected from each sample with fine needles and the clutch size (CS, number of eggs per sac) as well as the egg diameter (D, μm) were established. The egg carbon content was estimated from the egg diameter–egg carbon content relationship of Uye and Sano (1995).

Egg production rate (EPR, eggs female^{-1} day^{-1}) for *O. nana* was calculated using the egg-to-female ratio (E/F) estimated from the preserved samples and the embryonic time (ET, days).

$$EPR = (E/F)(1/ET)$$

ET was estimated from the equation reported by Temperoni *et al.* (2011) by using the mean temperature of the water column (T, °C):

$$ET = 4.694(T - 8)^{-0.462}$$

The specific-EPR (SEPR, day^{-1}) was then calculated as the product of EPR and the ratio of the egg and female carbon weight.

EPR for *O. helgolandica* was also calculated using the E/F ratio and the egg hatching rate (HR, % day^{-1}), because no ET was available for this species.

$$EPR = (E/F)\,HR/100$$

HR was estimated using the mean temperature from the linear equation reported by Nielsen *et al.* (2002).

$$HR = 4.217 + 1.754T$$

Secondary production of both species was then calculated by multiplying biomass by SEPR, assuming that SEPR of adult females is representative of juvenile's growth rates (Berggreen *et al.* 1988) and that adult growth is represented as egg production.

Data analysis

Temperature (T) and salinity (S) were averaged before statistical analysis, as the net tows were oblique. The surface salinity gradient (SSg, ΔS 10 km^{-1}) was calculated using the gradient operator routine available in SURFER software (Golden Software Inc., Golden, CO, USA)., as an indicator of the location of the surface salinity front. Prior to any statistical analysis, potential correlation among the environmental variables (T, S,

depth, SSg, Bt and Chl-*a* concentrations), as well as the reproductive rates (EPR, SEPR and SP) were tested.

The spatial distribution of *Oithona* was analysed in relation to the presence of the water masses (LSCW and MSW). A General Linear Model (GLM) (McCullagh and Nelder 1989) was applied to establish the relationship among the abundance and biomass of stages of different *Oithona* species and the environmental variables. To stabilise the variance of the data, a $\log(x+1)$ transformation was applied to abundance and biomass of *Oithona* spp. The Pearson's product moment correlation coefficient was used to measure the intensity of the association between EPR and the abiotic variables. A nested-ANOVA was applied to determine variation in mean CS and D between and along sections. The Tukey HSD test was applied *a posteriori* to our ANOVA results (Zar 1999). Statistica package ver. 7.0 was used to perform the statistical analysis.

Results

Environmental parameters

Surface temperature ranged between 11 and 19°C, with the highest records occurring at the inner stations of S1 and S2, whereas the lowest ones were near the 50-m isobath (Fig. 2*a*).

Surface salinity values ranged between 9 and 33.6, which indicated the coexistence of Low Salinity Coastal Waters (LSCW) and Middle Shelf Waters (MSW) (Fig. 2*b*). LSCW were widely distributed throughout the study area up to the 50-m isobath, where MSW occurred. The river drained towards the north-east, and the surface salinity front was detected at Stations 4, 7 and 8 (Figs 1, 2*b*).

Surface Chl-*a* concentration varied widely throughout the study area. The highest Chl-*a* value concentration (14 mg m^{-3}) occurred at the inner stations of S1, whereas throughout the study area Chl-*a* concentrations ranged between 0.8 and 3 mg m^{-3} (Fig. 2*c*). Higher Bt concentrations also occurred at the inner S1 station (2×10^6 cells m^{-3}) and at the deepest S2 (1×10^6 cells m^{-3}) and S3 ones (3×10^6 cells m^{-3}) (Fig. 2*c*).

Distribution, abundance, biomass and stage composition of Oithona *spp.*

O. nana and *O. helgolandica* were the only two *Oithona* species found in this study, and their abundances varied spatially. *O. nana* was mainly distributed in LSCW (Stations 1–8, 13–12) whereas *O. helgolandica* was restricted to stations with salinity values >30 (Stations 9–11) (Fig. 3).

The abundance and biomass of all oithonid stages as well as their relative composition is shown in Fig. 3. The lowest *O. nana* abundance (<1000 individuals m^{-3}; 0.06 mg C m^{-3}) was found at the deepest offshore S3 stations (11 and 10). A relative increase (between 11 000 and 17 000 individuals m^{-3}; 0.8–0.95 mg C m^{-3}) occurred at the frontal stations (4, 7 and 8), whereas at the other coastal stations (1–3, 5–6, 13–12), intermediate records (between 3000 and 8000 individuals m^{-3}; 0.3–0.5 mg C m^{-3}) were found (Fig. 3*a*, *b*). Overall, the proportion of eggs (50–70%) and copepodite stages (15–50%) of *O. nana* was higher than that of the adults, which accounted for less than 15% of the total abundance throughout the study area. All the copepodite stages were present at nearly every station, with the exception of the deepest ones, where only some stages were found (Fig. 3*c*).
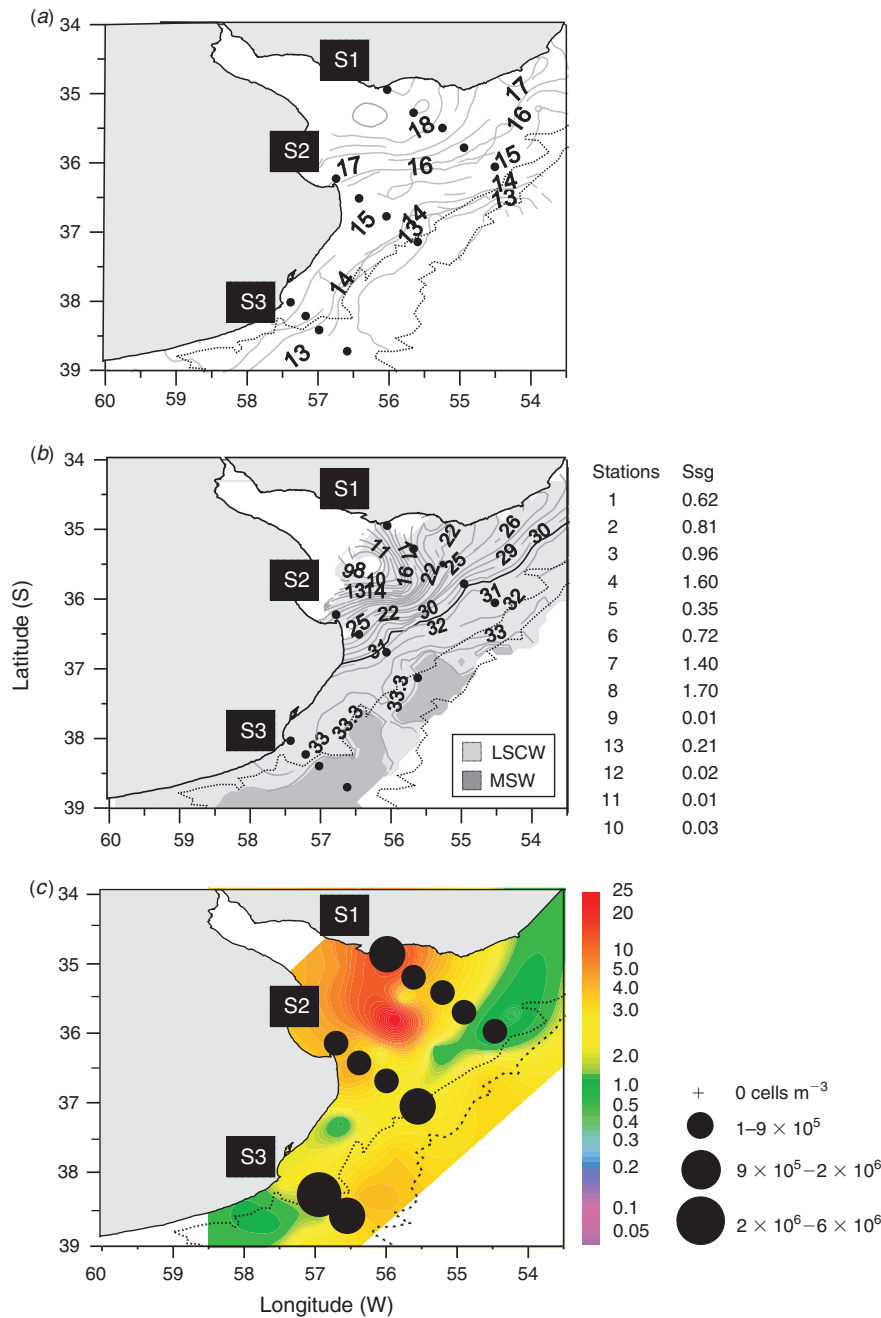
Fig. 2. Spatial distribution of surface temperature (*a*), salinity (*b*), and chlorophyll-*a* and bacterio-plankton concentration (*c*), during spring of 2002 in the La Plata estuary. SSg, surface salinity gradient; LSCW, Low Salinity Coastal Waters; MSW, Middle Shelf Waters; solid line, 30 isohaline. Please see stations numbers in Fig. 1.

Total abundance and biomass of *O. helgolandica* were lower than the values observed for *O. nana* and exhibited an inverse pattern to that of *O. nana*. The abundance of *O. helgolandica* increased (<1000 individuals m^{-3}; 0.1–0.2 mg C m^{-3}) from the coastal S3 stations (13 and 12) to the offshore stations (3000 individuals m^{-3}; 0.5–0.6 mg C m^{-3}) (Fig. 3*a, b*). *O. helgolandica* stages also increased in number towards the offshore stations. At Stations 9 and 10 all stages occurred, with

copepodite stages being the most abundant (55%), whereas eggs and adults both made up 20–25% (Fig. 3*c*).

Length, reproductive traits and production of Oithona *spp. females*

O. nana females were smaller at Station 5 (328.9 μm ± 29.3 μm) and larger at Station 13 (364.2 μm ± 11.3 μm). Along S1–S2 females were smaller than along S3 ($F_{2,177} = 43$, $P < 0.0001$)
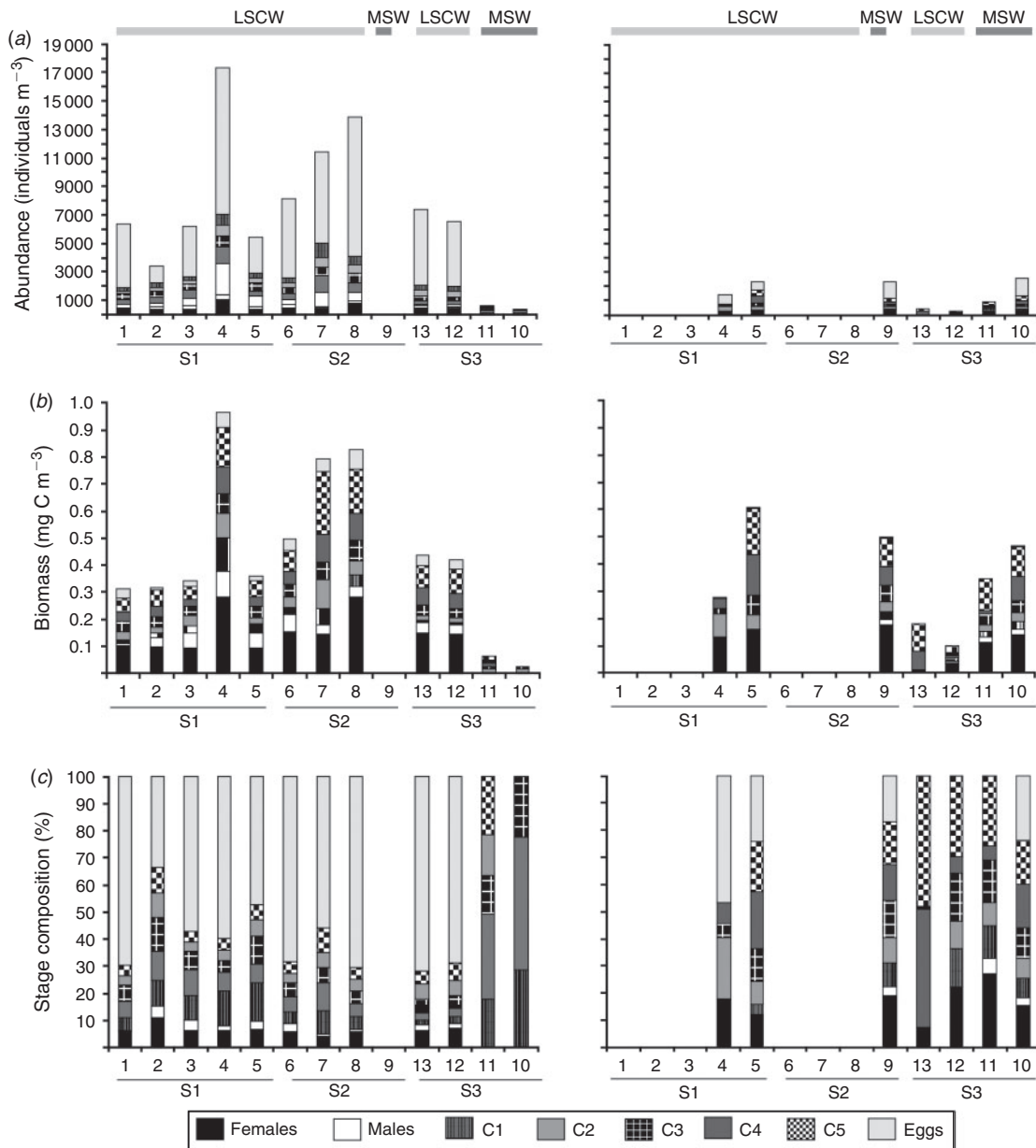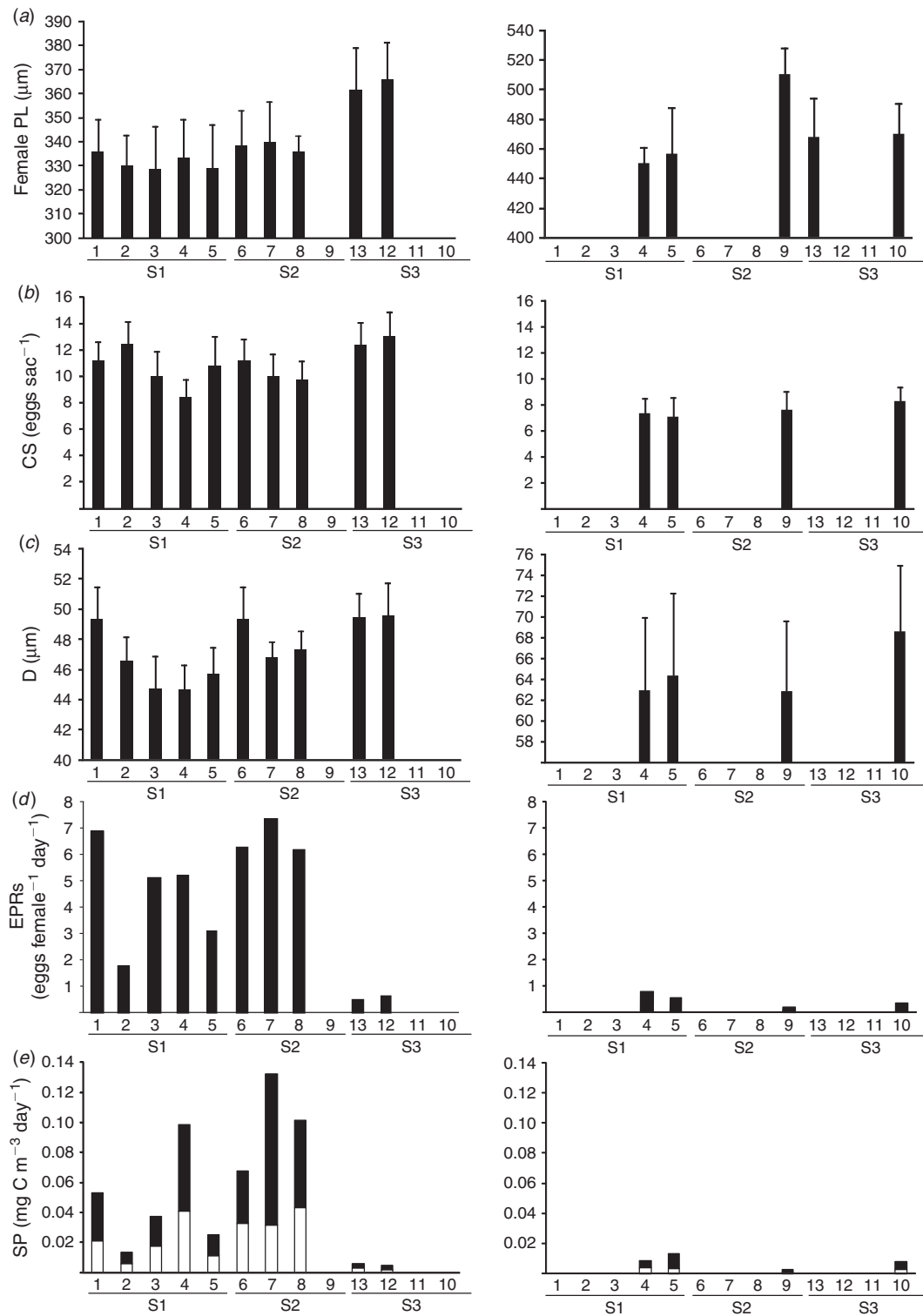
Fig. 3. *Oithona nana* (left column) and *O. helgolandica* (right column) in the La Plata estuary during spring 2002. Spatial variation of abundance (*a*), biomass (*b*) and stage composition (*c*) along the three sections (S1–S3) are shown. LSCW, Low Salinity Coastal Waters; MSW, Middle Shelf Waters. Blank spaces indicate no occurrence.

and no significant differences occurred within the sections ($F_{7,177} = 1$, $P = 0.40$) (Fig. 4*a*). Mean *O. nana* CS varied between 8.48 (± 1.3) at 4 and 12.43 (± 1.77) at Station 2 (Fig. 4*b*). CS was smaller along S1–S2 than along S3 (ANOVA, $F_{2,285} = 3.26$, $P < 0.05$). CS was significantly different among all S1 stations (ANOVA, $F_{7,285} = 19$, $P < 0.0001$) (Fig. 4*b*). Mean D varied between 44.6 μm (± 1.6 μm) at Station 4 and 49.8 μm (± 1.6 μm) at Station 8 (Fig. 4*c*). Mean D was significantly different both among ($F_{2,285} = 109$, $P < 0.0001$) and along ($F_{7,285} = 19$, $P < 0.00001$) the sections. In general, eggs were smaller along S1–S2 than along S3.

Reproductive activity of *O. nana* was more intense in the estuarine sections S1 and S2. EPR and SEPR varied from a minimum of less than 1 egg female^{-1} day^{-1} (0.01 day^{-1}) at S3 coastal stations (13, 12) to a maximum of 7.4 eggs female^{-1} day^{-1} (0.2 day^{-1}) at a frontal one (7) (Fig. 4*d*). Estimated secondary production followed the same biomass pattern. Secondary production of *O. nana* ranged between a minimum of 0.005 mg C m^{-3} day^{-1} at the coastal stations and maxima of 0.1–0.13 mg C m^{-3} day^{-1} at the frontal stations (Fig. 4*e*).

Females of *O. helgolandica* were smaller at Station 5 (450.23 μm ± 10.66 μm), but larger at Station 9 (510.68 μm ± 17.20 μm) (Fig. 4*a*). Mean CS varied between 7.1 (± 1.47) at Station 5 and 8.31 (± 1.07) at Station 10, and no significant differences were observed among stations (ANOVA, $F_{3,90} = 1.58$, $P = 0.19$) (Fig. 4*b*). Mean D varied between 66.41 μm

Fig. 4. *Oithona nana* (left column) and *O. helgolandica* (right column) in the La Plata estuary during spring 2002. Spatial variation of female prosome length (PL) (*a*), clutch size (CS) (*b*), eggs diameter (D) (*c*), egg production rates (EPRs) (*d*) and secondary production (SP) (*e*) along the three sections (S1–S3) are shown. Black bars, females; white bars, copepodites. Blank spaces indicate no occurrence.

Table 1. General Lineal Model analysis between (log+1)-transformed abundances of developmental stages of *Oithona* (individuals m^{-3})/biomasses (mg C m^{-3}) and bacterioplankton (Bt), chlorophyll-*a* (Chl-*a*), mean salinity (S) and surface salinity gradient (SSg)

GLM model: $\log(\text{stage abundance/biomass} + 1) = \mu + \beta_1 Bt + \beta_2 Chl\text{-}a + \beta_3 S + \beta_4 SSg$. Significance levels: **, $P < 0.01$; *, $P < 0.05$. F, female; M, male; C1–C5, copepodites 1–5; E, eggs

Species	Model	d.f.	β_1	β_2	β_3	β_4	F	R^2	P
O. nana	F	4	−0.41*/−0.40*	−0.05/−0.04	−0.35/−0.31	0.63*/0.66**	18.92/17.64	0.92/0.92	0.001/0.001
	M	4	−0.26/−0.24	−0.51/−0.52	−0.69/−0.69	0.51/0.50*	5.22/5.16	0.77/0.77	0.03/0.03
	C$_1$	4	−0.11/−0.11	−0.25/−0.25	−0.32/−0.31	0.83**/0.83**	13.87/13.30	0.90/0.89	0.003/0.004
	C$_2$	4	−0.17/−0.17	−0.34/−0.33	−0.50/−0.50	0.61*/0.61*	3.52/3.38	0.70/0.69	0.08/0.08
	C$_3$	4	−0.52*/−0.52*	−0.06/−0.05	−0.42/−0.41	0.50*/0.50*	18.85/18.72	0.92/0.92	0.001/0.001
	C$_4$	4	−0.45*/−0.44*	0.01/0.01	−0.20/−0.18	0.58*/0.58*	7.74/7.61	0.83/0.83	0.01/0.01
	C$_5$	4	–	–	–	–	2.78/2.54	0.65/0.62	0.12/0.14
	E	4	−0.27/−0.27	−0.27/−0.26	−0.47/−0.45	0.59/0.59*	4.76/4.66	0.76/0.75	0.04/0.04
O. helgolandica	F	4	–	–	–	–	2.74/2.28	0.64/0.60	0.12/0.17
	M	4	0.13**/0.36	0.06/0.05	0.15/0.81	−0.13/−0.35*	28.26/70.16	0.94/0.98	0.0004/0.0005
	C$_1$	4	0.28/0.13	−0.08/−0.06	0.74*/0.90**	−0.43*/−0.54**	9.50/28.93	0.86/0.95	0.009/0.001
	C$_2$	4	–	–	–	–	2.06/1.29	0.57/0.47	0.2/0.37
	C$_3$	4	–/−0.52*	–/−0.05	–/−0.41*	–/0.50*	2.79/18.72	0.65/0.92	0.12/0.001
	C$_4$	4	–	–	–	–	1.93/1.48	0.56/0.49	0.22/0.31
	C$_5$	4	0.23/–	−0.11/–	0.71*/–	−0.45*/–	6.67/3.36	0.81/0.79	0.02/0.1
	E	4	–/−0.27	–/−0.26	–/−0.45	–/0.59*	0.36/0.75	0.86/4.66	0.53/0.04

(±7.9) at Station 5 and 70.67 μm (±6.35) at Station 10. Significant differences in mean D were observed among stations (ANOVA, $F_{3,741} = 6.2$, $P < 0.005$). Eggs were smaller at Station 4 than at Station 10 (Fig. 4*c*). Practically no *O. helgolandica* EPR and SEPR were registered (0–0.8 eggs sac^{-1} female^{-1}; 0.006–0.03 day^{-1}) at any station (Fig. 4*d*). Also, secondary production was low (0.003 and 0.014 mg C m^{-3} day^{-1} at the offshore Stations 9 and 5 respectively) (Fig. 4*e*).

Relationships between demographic and reproductive traits of Oithona *spp. and environmental variables during spring 2002*

Some of the environmental variables were strongly correlated with each other so, in order to avoid collinerity, we included only S, SSg, Chl-*a* and Bt concentrations in the analyses.

The abundance and biomass of all *O. nana* stages (with the exception of C5) were positively related to SSg, which explained more than 50% of the total variation of each stage. Females, C3 and C4 abundance and biomass were also negatively related to Bt (Table 1). Mean PL of *O. nana* females was positively related with S ($r = 0.60$, $P < 0.001$, $n = 10$) and no relationship existed with Chl-*a* ($r = 0.3$, $P = 0.60$, $n = 10$) nor with Bt ($r = 0.2$, $P = 0.50$, $n = 10$). Mean CS weakly negatively correlated with S ($r = -0.23$, $P < 0.0001$, $n = 295$) and positively with female PL ($r = 0.68$, $P < 0.05$, $n = 10$). Mean D was positively correlated with female PL ($r = 0.82$, $P < 0.0001$, $n = 10$), whereas there was no significant relationship with S ($r = 0.004$, $P = 0.93$, $n = 295$), Chl-*a* ($r = 0.002$, $P = 0.80$, $n = 295$) and Bt ($r = 0.001$, $P = 0.70$, $n = 295$). EPR of *O. nana* significantly increased when S decreased ($r = -0.64$, $P < 0.05$, $n = 10$).

The abundance and biomass of all stages of *O. helgolandica* presented no clear relationship with the environmental variables. Only the abundance and biomass of CI increased with both the S increase and the SSg decrease (Table 1). PL of *O. helgolandica* females was positively related with S ($r = 0.24$,

$P < 0.001$, $n = 5$). On the other hand, mean D was not significaticantly correlated with S ($r = -0.068$, $P = 0.85$, $n = 7$). No pattern or correlation for *O. helgolandica* was detected between EPR and the environmental variables.

Discussion

This paper reports for the first time data about the distribution, demography and secondary production of *Oithona* species in the La Plata estuarine system, a highly productive environment within the south-west Atlantic Ocean.

O. nana was broadly distributed in the study system in the low salinity waters (LSCW, 9–33.3), which clearly dominated throughout the area. This agrees with previous results reported for this species in other estuarine systems (Vieira *et al.* 2003; Williams and Muxagata 2006; Miyashita *et al.* 2009). In contrast, *O. helgolandica* had a more restricted distribution due to the scant occurrence of saltier waters (MSW, 33.4–33.6) in the study area. Nevertheless, it is clear that the distribution of *O. helgolandica* is related to the halophilic character attributed to this species (Hansen *et al.* 2004; Castellani *et al.* 2007; Elliott and Kaufmann 2007). In addition, our results are also supported by previous observations performed in the area during the spring of 1995 when, under the effects of 'La Niña', high-salinity waters (>30) broadly dominated in the area due to local wind forcing (Guerrero *et al.* 1997). This resulted in the widespread prevalence of *O. helgolandica* over *O. nana* (Viñas *et al.* 2002) and highlighted the important role that the hydrodynamics of this frontal system plays upon these small copepods.

An increase of biomass of *O. nana* of up to two-fold occurred at the surface salinity front, as has been reported for other zooplankters (Mianzan *et al.* 2001; Viñas *et al.* 2002; Marrari *et al.* 2004). Similarly, high biomass of *Oithona* spp. has been reported in other coastal or oceanic fronts, which is closely associated with high food availability (Nielsen and Sabatini

1996; Castellani *et al.* 2007; Zervoudaki *et al.* 2007). Although it is well accepted that high primary production typically occurs at estuarine fronts (Largier 1993, and references therein), our results indicated that neither chlorophyll-*a* nor bacteria concentration increased at the surface salinity front. In fact, lower phytoplankton biomass typically occurs in this front because nitrate is mostly consumed inside the estuary (Carreto *et al.* 2007, and references therein). In general, egg production rate is a much more sensitive indicator of oceanographic-mediated changes in copepod feeding conditions than biomass (Tester and Turner 1990). In this regard, although the biomass of *O. nana* increased at the surface salinity front, EPR was not particularly high at the surface salinity front. Therefore, our results suggest that the observed increase in the biomass of *O. nana* at the surface salinity front could potentially be the product of passive convergent transport towards the front (Mann and Lazier 2006) rather than a high local productivity precisely there.

In spite of these findings, our estimates of EPR for *O. nana* were higher under the direct influence of the La Plata River plume (2–7 eggs female^{-1} day^{-1} along S1–S2) than outside of it (less than 1 egg female^{-1} day^{-1} at S3). These values and those of SEPR agree with *in situ* reports for *O. davisae* (Uye and Sano 1995; Zamora-Terol and Saiz 2013), which show 5.6 eggs female^{-1} day^{-1} under food-satiated conditions. The lack of correlation between EPR and chlorophyll-*a* and bacteria concentrations may indicate therefore that *O. nana* was not food-limited at the La Plata estuarine system. In fact, although phytoplankton can make up a considerable fraction of the diet of *Oithona* spp., it is well known that these cyclopoids prefer motile prey, feeding preferentially on ciliates rather than diatoms (Atkinson 1996). Unfortunately, no data on protozooplankton were available to corroborate our findings and we strongly suggest further studies on the distribution, abundance and biomass of this microplankton fraction given the important trophodynamic role of these organisms in the study area. In this context, the La Plata estuary appears to be a more suitable environment for the proliferation of *O. nana* populations, similar to other highly eutrophic systems (Jamet *et al.* 2001, 2005; Williams and Muxagata 2006; Miyashita *et al.* 2009) rather than the MSW system surrounding it.

Significant spatial variations in female size were observed. The presence of smaller females under estuarine conditions agreed with recent experimental reports on *O. rigida*, which demonstrated that low salinity causes slower development times mainly because of osmoregulation costs, which reduce the final size of the copepods (Santhanam and Perumal 2012). A reduction in copepod size in estuarine systems seems to be restricted to neritic species that penetrate estuaries (Uriarte and Villate 2006), as is seen in *O. nana* in the La Plata estuary.

The occurrence of smaller clutch sizes and eggs, associated with smaller females, concur with experimental reports regarding other *Oithona* species (Sabatini and Kiørboe 1994; Castellani *et al.* 2007; Zamora-Terol and Saiz 2013). Our results, however, conflict with other *in situ* studies, which report larger clutches typically occurring under favourable food conditions (Uye and Sano 1995; Castellani *et al.* 2005). In this sense, further experimental studies focussed on the biological mechanisms that control the balance between size reduction and egg production rate under stress conditions (such as low salinity)

in *Oithona*, as well as the individual effect of each variable (i.e. temperature, salinity, food quality), are necessary. Nevertheless, the general size reduction occurring at the La Plata estuarine system would probably represent an ecological advantage for this cyclopoid, potentially contributing to reduced predation risk by fish (Berasategui *et al.* 2004; Acha *et al.* 2008).

The present investigation represents an advance in the study of the life history of small copepods in one of the largest shallow temperate estuary systems within the south-west Atlantic Ocean. We provide valuable information to better understand the life strategies of *Oithona* species and the capacity of the species to cope with environmental variability. We have highlighted the effects of the estuarine influence on reproductive traits of *O. nana*, which contribute towards this species' fitness. Further studies aimed at generating estimates of production rates of other dominant copepod species are need in order (1) to fully understand the role and relative importance of these cyclopoids in the estuarine planktonic community, and (2) to assess the role of the Northern Argentine Continental Shelf as a nutrient and biotic source for the continental shelf of eastern South America.

Acknowledgements

References

Acha, E. M., Mianzan, H. W., Guerrero, R., Carreto, J. I., Giberto, D., Montoya, N., and Carignan, M. (2008). An overview of physical and ecological processes in the Río de la Plata estuary. *Continental Shelf Research* **28**, 1579–1588. doi:10.1016/J.CSR.2007.01.031

Atkinson, A. (1996). Subantartic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. *Marine Ecology Progress Series* **130**, 85–96. doi:10.3354/MEPS130085

Berasategui, A. D., Acha, E. M., and Fernández Araoz, N. C. (2004). Spatial patterns of ichthyoplankton assemblages in the Rio de la Plata estuary (Argentina, Uruguay). *Estuarine, Coastal and Shelf Science* **60**, 599–610. doi:10.1016/J.ECSS.2004.02.015

Berggreen, U., Hansen, B., and Kiørboe, T. (1988). Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Marine Biology* **99**, 341–352. doi:10.1007/BF02112126

Carreto, J. I., Lutz, V., Carignan, M. O., Cucchi Colleoni, A. D., and De Marco, S. (1995). Hydrography and chlorophyll *a* in a transect from the coast to the shelf-break in the Argentinean Sea. *Continental Shelf Research* **15**, 315–336. doi:10.1016/0278-4343(94)E0001-3

Carreto, J. I., Carignan, M., Montoya, N., and Cucchi Colleoni, A. (2007). Ecología del fitoplancton en los sistemas frontales del Mar Argentino. *El Mar Argentino y sus recursos pesqueros* **5**, 11–31.

Carreto, J. I., Montoya, N. G., Akselman, R., Carignan, M. O., Silva, R. I., and Cucchi Colleoni, A. (2008). Algal pigment patterns and phytoplankton assemblages in different water masses of the Río de la Plata maritime front. *Continental Shelf Research* **28**, 1589–1606. doi:10.1016/J.CSR.2007.02.012

Castellani, C., Robinson, C., Smith, T., and Lampitt, R. S. (2005). Temperature affects respiration rate of *Oithona similis*. *Marine Ecology Progress Series* **285**, 129–135. doi:10.3354/MEPS285129

Castellani, C., Irigoyen, X., Harris, R., and Holliday, N. (2007). Regional and temporal variation of *Oithona* spp. biomass, stage structure and productivity in the Irminger Sea, North Atlantic. *Journal of Plankton Research* **29**, 1051–1070. doi:10.1093/PLANKT/FBM079

Cepeda, G. D., Di Mauro, R. P., Martos, P., and Viñas, M. D. (2012). A section-sampling design to assess zooplankton distribution in a highly complex hydrographic region (SW Atlantic Ocean, 34–41°S): insights for a new time series? *Brazilian Journal of Oceanography* **60**, 381–390. doi:10.1590/S1679-87592012000300010

Chisholm, L. A., and Roff, J. C. (1990). Size–weight relationships and biomass of tropical neritic copepods off Kingston, Jamaica. *Marine Biology* **106**, 71–77. doi:10.1007/BF02114676

Cushing, D. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology* **26**, 249–293. doi:10.1016/S0065-2881(08)60202-3

Di Mauro, R. P., Capitanio, F. L., and Viñas, M. D. (2009). Capture efficiency for small dominant mesozooplankters (Copepoda, Appendicularia) off Buenos Aires Province (34°S–41°S), Argentine Sea, using two plankton mesh sizes. *Brazilian Journal of Oceanography* **57**, 205–214. doi:10.1590/S1679-87592009000300004

Elliott, D. T., and Kaufmann, R. S. (2007). Spatial and temporal variability of mesozooplankton and tintinnid ciliates in a seasonally hypersaline estuary. *Estuaries and Coasts* **30**, 418–430. doi:10.1007/BF02819388

Framiñan, M., and Brown, O. (1996). Study of the Río de la Plata turbidity front. Part I: Spatial and temporal distribution. *Continental Shelf Research* **16**, 1259–1282. doi:10.1016/0278-4343(95)00071-2

Guerrero, R., Acha, E. M., Framiñan, M., and Lasta, C. (1997). Physical oceanography of the Río de la Plata Estuary, Argentina. *Continental Shelf Research* **17**, 727–742. doi:10.1016/S0278-4343(96)00061-1

Hansen, F. C., Mollmann, C., Schutz, U., and Hinrichsen, H. H. (2004). Spatio-temporal distribution of *Oithona similis* in the Bornholm Basin (central Baltic Sea). *Journal of Plankton Research* **26**, 659–668. doi:10.1093/PLANKT/FBH061

Hopcroft, R. R., Roff, J. C., and Lombard, D. (1998). Production of tropical copepods in Kingston Harbour, Jamaica: the importance of small species. *Marine Biology* **130**, 593–604. doi:10.1007/S002270050281

Jamet, J., Bogé, G., Richard, S., Geneys, C., and Jamet, D. (2001). The zooplankton community in bays of Toulon area (northwest Mediterranean Sea, France). *Hydrobiologia* **457**, 155–165. doi:10.1023/A:1012279417451

Jamet, J., Jean, N., Bogé, G., Richard, S., and Jamet, D. (2005). Plankton succession and assemblage structure in two neighbouring littoral ecosystems in the north-west Mediterranean Sea. *Marine and Freshwater Research* **56**, 69–83. doi:10.1071/MF04102

Kuwae, T., and Hosokama, Y. (1999). Determination of abundance and biovolume of bacteria in sediments by dual staining with 4′,6-diamidino-2-phenylindole and acridine orange: relationship to dispersion treatment and sediment characteristics. *Applied and Environmental Microbiology* **65**, 3407–3412.

Largier, J. L. (1993). Estuarine fronts: how important are they? *Estuaries* **16**, 1–11. doi:10.2307/1352760

Leonarduzzi, E., Brown, D. R., and Sánchez, R. (2010). Seasonal variations in the growth of anchovy larvae (*Engraulis anchoita*) on the Argentine coastal shelf. *Scientia Marina* **74**, 267–274. doi:10.3989/SCIMAR.2010.74N1267

Lucas, A., Guerrero, R., and Mianzan, H. (2005). Coastal oceanographic regimes of the northern argentine continental shelf (34–43°S). *Estuarine, Coastal and Shelf Science* **65**, 405–420. doi:10.1016/J.ECSS.2005.06.015

Lutz, V. A., Segura, V., Dogliotti, A. I., Gagliardini, D. A., Bianchi, A. A., and Balestrini, C. F. (2010). Primary production in the Argentine Sea during spring estimated by field and satellite models. *Journal of Plankton Research* **32**, 181–195. doi:10.1093/PLANKT/FBP117

Mann, K. H., and Lazier, J. R. (2006). 'Dynamics of Marine Ecosystems: Biological–Physical Interactions in the Oceans', 3rd edn. (Blackwell Science Publications: Cambridge, MA, USA.)

Marrari, M., Viñas, M. D., Martos, P., and Hernández, D. (2004). Spatial patterns of mesozooplankton distribution in the southwestern Atlantic Ocean (34°S–41°S) during austral spring: relationship with the hydrographic conditions. *ICES Journal of Marine Science* **61**, 667–679. doi:10.1016/J.ICESJMS.2004.03.025

Martos, P., Hansen, J. E., Negri, R. M., and Madirolas, A. (2005). Factores oceanográficos relacionados con la abundancia relativa de anchoíta sobre la plataforma bonaerense (34–41°S) durante la primavera. *Revista de Investigación y Desarrollo Pesquero* **17**, 5–33.

Mauchline, J. (Ed.) (1998). 'The Biology of Calanoid Copepods. Advances in Marine Biology, Vol. 33.' (Academic Press: San Diego, CA, USA.)

McCullagh, P., and Nelder, J. A. (1989). 'Generalized Linear Models', 2nd edn. (Chapman and Hall: London.)

Mianzan, H. W., Lasta, C., Acha, E. M., Guerrero, R., Macchi, G., and Bremec, C. (2001). The Río de la Plata Estuary, Argentina, Uruguay. In 'Ecological Studies: Coastal Marine Ecosystems of Latin America'. (Eds U. Seeliger, L. de Lacerda, and B. Kjerve.) pp. 185–204. (Springer: Berlin.)

Miyashita, L. K., De Melo Junior, M., and Lopes, R. M. (2009). Estuarine and oceanic influences on copepod abundance and production of a subtropical coastal area. *Journal of Plankton Research* **31**, 815–826. doi:10.1093/PLANKT/FBP039

Nielsen, T. G., and Sabatini, M. E. (1996). Role of cyclopoid copepods *Oithona* spp. in North Sea plankton communities. *Marine Ecology Progress Series* **139**, 79–93. doi:10.3354/MEPS139079

Nielsen, T. G., Møller, E. F., Satapoomin, S., Ringuette, M., and Hopcroft, R. R. (2002). Egg hatching rate of the cyclopoid copepod *Oithona similis* in arctic and temperate waters. *Marine Ecology Progress Series* **236**, 301–306. doi:10.3354/MEPS236301

Padovani, L. N., Viñas, M. D., and Pájaro, M. (2011). Importance of the Rio de la Plata estuarine front (southwest Atlantic Ocean) in the feeding ecology of Argentine anchovy, *Engraulis anchoita* (Cupleiformes, Cupleidae). *Latin American Journal of Aquatic Research* **39**, 205–213. doi:10.3856/VOL39-ISSUE2-FULLTEXT-2

Piola, A. R., Matano, R. P., Palma, E. D., Möller, O. O. Jr, and Campos, E. J. D. (2005). The influence of the Plata River discharge on the western South Atlantic shelf. *Geophysical Research Letters* **32**, 1603–1606. doi:10.1029/2004GL021638

Postel, L., Fock, H., and Hagen, W. (2000). Biomass and abundance. In 'ICES Zooplankton Methodology Manual'. (Eds R. Harris, P. Wiebe, J. Lenz, H. Skjoldal, and M. Huntley.) pp. 83–192. (Academic Press: London.)

Ramírez, F. C. (1970). Copépodos planctónicos del sector patagónico: resultados de la campaña 'Pesquería XI'. Buenos Aires: Asociación Argentina de Ciencias Naturales. *Physis (Rio de Janeiro, Brazil)* **29**, 473–476.

Sabatini, M. E., and Kiørboe, T. (1994). Egg production, growth and development of the cyclopoid copepod *Oithona similis*. *Journal of Plankton Research* **16**, 1329–1351. doi:10.1093/PLANKT/16.10.1329

Santhanam, P., and Perumal, P. (2012). Effect of temperature, salinity and algal food concentration on population density growth and survival of marine copepod *Oithona rigida* Giesbrecht. *Indian Journal of Geo-Marine Sciences* **41**, 369–376.

Sato, N., Hernández, D., and Viñas, M. D. (2011). Hábitos alimentarios de las larvas de *Engraulis anchoita* (Hubbs & Marini, 1935) en las aguas costeras de la Provincia de Buenos Aires, Argentina. *Latin American Journal of Aquatic Research* **39**, 16–24.

Simionato, C. G., Dragani, W. C., Meccia, V. L., and Nuñez, M. (2004). A numerical study of the barotropic circulation of the Rio de la Plata estuary: sensitivity to bathymetry, earth's rotation and low frequency wind variability. *Estuarine, Coastal and Shelf Science* **61**, 261–273. doi:10.1016/J.ECSS.2004.05.005

Temperoni, B., Viñas, M. D., Diovisalvi, N. R., and Negri, R. N. (2011). Seasonal production of *Oithona nana* Giesbrecht, 1893 (Copepoda: Cyclopoida) in temperate coastal waters off Argentina. *Journal of Plankton Research* **33**, 729–740. doi:10.1093/PLANKT/FBQ141

Tester, P., and Turner, J. (1990). How long does it take copepods to make eggs? *Journal of Experimental Marine Biology and Ecology* **141**, 169–182. doi:10.1016/0022-0981(90)90222-X

Turner, J. (2004). The importance of small planktonic copepods and their roles in pelagic marine food webs. *Zoological Studies* **43**, 255–266.

Uriarte, I., and Villate, F. (2006). Spatial variations in size, weight and condition factor of the females of *Acartia clausi* (Copepoda: Calanoida) along a salinity gradient in two contrasting estuaries of of the Basque coast (Bay of Biscay). *Hydrobiologia* **571**, 329–339. doi:10.1007/S10750-006-0258-1

Uye, S., and Sano, K. (1995). Seasonal reproductive biology of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. *Marine Ecology Progress Series* **118**, 121–128. doi:10.3354/MEPS118121

Uye, S., Nagano, N., and Shimazu, T. (2000). Abundance, biomass, production and trophic roles of micro- and net-zooplankton in Ise Bay, central Japan, winter. *Journal of Oceanography* **56**, 389–398. doi:10.1023/A:1011172221257

Vieira, L., Azeiteiro, U., Re, P., Pastorinho, R., Marques, J. C., and Morgado, F. (2003). Zooplankton distribution in a temperate estuary (Mondego estuary southern arm: western Portugal). *Acta Oecologica* **24**, S163–S173. doi:10.1016/S1146-609X(03)00038-9

Viñas, M. D., Negri, R. N., Ramírez, F. C., and Hernández, D. (2002). Zooplancton assemblages and hydrography in the spawning area of anchovy (*Engraulis anchoita*) off Rio de la Plata estuary (Argentina–Uruguay). *Marine and Freshwater Research* **53**, 1031–1043. doi:10.1071/MF00105

Viñas, M. D., Negri, R. M., Cepeda, G. D., Hernández, D., Silva, R., Daponte, M. C., and Capitanio, F. L. (2013). Seasonal succession of zooplankton in coastal waters of the Argentine Sea (southwest Atlantic Ocean): prevalence of classical or microbial food webs. *Marine Biology Research* **9**, 371–382. doi:10.1080/17451000.2012.745003

Williams, J., and Muxagata, E. (2006). The seasonal abundance and production of *Oithona nana* (Copepoda: Cyclopoida) in Southampton Water. *Journal of Plankton Research* **28**, 1055–1065. doi:10.1093/PLANKT/FBL039

Zamora-Terol, S., and Saiz, E. (2013). Effects of food concentration on egg production and feeding rates of the cyclopoid copepod *Oithona davisae*. *Limnology and Oceanography* **58**, 376–387. doi:10.4319/LO.2013.58.1.0376

Zar, J. (1999). 'Biostatistical Analysis', 4th edn. (Prentice-Hall: Upper Saddle River, NJ, USA.)

Zervoudaki, S., Christou, E., Nielsen, T., Siokou-Frangou, I., Assimakopoulou, G., Giannakourou, A., Maar, A., Pagou, K., Krasakopoulou, E., Christaki, U., and Moraitou-Apostolopoulou, M. (2007). The importance of small-sized copepods in a frontal area of the Aegean Sea. *Journal of Plankton Research* **29**, 317–338. doi:10.1093/PLANKT/FBM018