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Egg production and hatching success of *Calanus chilensis* and *Acartia tonsa* in the northern Chile upwelling zone (23°S), Humboldt Current System

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ABSTRACT

Oxygen Minimum Zones (OMZ's) are expanding and intensifying as result of climate change, affecting Eastern Boundary Upwelling Systems. Local effects of vertical movements of OMZ's that result from changes in upwelling intensity could reduce or expand the oxygenated surface layer that most zooplanktonic species inhabit in coastal areas. Using the copepods *Calanus chilensis* and *Acartia tonsa* as model organisms, an experimental test of the impact of different dissolved oxygen (DO) concentrations (between 0.5 and 5 ml L^{-1}) on egg production and hatching success was carried out and compared with field estimations of egg production, female and egg abundance in Mejillones Bay (23°S). Abundance of *C. chilensis* was highly variability and no consistent pattern in egg production and hatching success was found across DO levels, whereas *A. tonsa* egg production had maximum values between 2.6 and 4.7 ml O_2 L^{-1} and hatching success was positively correlated with DO ($r = 0.75$). In the field, temperature was the main factor controlling the dynamics of both species, while Chl-*a* and DO were also correlated with *C. chilensis* and *A. tonsa*, respectively. Principal Component Analysis showed that abundances of both copepods were controlled by temperature, stratification, OMZ depth, and Ekman transport, which together explained more than 70% of the total variance and were the main factors that modulated the populations of *C. chilensis* and *A. tonsa* in the upwelling zone of northern Chile (23°S). The differential responses of *C. chilensis* and *A. tonsa* to changes in DO concentrations associated with vertical movements of the OMZ suggest that *C. chilensis* may be better adapted to hypoxic conditions than *A. tonsa*, however both species are successful and persistent all year-round. We suggest that physiological responses of copepods could be used to evaluate population dynamics affected by the shoaling of OMZ's and the repercussions to trophic food webs of eastern boundary current systems.

1. Introduction

Nowadays, one of the main focuses of study in marine sciences is to understand how climate change affects the chemistry, physics and biology of the ocean. As a result, we known that chemical transformations occur in the ocean, principally acidification, deoxygenation, and expansion of Oxygen Minimum Zones (OMZ's) (Keeling et al., 2010; Pelejero et al., 2010). Physical conditions in the oceans that are affected by climate change include an increase in stratification due to the warming of the ocean surface (Bograd et al., 2008; Richardson, 2008) and favorable intensification of upwelling winds at eastern boundary currents, which cools coastal areas and shoals the low oxygen water of OMZ's (Bakun, 1990; Rykaczewski and Checkley, 2008). The changes in the ocean biology could be expressed as a consequence of the physico-chemistry shifts of the ocean, where plankton distribution, abundance, and phenology are important aspects of the ecosystems that can be used to understand and quantify ecological changes caused by CO_2-induced climate change (Hays et al., 2005).

The Humboldt Current System (HCS) in northern Chile has high biological productivity throughout the year. This production is driven by frequent upwelling events, which cause shoaling of the oxygen poor Equatorial Subsurface Water (ESSW) that forms the OMZ in the South Eastern Pacific, resulting in low levels of dissolved oxygen (<0.5 ml L^{-1}) near the surface (Escribano, 1998; Mann and Lazier,

Abbreviations: HCS, Humboldt Current System; DO, dissolved oxygen; OMZ, Oxygen Minimum Zone; EPR, Egg Production Rate; EPR_{exp}, Experimental Egg Production Rate; HS, Hatching Success.

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1991; Morales et al., 1999). The upper boundary of the OMZ is defined as the depth with dissolved oxygen (DO) concentrations of 1 ml L^{-1}, which in this area, is normally close to surface waters (<50 m) (Fuenzalida et al., 2009; Hidalgo et al., 2005; Morales et al., 1999). Below these depths, the dissolved oxygen concentration decreases markedly to very low values (<0.1 ml L^{-1}) and even to the total absence of oxygen (Ulloa et al., 2012). The upper boundary of the OMZ acts as a physical–chemical barrier to vertical distribution of most aerobic planktonic organisms in the water column (Hidalgo and Escribano, 2001; Morales et al., 1999). The OMZ is considered an Oxygen-Deficient Ecological Barrier (BEDOX) that concentrates the abundance, biomass and diversity of plankton into the oxygenated and food-rich surface water within the photic zone (Donoso and Escribano, 2014), promoting more efficient carbon flux through pelagic food webs due to higher overlap of primary production, grazers, and predators (Escribano et al., 2009; Hidalgo and Escribano, 2001; Hidalgo et al., 2010; Manríquez et al., 2009), either by direct herbivory or a heterotrophic pathway (Vargas et al., 2007).

Copepods constitute about 80% of the zooplankton biomass in the HCS (Escribano and Hidalgo, 2000; Escribano et al., 2007; Hidalgo and Escribano, 2001). About 118 species have been identified in this system; the calanoid copepods *Acartia tonsa*, *Calanus chilensis*, *Centropages brachiatus* and *Paracalanus cf. indicus* are the most abundant species (Hidalgo and Escribano, 2001; Hidalgo et al., 2010). Production and growth rates of copepods in the HCS show high temporal and spatial variability and are controlled by temperature (Escribano et al., 1998), food quality and quantity (Poulet et al., 2007; Torres and Escribano, 2003; Vargas et al., 2006), advection (Escribano, 1998), and upwelling intensity (Escribano et al., 2012). Previous studies in other systems have shown that hypoxic conditions could negatively affect *A. tonsa* egg production, hatching success and survival (Marcus et al., 2004; Richmond et al., 2006) while other copepods and their different developmental stages, like *Calanoides carinatus*, are able to tolerate low DO concentrations (Auel and Verheye, 2007). Understanding how copepod growth and reproduction respond to stress and environmental variability would greatly improve our understanding of how populations and ecosystems function (Runge and Roff, 2000).

The Antofagasta Peninsula and Mejillones Bay are characterized by cold and oxygen-poor subsurface waters due to semi-permanent upwelling throughout the year (Marín and Olivares, 1999) and an upper boundary of the OMZ located on average at 26 m depth (Hidalgo et al., 2005). In this area, year-round copepod reproduction indicates they may not be limited by food availability; the main factors affecting their life cycles and distribution are changes in temperature and DO associated with upwelling intensity (Escribano et al., 1998, 2012; Hidalgo and Escribano, 2008; Hidalgo et al., 2010). However, direct effects of the vertical displacement of the OMZ on the abundance and production rates of different copepod species remain unknown. Also, no direct measurements of the effect of different DO concentrations on production, and specifically, on copepod egg production and egg viability, are available in the HCS.

The main goal of this study was to determine whether vital rates of dominant copepods in the HCS are affected by DO concentrations that occur in the field. Vertical movements of the OMZ that result from changes in upwelling intensity could reduce or expand the oxygenated surface layer that most copepods inhabit, especially in areas where upwelling intensity and the OMZ's extension is increasing due to climate change. We hypothesize that these vertical movements would expose the copepods to lower oxygen conditions than necessary for their physiological requirements, and therefore reduce production and growth rates. To evaluate this hypothesis, several oxygen-controlled experiments were conducted to test the Egg Production Rates (EPR) and hatching success (HS) of *C. chilensis* and *A. tonsa* during spring and summer 2010. The laboratory results were complemented with a one-year time series of monthly *in situ* abundances of females and eggs, *in situ* egg production rates, and oceanographic parameters.

2. Materials and methods

2.1. Field studies

In order to study the temporal and spatial variability of zooplankton in the coastal upwelling zone of Mejillones Bay (23°S), we conducted monthly hydrographic surveys, net tows and Niskin samples during 2010. Three stations located along a coast-to-ocean transect were sampled monthly from January to December (St-1: 23° 04.2′ S, 70° 25.8′ W, station depth (z_{max}) = 60 m; St-2: 23° 02.4′ S, 70° 27.0′ W, z_{max} = 90 m; and St-3: 23° 00.2′ S, 70° 28.2′ W, z_{max} = 120 m) (Fig. 1). At each station, an autonomous oceanographic profiler CTD-O SeaBird 19 was used to obtain conductivity, temperature, density, and DO measurements.

To assess zooplankton abundance and composition, samples were obtained at each station using vertical hauls of a WP-2 net (57 cm ring diameter and 200 μm mesh) equipped with a flowmeter. The vertical hauls were conducted from 30 m to surface to ensure sampling of the upper boundary of the OMZ layer, which has been shown to have an average depth of 26 m (Hidalgo et al., 2005). Zooplankton samples were preserved in 4% buffered formalin solution for later composition analysis.

Water samples at 10 m depth were collected with a Niskin bottle (10 L) to obtain microplankton samples to estimate copepod egg abundance and Chlorophyll-*a* (Chl-*a*). This depth was chosen based on studies that showed that the Chl-*a* maximum is typically located above 15 m depth (Iriarte et al., 2000) and that changes in copepod egg abundance between 10 and 15 m is a good index of overall changes in eggs throughout the water column due to the presence of very low oxygen waters below those depths (Hidalgo and Escribano, 2007, 2008). Approximately 9.6 L of the sample was 20-μm sieved to collect microplankton samples that were preserved in 2% buffered formalin solution for later composition analysis. Chl-*a* was determined by filtering 200 ml subsamples onto GF/F filters (0.7 μm pore diameter) and analyzing by the fluorometric method (Anabalón et al., 2014; Holm-Hansen et al., 1965).

2.2. Oceanographic data

To assess the relationship between upwelling intensity and the vertical distribution of the OMZ, upwelling was estimated from Ekman transport using the equation described by Mann and Lazier (1991):

$$M_x = \tau_y / f$$

where, M_x is Ekman transport ($m^3\ s^{-1}\ km^{-1}$), f is the Coriolis parameter, and τ_y is the along-shore wind stress (Pa). M_x is positive for south winds (upwelling) and negative for north winds (downwelling), and Tau (τ) was estimated as:

$$\tau_y = \rho_a * C_d * (V_y|V_y|)$$

where, ρ_a correspond to air density (1.21 kg m^{-3}), C_d is the empirical constant known as drag coefficient (= 0.0014) and V_y represents the along-shore wind velocity ($m\ s^{-1}$). Daily wind data were taken from the Meteorological Station of Cerro Moreno (latitude–longitude: 23° 27′ S–70° 26′ W) maintained by the Dirección Meteorológica de Chile (http://164.77.222.61/climatologia/).

Water column stratification was estimated from density profiles obtained with the CTD-O as the geopotential energy anomaly (ϕ_{50}) ($J\ m^{-3}$) described by Bowden (1983) and applied in the HCS (Hidalgo and Escribano, 2007, 2008; Torres et al., 2002):

$$\phi_{50} = 1/H \int_{-H}^{0} (\rho_m - \rho) g z \ dz$$

where, ρ_m is the mean density of the water column, ρ is the density at a given depth z, g is acceleration due to gravity, and H = 50 m. The

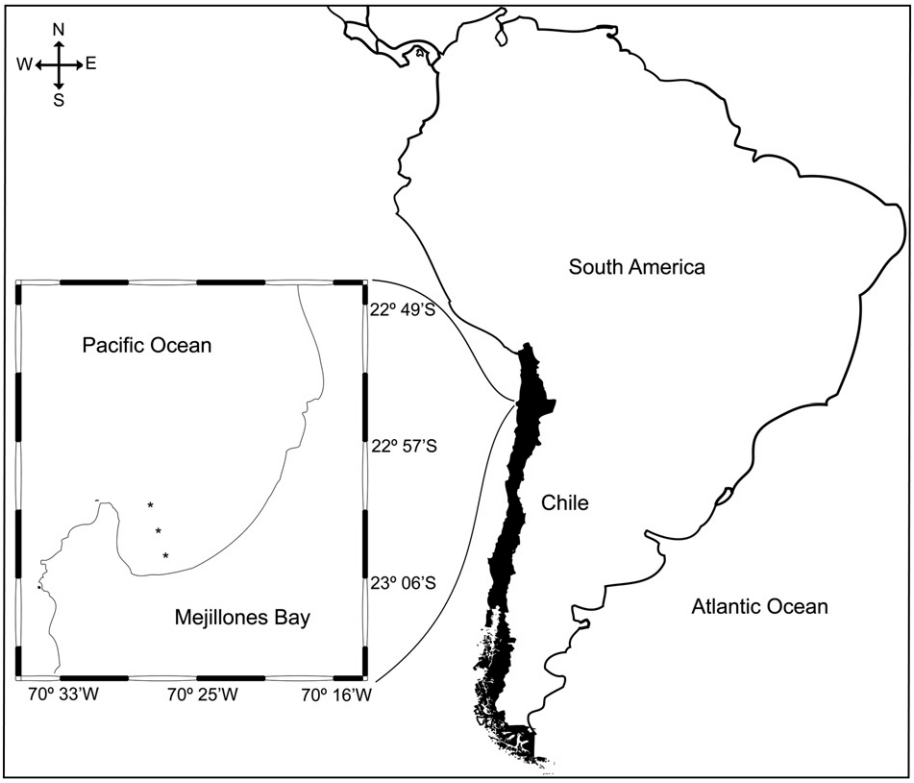

Fig. 1. South America context and study area in Mejillones Bay, northern Chile. The stations (St-1, St-2, St-3) sampled monthly during 2010 are denoted by stars: St-1 is the most southern station, closest to the coast.

geopotential energy anomaly is used to quantify the depth-averaged deficit of potential energy resulting from stratification compared to the potential energy of a totally mixed water column (Torres et al., 2002).

The depth of the upper OMZ boundary was determined as the depth where DO was 1 ml O_2 L^{-1} (Fuenzalida et al., 2009; Hidalgo et al., 2005; Morales et al., 1999). The mean and standard deviation of sea surface temperature (SST) were obtained. Vertical changes of the OMZ were also assessed using the mean and standard deviation of oceanographic variables (T, S, DO, Chl-*a*) at 10 m depth as this depth has been shown to be representative of environmental changes in the upper mixed layer that copepods inhabit (Escribano, 1998). To characterize the water column conditions where zooplanktonic samples were obtained, the average and standard deviation between 0 and 30 m depth were calculated for temperature ($T_{0–30}$), salinity ($S_{0–30}$) and dissolved oxygen ($DO_{0–30}$) at all sampling stations.

2.3. Egg production and hatching success experiments

Egg production and hatching success experiments were conducted on *C. chilensis* in December and on *A. tonsa* in February, March, and December. Copepods were obtained using oblique hauls from 30 to 0 m depth collected between stations St-3 and St-2. Live copepods were diluted with ambient seawater, stored in an insulated cooler, and transported to the laboratory within 2 h. Immediately after arrival at the laboratory, mature and healthy females of *C. chilensis* and *A. tonsa* were carefully sorted into 23-μm filtered seawater. In the meantime, seawater was bubbled with N_2 to create six experimental DO concentrations of 0.5, 1, 2, 3, 4 and 5 ml O_2 L^{-1}, to test the range of oxygen found *in situ* across the vertical gradient. A thermoregulated bath was used to maintain the temperature at 15 °C, a value close to the annual mean temperature at 10 m depth measured by Hidalgo and Escribano (2008) during a typical non-El Niño year in Mejillones Bay. *C. chilensis* or *A. tonsa* females were carefully transferred to 1-L polycarbonate bottles filled with treatment water at a density of 10 females L^{-1}. At this density, no crowing effects on production were expected, as suggested for *Centropages typicus* (Miralto et al., 1996). Incubations were conducted in triplicate at each of the six DO concentrations. Some experiments were repeated (also with 3 replicates). To remove food limitation and minimize egg cannibalism as factors in experiments, unlimited food (10^3–10^4 cell mL^{-1}) was provided by adding concentrated *in situ* water to each bottle until a greenwish color was attained (Runge and Roff, 2000; Torres and Escribano, 2003).

To avoid oxygenation, each bottle was sealed with Parafilm without bubbles before capping and incubating for 24 h. DO concentrations were measured at the beginning and the end of each incubation with an optical sensor type OXY-4 Micro Optode PreSens. Needle-type optical probes were set up using two-point calibration (0% and 100% of oxygen saturation) following the manufacturer's instructions. After 24 h, the content of each bottle was gently filtered with a 23-μm sieve attempting to leave the sieve submerged in treatment water to ensure the females and eggs remained in the water during sieving. Eggs were immediately incubated for hatching success experiments as described below; females were preserved in 4% buffered formalin solution for later measurements of the prosome length (μm) using a stereoscopic microscope equipped with an ocular micrometer (±0.01 mm).

Egg production rates (egg $females^{-1}$ day^{-1}) were calculated as:

$$EPR_{exp} = N_{eggs} * (24/N_{females} * t)$$

where EPR_{exp} is the experimental egg production rate, N_{eggs} corresponds to the number of eggs released during the incubation time *t* (in hours), and $N_{females}$ is the number of females under observation (Runge and Roff, 2000).

Hatching success (%) was determined by calculating the percentage of eggs produced in EPR_{exp} that hatched into nauplii by 24 h after spawning. The eggs laid in each EPR_{exp} bottle were pipetted from the sieve into 250-ml polycarbonate bottles and incubated under the same DO conditions in which they had been laid. After 24 h, unhatched

eggs and nauplii were collected with a 23-μm sieve, counted and preserved for later diameter (μm) measurements. The mean of the initial and final DO concentrations was used as the concentration that females and eggs were exposed to during experiments.

2.4. Zooplankton and microplankton sample analysis

In the laboratory, all females of *C. chilensis* and *A. tonsa* were identified and counted from one half of each sample, using a stereomicroscope at 2× and 4× magnification. Their abundances were calculated as number of individuals m^{-3} of water filtered. Eggs of *C. chilensis* and *A. tonsa* from the seawater samples were identified and counted at 10× and 40× magnification. Eggs were identified using size (mean diameters of 160 μm and 81 μm for *C. chilensis* and *A. tonsa*, respectively) and morphological characteristics (e.g., spiny or smooth surface shape) according to previous eggs production experiments for both species (Ruz, unpublished data) and morphological characteristics from published data of *A. tonsa* (Hansen et al., 2010). These features allowed the eggs of *C. chilensis* and *A. tonsa* to be distinguished from eggs of other calanoid copepods found in the region. Eggs abundances were calculate as number of eggs L^{-1}.

Abundances of females and eggs were used to estimate *in situ* EPR using the approach of Edmonson (1968) in order to compare to experimental results. This method has been used to estimate EPR of other broadcast spawning calanoids, including *Clausucalanus forcatus* (Bi and Enfield, 2006), *C. chilensis* and *C. brachiatus* (Hidalgo and Escribano, 2007, 2008) and it is defined as:

$$EPR = \left(N_{eggs}/N_{females}\right)/D$$

where EPR is the *in situ* egg production rate (eggs $female^{-1}$ day^{-1}), N_{eggs} corresponds to egg abundance (number m^{-3}), $N_{females}$ is the female abundance (number m^{-3}), and *D* represents the embryonic development time (day^{-1}) as a function of temperature described by Bělehrádek (1935) and denoted by the following equation:

$$D = a * (T - t_0)^{-b}$$

where *T* is the temperature (°C) and *a*, t_0 and *b* are constants. Parameters *a* and t_0 are species-specific, while *b* corresponds to a fixed value for copepods (=2.05). Species-specific equations of Bělehrádek embryonic development time were obtained from published data for *C. chilensis* (Escribano et al., 1998; Hidalgo and Escribano, 2007, 2008) and *A. tonsa* (Mauchline, 1998; McLaren et al., 1969), denoted by the following equations:

$$D = 947.7 * (T + 11.0)^{-2.05} \qquad C.\ chilensis$$

and

$$D = 489 * (T - 1.8)^{-2.05} \qquad A.\ tonsa$$

For both species, *D* values were estimated using the mean temperature over the 0–30 m layer of the water column.

2.5. Data analysis

The effects of DO and female size (as a covariable) on EPR_{exp} and HS were statistically analyzed through Analysis of Covariance (ANCOVA), used with a level of significance of $\alpha = 0.05$. A post-hoc Tukey test was done to identify significant differences between treatments (Quinn and Keough, 2002). When assumptions of parametric tests were not met, a non-parametric ANCOVA based on ranks was performed (Olejnik and Algina, 1984). Experimental data values are reported as mean ± 1SE (Standard Error).

Non-parametric Spearman correlations were used to test statistical relationships between experimental parameters (EPR_{exp} and HS) and DO concentrations as well as relationships between *in situ* parameters (female, eggs abundance and EPR) and oceanographic parameters and indices. Principal Component Analysis (PCA) with Varimax rotation (Quinn and Keough, 2002) was used to explore patterns in *in situ* parameters of *C. chilensis* and *A. tonsa*, physical, chemical, biological variables and oceanographic indices.

Finally, to evaluate whether *C. chilensis* and *A. tonsa* demography in Mejillones Bay were consistent with the experimental results (EPR_{exp} and HS) under different oxygen levels, we made graphical comparisons between these variables and DO_{10} and the DO concentrations used in the experiments. *In situ* data values are reported as mean ± 1SD (Standard Deviation).

3. Results

3.1. Oceanographic conditions

The water temperature throughout the whole sampling period (2010) ranged between 11.9 and 17.7 °C. The monthly means suggest the presence of two periods with a transition between June and July (Fig. 2a). The first six months, the summer/autumn period, was significantly warmer (14.9 ± 0.35 °C) (mean ± 1 SD) than the second, winter/spring period (13.6 ± 0.82 °C) (Two sample *T*-test, T = 6.1, DF = 34, $p < 0.001$).

Dissolved oxygen (DO) profiles showed a thin oxygenated surface layer above ~20 m, except in August and November when a deeper oxycline (>80 m) was found at St-2 and St-3, and St-1, respectively (Fig. 2b). In the subsurface layer, oxygen-poor water prevailed during the most of the year (Fig. 2b). Coincidently, the upper limit of the OMZ (1 ml O_2 L^{-1}) was found at an overall average depth of 21.5 m.

Relatively small salinity changes were observed during the year. However, an intrusion of high-salinity subsurface waters to the surface was observed in January, April and October (Fig. 2c), coincident with the highest values of the upwelling index (Ekman transport) (Fig. 3a). Salinity followed the trend of the upwelling, except during the end of the year when the opposite relation occurred, probably due to changes in water masses in the season (Fig. 3a and d). The upwelling index indicated that winds were upwelling favorable year-round, with intensified upwelling in spring and summer and more variable upwelling in autumn and winter. The stratification index showed the highest values from September to December, whereas the rest of the year stratification was highly variable (Fig. 3a). These indices were significantly correlated ($r = 0.57$; $p < 0.01$; $n = 12$).

Chl-*a* ranged from 1.3 to 106.7 mg m^{-3} with an annual average of 22.8 ± 22.6 mg m^{-3}. Two major peaks were measured in January (>80 mg Chl-*a* m^{-3}) and May (40 mg Chl-*a* m^{-3}) whereas from June to December, mean values of Chl-*a* were close to 20 mg m^{-3} (Fig. 3e). Chl-*a* at 10 m depth was positively correlated with T_{10} ($r = 0.59$, $p < 0.001$), indicating that higher values of Chl-*a* occurred in summer and autumn when surface waters were warmer and upwelling was more intense (Fig. 3a; b; e).

During January, February, March, and December, experimental activities were carried out (white arrows in Fig. 2c). In these months, vertical profiles showed a strong thermocline (Fig. 4a) and, shallow oxycline with the OMZ upper boundary (1 ml O_2 L^{-1}) at 19, 12, 13, and 31 m for January, February, March, and December, respectively (Fig. 4b). The salinity showed well mixed subsurface water from the OMZ (Fig. 4a; c).

3.2. Egg Production Rate (EPR_{exp}) and Hatching Success (HS) experiments

Survival of *C. chilensis* females was 99.7% by the end of the experiments, with only one death in treatment 3 (Table 1). The mean Prosome Length (PL) (±1 SE) of incubated females was 2476 ± 3.8 μm (range = 2143–2876 μm, n = 320) and the mean egg diameter was 160 ± 0.3 μm

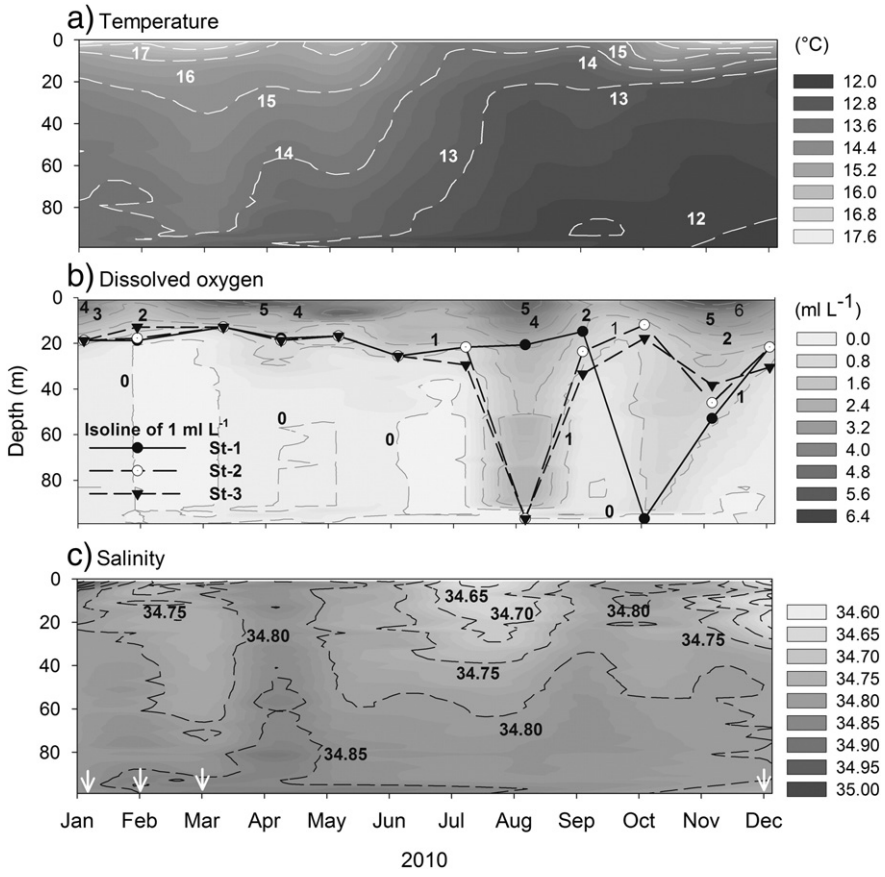

Fig. 2. Mean conditions at the three stations during 2010: a) temperature (°C); b) dissolved oxygen (ml L^{-1}) with the isoline of 1 ml L^{-1} (OMZ upper limit) by station (1, 2 and 3) and; c) salinity. White arrows show the months where egg production and hatching success experiments were conducted.

(range = 143–176 μm, n = 469). Among different oxygen levels, we were not able to identify significant differences in EPR_{exp} (ANCOVA, F = 2.06, p = 0.10) nor a significant correlation between EPR_{exp} and DO (r = 0.24, p = 0.18). However, EPR_{exp} showed high variability within and among DO concentrations (Fig. 5a). The lowest mean EPR_{exp} was measured in the 1.33 ml O_2 L^{-1} concentration, with 4 ± 1 eggs per female, while the maximum was measured at 4.30 ml O_2 L^{-1} with 41 ± 6 eggs per female (Table 1). Female size had a significant effect on EPR_{exp} (ANCOVA, F = 7.34, p = 0.01) and was positively correlated with it (r = 0.49, p < 0.01).

Hatching success (HS) of *C. chilensis* also showed no significant differences among DO levels and high variability (averaging 40.9–86.6%; Fig. 5b; Table 1) (ANCOVA, F = 0.27, p = 0.93) and, no significant correlation with DO (r = −0.15, p = 0.43). A negative correlation between HS and females size was observed (r = −0.41, p = 0.03). However, ANCOVA results show that female size had no significant effect on HS (ANCOVA, T = −1.87, p = 0.08).

Overall, *A. tonsa* survival was 85.4% at the end of the experiments. However, mortality occurred only in the lowest DO treatment (0.91 ± 0.12 mL O_2 L^{-1}) (mean ± SE) in which 52.4% of the females survived for 24 h (Table 2). The mean PL of incubated *A. tonsa* females was 1016 ± 16.3 μm (range = 762–1167 μm, n = 224) and mean egg diameter was 75 ± 1.2 μm (range = 71–83 μm, n = 230). Significant differences in EPR_{exp} among DO concentrations were observed (ANCOVA, F = 6.69, p < 0.01) (Fig. 5a; Table 2) without a significant correlation between these parameters (r = 0.07, p = 0.73). The lowest value of EPR_{exp} was found in the 5.00 ml O_2 L^{-1} treatment with 3 ± 2 eggs per female while the maximum was found at 4.66 O_2 L^{-1} with 21 ± 4 eggs per female (Table 2). In contrast to *C. chilensis*, the body size of *A. tonsa* did not affect their EPR_{exp} (ANCOVA, F = 0.24, p = 0.63). Mean HS ranged from 38.1%–73.5% (Table 2) with significantly increased HS at higher DO (ANCOVA, F = 3.90, p = 0.03) and a significant positive correlation (r = 0.76, p < 0.001) (Fig. 5b; Table 2). HS was not affected by, nor correlated with, female size (ANCOVA, T = −0.84, p = 0.42).

3.3. In situ variability of *C. chilensis* and *A. tonsa*

In the field, *C. chilensis* females were present at all stations throughout 2010, although with high seasonal variability (Fig. 6a). Lower abundances (mean ± 1SD) were found from January to March, June, and November to December, with a mean of 0.6 ± 0.65 (range = 0–2.4, n = 15) females m^{-3}. Higher female abundances were observed in April, May, and July to October, with a mean of 45 ± 58.9 (range = 0.2–242.1, n = 21) females m^{-3}. *C. chilensis* egg abundance averaged 0.4 ± 1.3 (range = 0–6.2) eggs L^{-1} across all months, but were present only during September, October, and December. Monthly mean of *in situ C. chilensis* EPR was 30 ± 88.5 (range = 0–438) eggs $female^{-1}$ day^{-1} (Fig. 6a).

The non-parametric correlation analysis showed that temperature ($T_{0–30}$) was negatively related to *C. chilensis* eggs abundance (r = −0.43, p = 0.01) and EPR (r = −0.39, p = 0.03), but positively correlated with female abundance (r = 0.43, p = <0.01). Female abundance, egg abundance, and EPR were all negatively correlated with Chl-*a* (r = −0.46, p < 0.01; r = −0.44, p < 0.01 and; r = −0.53, p < 0.01, respectively). Female abundance was negatively, but weakly, related to upwelling (r = −0.35, p = 0.03) and stratification (r = −0.40, p = 0.02), whereas eggs (r = 0.42, p = 0.01) and EPR (r = 0.42, p = 0.02) showed a positive relationship with the stratification index.

In order to explore relationships among oceanographic conditions and *C. chilensis* biological metrics (abundances and EPR), a Principal Component Analysis (PCA) was performed. The first three principal components explained 73% of the total variance in the dataset. The

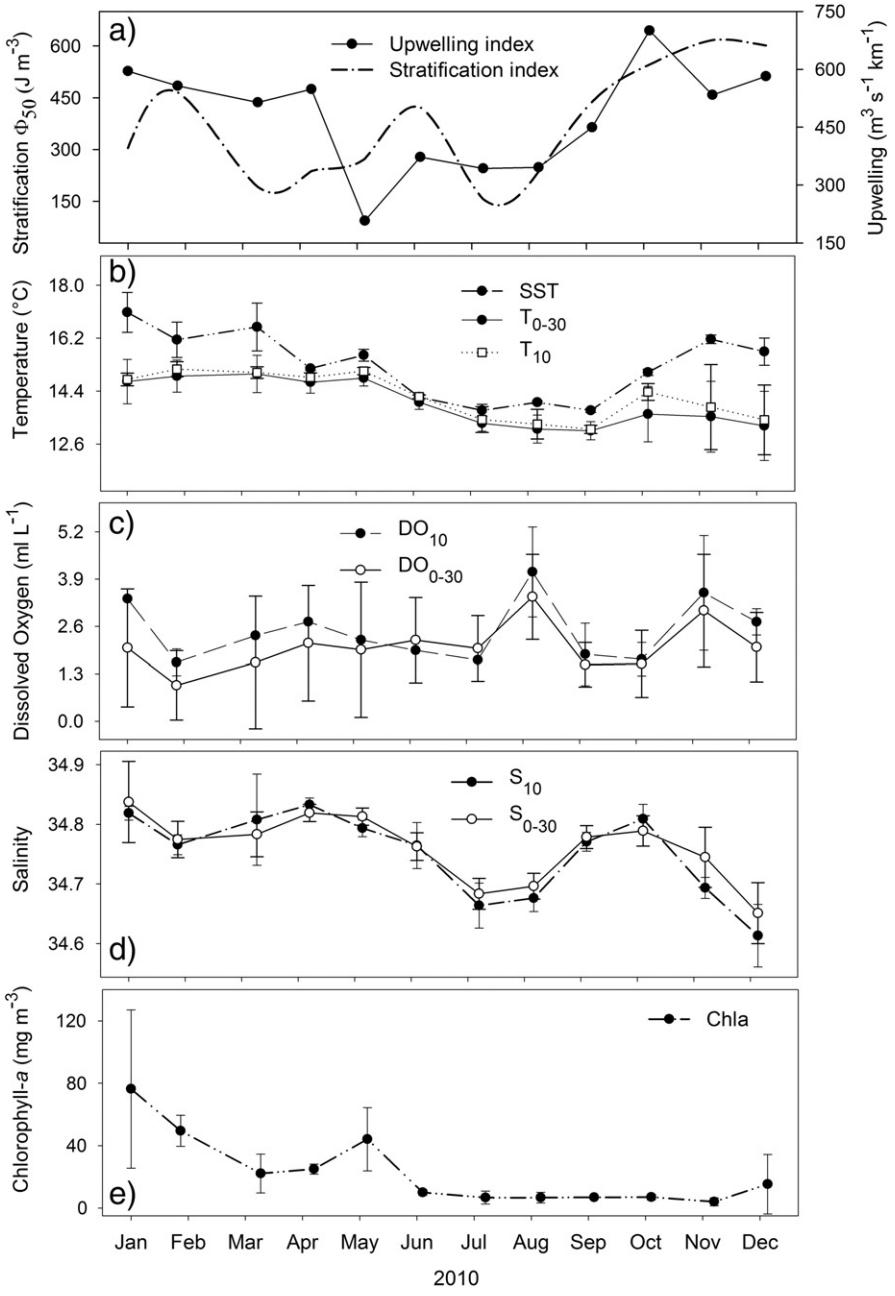

Fig. 3. Temporal variability of oceanographic conditions during 2010: a) Stratification index (J m^{-3}) and Upwelling index (m^3 s^{-1} km^{-1}); b) temperature (°C) at 1 m (SST), at 10 m (T_{10}), and between 0–30 m depth (T_{0-30}); c) dissolved oxygen (ml L^{-1}) at 10 m depth (DO_{10}) and between 0–30 m depth (DO_{0-30}); c) Salinity at 10 m (S_{10}) and between 0–30 m depth (S_{0-30}) and; e) Chl-*a* (mg m^{-3}) at 10 m depth. Index values in panel *a*, have only one value per month; values in *b*, *c*, *d* and *e* correspond to the mean and standard deviation of the three stations.

first component (PC1) represented the temperature (T_{0-30} and T_{10}), Chl-*a*, water stratification, and female abundance and explained 29% of the variance. The second component (PC2) was related to DO (DO_{10} and DO_{0-30}) and OMZ depth, with 23% of explained variance. The third component (PC3) represented egg abundance and EPR, plus the upwelling index (Ek) with 21% of explained variance (Fig. 7a; Table 3).

A. tonsa females were also present in the field year-round, with higher abundances during spring and summer than in winter. The lowest abundance was observed in June with 2 ± 1.0 (range = 1–2, n = 3) females m^{-3}; moderate abundances were found in January, February, April, May, and July to November with a mean of 45 ± 46.3 (range = 0–187, n = 27) females m^{-3}; high abundances were present in March, October, and December with a mean of 258 ± 298.7 (range = 19–944, n = 9) females m^{-3} (Fig. 6b). The overall mean value of *A. tonsa* eggs was 9 ± 19.1 (range = 0–96) eggs L^{-1}, but eggs only were present from January to March and October to December. Average monthly estimate of *in situ A. tonsa* EPR was 120 ± 289.3 (range = 0–1108) eggs $female^{-1}$ day^{-1} (Fig. 6b). Non-parametric correlations between *A. tonsa* and oceanographic parameters showed that EPR increased with DO at 10 m depth (DO_{10}) ($r = 0.39$, $p = 0.02$), whereas no correlations were found with temperature, salinity, Chl-*a*, OMZ depth, or dissolved oxygen from 0 to 30 m depth (DO_{0-30}). Upwelling and stratification indices were positively correlated with egg abundance ($r = 0.44$, $p < 0.01$; $r = 0.45$, $p < 0.01$; respectively) and with EPR ($r = 0.44$, $p < 0.01$; $r = 0.46$, $p < 0.01$; respectively). However, abundance of females was not related with the indices mentioned above.

Similar to C. *chilensis* the Principal Component Analysis (PCA) of *A. tonsa* population and oceanographic variables revealed three significant components explaining 75% of the total variance. The first component (PC1) was represented by the temperature (T_{0-30} and T_{10}), Chl-*a*,

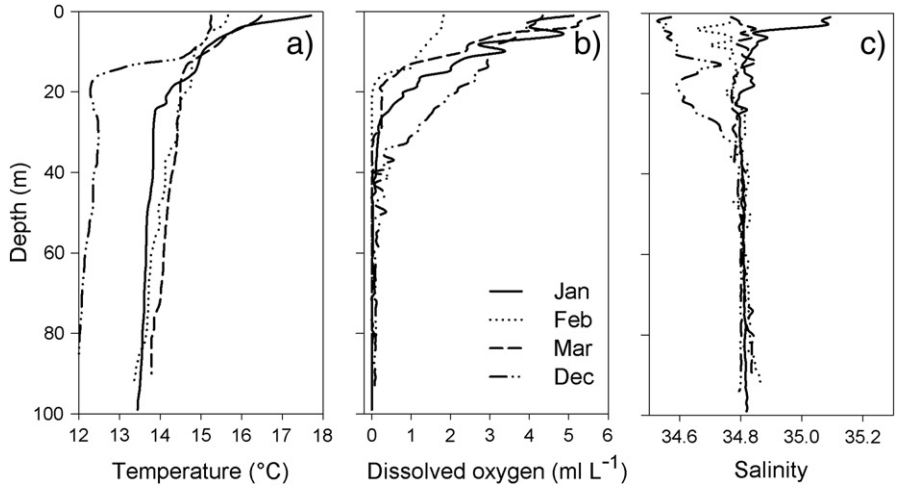

Fig. 4. Vertical profiles at St-3 during the experimental period: a) temperature (°C); b) dissolved oxygen (ml L^{-1}) and; c) salinity.

and stratification, with 28% of explained variance. The second component (PC2) showed that egg abundance and EPR of *A. tonsa* plus the upwelling index (Ek) explained 24% of the variance. Finally, the third component (PC3) was represented by DO (DO_{10} and $DO_{0–30}$) and OMZ depth, with 23% of explained variance (Fig. 7b; Table 3).

3.4. Comparison between field and experimental data

A comparison between *in situ* EPR and EPR_{exp} showed high *C. chilensis* EPR in the field when DO_{10} was between ~1–3 ml O_2 L^{-1}, but maximum EPR_{exp} were found between ~3–4.5 ml O_2 L^{-1} (Fig. 8a; Table 1). In contrast, *A. tonsa* showed highest *in situ* EPR and EPR_{exp} between ~2–4 ml O_2 L^{-1} (Fig. 8b; Table 2).

For *C. chilensis*, high *in situ* EPR were found between 1 to 3 mL O_2 L^{-1}, whereas hatching success from experiments were not be affected by DO (Fig. 9a; Table 1). By contrast, *A. tonsa* showed the higher *in situ* EPR at mid DO concentrations in a similar range of DO where HS showed high but no the highest values (Fig. 9b; Table 2).

4. Discussion

We assessed the effect of different DO concentrations, from hypoxia to well-oxygenated waters, on experimental egg production rate (EPR_{exp}) and hatching success (HS) of two dominant copepods off northern Chile, *C. chilensis* and A. *tonsa*. The two species showed different responses to DO: *C. chilensis* EPR_{exp} and HS were not affected by DO whereas *A. tonsa* EPR_{exp} and HS were (Fig. 5). Our results only indicate the short-term effects of DO on these species since, as in many experimental studies (see Runge and Roff, 2000), we were not able to acclimate females to treatment conditions prior to spawning, but the results do indicate an interesting difference in sensitivity among species which co-occur in a region that chronically experiences low oxygen levels. These short-term effects of DO could be relevant in the field, for example, due to DVM, vertical movements of the OMZ during upwelling, or as eggs sink through the water column.

Mejillones Bay in northern Chile presents a highly heterogeneous environment due to semi-permanent coastal upwelling all year-round, allowing the presence of physico-chemical processes that modulate copepod dynamics and community structure; as changes in upwelling intensity (Escribano et al., 2012), the presence of thermal fronts, upwelling shadows acting as retention areas (Giraldo et al., 2002; Marín et al., 1993) and shallow OMZ that could aggregate and increases copepod diversity in the food-rich photic zone (Hidalgo and Escribano, 2008; Hidalgo et al., 2010). These factors plus changes in food concentrations affect growth and development of copepods (Escribano et al., 1998; Poulet et al., 2007) and may explain the high variability in abundances and EPR (experimental and *in situ*) estimated in this study for *C. chilensis* and *A. tonsa*.

The EPR_{exp} values of *C. chilensis* were in a similar range (~0–85 eggs female^{-1} day^{-1}) with previous studies where factors as temperature (Escribano et al., 1996, 1998, 2014) and food (Poulet et al., 2007) were analyzed in the HCS. Considering all data, mean EPR was slightly lower (18.8 ± 3.57 eggs female^{-1} day^{-1}) than previous records of *C. chilensis* and other *Calanus* spp. with ~30 eggs female^{-1} day^{-1} (Escribano et al., 2014; Mauchline, 1998). Regressions analyses between prosome length (PL) and the number of eggs spawned showed no significant relationship (Escribano, 1998; Escribano et al., 1996) in contrast with our results where a positive relation was found ($r = 0.49$, $p < 0.01$).

Vargas et al. (2006) found higher *A. tonsa* EPR (50–10 eggs female^{-1} day^{-1}) in central-southern Chile (36°S) than other studies on the reproductive performance of *A. tonsa* in the HCS. EPR values for *A. tonsa* range between 4 and 6 eggs female^{-1} day^{-1} (Aguilera et al., 2011) in northern and southern Chile. Aguilera et al. (2013) showed that *A. tonsa* EPR ranged from 39 and 5 eggs female^{-1} day^{-1} from coastal to

Table 1
Summary of experimental results for *Calanus chilensis* during spring/summer 2010.

T	N_e	DO_i	DO_f	DO_t	EPR_{exp}	HS	PL	$Ø_{eggs}$	$N_{females}$
1	4	0.48 ± 0.06	0.50 ± 0.23	0.49 ± 0.15 (0.13–1.10)	14 ± 4	73.4 ± 5.20	2487 ± 13.25	159 ± 1.83	129
2	1	0.88 ± 0.02	1.79 ± 0.05	1.33 ± 0.04 (1.75–2.17)	4 ± 1	86.6 ± 5.09	2400 ± 46.16	162 ± 0.52	26
3	3	2.28 ± 0.13	2.80 ± 0.15	2.54 ± 0.14 (2.00–3.87)	21 ± 7	66.2 ± 11.80	2474 ± 15.47	159 ± 0.72	92 (1)
4	1	3.33 ± 0.09	3.30 ± 0.21	3.31 ± 0.15 (3.07–4.00)	35 ± 13	55.1 ± 20.41	2541 ± 41.86	165 ± 4.25	32
5	1	4.50 ± 0.01	4.09 ± 0.24	4.30 ± 0.12 (4.42–4.98)	41 ± 6	40.9 ± 13.23	2500 ± 5.70	159 ± 2.73	25
6	1	5.00 ± 0.02	4.46 ± 0.06	4.73 ± 0.04 (4.54–5.15)	7 ± 2	74.8 ± 16.23	2493 ± 23.94	162 ± 0.65	27

Incubation treatments (T) and number of experiments (N_e) run per treatment; each experiment had three replicates. This table shows mean, standard error (SE) of initial dissolved oxygen (DO_i), final (DO_f), treatment DO (DO_t) plus range (ml L^{-1}) and, SE of experimental egg production rate (EPR_{exp}) (eggs females^{-1} day^{-1}), hatching success (HS) (%), prosome length (μm) (PL) and egg diameter (Ø eggs) (μm) of *C. chilensis*. The number of females ($N_{females}$) alive versus dead (value in parentheses) at the end of experiments are shown. ANCOVA test did not show significant differences among treatments for EPR_{exp} or HS.

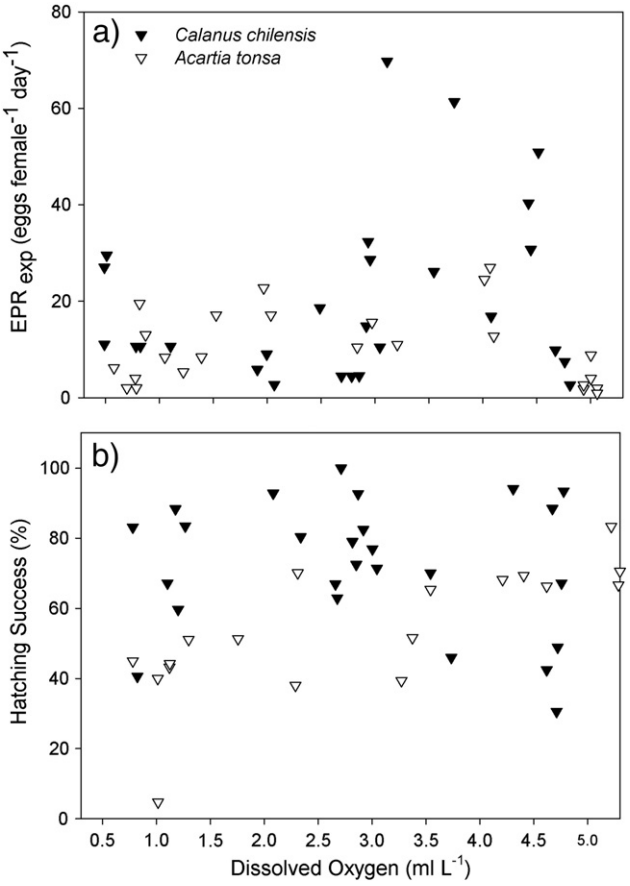

Fig. 5. Experimental variables: a) egg production rates (EPR_{exp}) (egg females^{-1} day^{-1}) and; b) hatching success (%) at different dissolved oxygen levels (DO) (ml L^{-1}) for *Calanus chilensis* and *Acartia tonsa*.

estuarine waters (Aguilera et al., 2013). Our estimates of EPR (21–3 eggs female^{-1} day^{-1}) were within the range of previous data for this specie in different environments of the HCS. In other seasonal environments where *A. tonsa* inhabits, also high values of EPR could be found ranging between ~100 and 20 (Marcus et al., 2004) and ~70 and 30 eggs female^{-1} day^{-1} (Sedlacek and Marcus, 2005) in control treatments at 20 °C, in difference to our estimates carried out at 14 °C. Considering a Q_{10} of 3 for EPR (Kiørboe and Sabatini, 1995), *A. tonsa* EPR estimated in this study mostly was within the theoretical value (~10 to 36 eggs female^{-1} day^{-1}) estimated at 14 °C. Similar to previous studies we observed no relation between female size and egg production in *A. tonsa* (Durbin et al., 1983).

Previous *in situ* EPR estimated by Edmonson method (Edmonson, 1968) showed that *C. chilensis* in the HCS have sporadic reproductive events with values ranging between 2.7 and 403 eggs female^{-1} day^{-1}, mainly concentrated in spring/summer and associated with abundances of 400 eggs m^{-3} in south-central Chile (Hidalgo and Escribano, 2007), whereas in northern Chile, the reproductive pulses could take place at any time of the year with a mean value of 4.22 eggs female^{-1} day^{-1} and abundances up to ~2000 eggs m^{-3} (Hidalgo and Escribano, 2008). Coincidently, our data showed high variability for *C. chilensis* and *A. tonsa* with values ranging between 0–438 eggs female^{-1} day^{-1} and 0–1108 eggs female^{-1} day^{-1}, respectively. Other species have been studied using this method, EPR of *C. forcatus* in the Gulf of Mexico was 1.93 ± 0.38 eggs female^{-1} day^{-1} (Bi and Enfield, 2006), showing lower values than *C. chilensis* and *A. tonsa* in the HCS. Peterson and Kimmerer (1994) suggest that the egg-ratio method could underestimate EPR due to high egg mortality rates produced by factors like predation and physiological causes that affect the embryonic development in nature (Laabir et al., 1995).

The experimental estimates of EPR were at least one order of magnitude smaller than *in situ* EPR. Analyzing both estimates by separated they are similar to previous data for *C. chilensis* and *A. tonsa* as we described above. However, the difference between these two approaches could be caused by a reduction of EPR due to female cannibalism over the eggs (Kang and Poulet, 2000), or differences in the timing and the number of eggs per clutch released (Peterson, 1988). It has been observed that EPR of *C. marshallae* changes (lower) during active to relaxation phase of the upwelling, due to a decoupling between egg production and food supply. Therefore the high heterogeneity of the upwelling system of Mejillones Bay may play a key role in the high variability of eggs abundances and *in situ* EPR.

On the other hand, high variability of HS of *C. chilensis* could be more attributed to maternal effects (Escribano et al., 1998; Poulet et al., 2007) than DO; where our results showed high values (40–87%) and variability at all experimental levels. For other copepods species such as *A. tonsa* and *Oithona colcarva* it has been observed that DO concentrations below 1 mg O_2 L^{-1} (~0.7 ml O_2 L^{-1}) could inhibit hatching (Roman et al., 1993). We only observed a reduction in the HS of *A. tonsa* under similar DO conditions, suggesting that *A. tonsa* from the northern area of HCS could be more tolerant to hypoxia than *A. tonsa* inhabit systems with seasonal bottom hypoxia, like Chesapeake Bay (see Roman et al., 1993). The *in situ* data showed a strong seasonal pattern of EPR, and to a lesser extent female abundances, that differed between species. *C. chilensis* eggs were present only at the beginning of the spring and early summer when upwelling-favorable winds intensified (Figs. 3a and 6a). This contrasts with previous studies in which eggs were found through most of the year (Escribano, 1998; Escribano and McLaren, 1999; Hidalgo and Escribano, 2008). High abundance of *A. tonsa* eggs occurred through spring and summer when upwelling and stratification was intensified (Fig. 3a) and a shallower OMZ was present; this is the first report of the temporal distribution of *A. tonsa* eggs in Mejillones Bay. However, experimental EPR estimates of *A. tonsa* in southern-central Chile (Vargas et al., 2006) showed that this specie is able to lay eggs all year-round, with higher EPR in spring and summer than the rest of the year. Females of *C. chilensis* and *A. tonsa* were found all year-round. *C. chilensis* showed high abundances

Table 2
Summary of experimental results for *Acartia tonsa* during spring/summer 2010.

T	N_e	DO_i	DO_f	DO_t	EPR_{exp}	HS	PL	$Ø_{eggs}$	$N_{females}$
1	2	0.63 ± 0.09	1.18 ± 0.15	0.91 ± 0.12 (0.50–1.23)	$6^{a, b, c, d, f}$ ± 3	38.1^{a} ± 6.8	1043 ± 9.4	79 ± 1.5	63 (30)
2	1	1.51 ± 0.02	1.83 ± 0.28	1.67 ± 0.14 (1.05–1.76)	$7^{a, b, c, d, e, f}$ ± 1	$53.2^{a,b}$ ± 9.3	957 ± 20.4	74 ± 0.4	32
3	1	2.50 ± 0.05	2.73 ± 0.24	2.62 ± 0.12 (1.53–2.85)	$19^{a, b, c, d, e}$ ± 2	$52.1^{a,b}$ ± 7.5	1027 ± 4.5	74 ± 0.7	37
4	1	3.50 ± 0.02	3.92 ± 0.23	3.71 ± 0.11 (2.67–4.08)	$12^{a, b, c, d, e, f}$ ± 2	$68.0^{a,b}$ ± 0.9	1014 ± 15.4	77 ± 0.2	28
5	1	4.51 ± 0.10	4.82 ± 0.05	4.66 ± 0.03 (4.01–4.85)	$21^{b,c, d, e}$ ± 4	73.5^{b} ± 5.0	1045 ± 1.7	74 ± 0.5	22
6	1	4.99 ± 0.01	5.01 ± 0.33	5.00 ± 0.17 (4.91–5.59)	$3^{a, b, d, f}$ ± 2	–	1001 ± 4.9	77 ± 2.2	24

Different letter superscript denotes significant differences (pairwise comparison Tukey test; $p < 0.05$) between treatments.
Incubation treatments (T) and number of experiments (N_e) run per treatment; each experiment had three replicates. This table shows mean, standard error (SE) of initial dissolved oxygen (DO_i), final (DO_f), treatment DO (DO_t) plus range (ml L^{-1}) and, SE of experimental egg production rate (EPR_{exp}) (eggs females^{-1} day^{-1}), hatching success (HS) (%), prosome length (μm) (PL) and egg diameter (Ø eggs) (μm) of *A. tonsa*. ANCOVA results show significant differences among treatments for egg production rate and hatching success. A posteriori pairwise comparison Tukey test denotes where the significant differences were found, specified with different letter superscript.

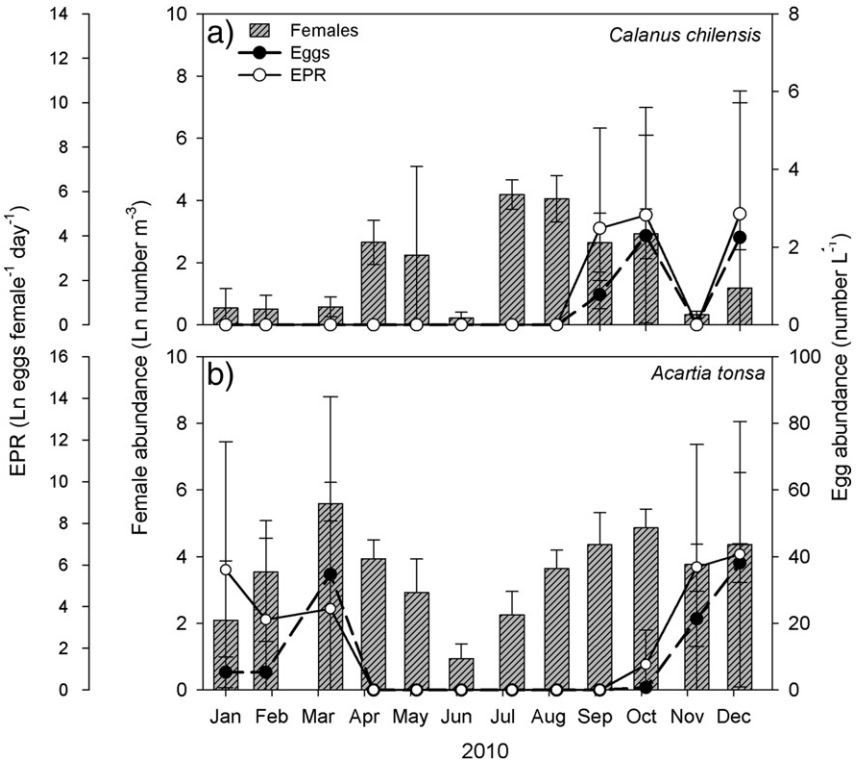

Fig. 6. Temporal changes of female abundance (Ln number m^{-3}), egg abundance (number L^{-1}) and egg production rate from *in situ* samples (EPR) (Ln eggs $female^{-1}$ day^{-1}) for a) *Calanus chilensis* and b) *Acartia tonsa* during 2010.

in winter and spring, in contrast with previous reports of the specie that had reported the highest female abundances during autumn and winter (Hidalgo and Escribano, 2008). Females of *A. tonsa* decreased during winter with higher abundance the rest of the year, corresponding to the first report on temporal variability of *A. tonsa* in Mejillones Bay. These differences are likely to be due to the increasing of upwelling during the last decade (2000–2008) that may be unfavorable for copepod populations (Escribano et al., 2012) plus changes in the timing of the upwelling as results of the oceanographic interannual variability of northern Chile and the influence of the Mejillones Peninsula that plays a key role on circulation and retention processes in Mejillones Bay (Letelier et al., 2012).

In the coastal upwelling zone of Mejillones Bay where food limitation potentially does not exist, copepod population dynamics are expected to be controlled mainly by temperature (Escribano et al., 2014). Copepods that inhabit these areas typically exhibit nearly continuous reproduction during the upwelling season, resulting in the coexistence of multiple generations and cohorts (Escribano et al., 2014; Hidalgo et al., 2005; Peterson, 1998). Therefore, the stage-specific copepod abundance found at any particular sampling period is at least partially affected by the specie's life cycle. For example, *C. chilensis* development is 38.1 days at 15 °C (Escribano et al., 1998), but in the field its populations have shown *ca.* 15 abundance peaks of adults in one year, with average time intervals of 20 days (Hidalgo and Escribano, 2008). As a consequence, a mismatch between the timing of a peak of adults and the cruises could lead to underestimated female abundances. However, maximal periods of female abundance of C. *chilensis* were higher than previous estimates in the bay (Escribano and McLaren, 1999; Hidalgo and Escribano, 2008) reaching values up two-fold during late winter and spring, indicating that our monthly sampling did not miss peaks of females abundances. *A. tonsa* female abundances were less variable than *C. chilensis*, possibly due to faster development associated with their smaller size, 25 days at 17 °C (McLaren et al., 1969), and so relatively higher adult production. Faster development also permits easier population maintenance within highly advective environments, as has been shown for *A. tonsa* off southern Europe (Leandro et al., 2006).

With our index of egg production taken only from 10 m depth, we may have underestimated abundances during autumn and winter when lower stratification occurs, leading to a deeper distribution of eggs than in spring and summer. Also, advection or a mismatch between sampling times and reproductive events could influence the abundance of eggs sampled. Our data do not allow us to assess these potential sources of variability; intensive sampling (e.g., weekly) of eggs and nauplii over a broader depth range would be necessary to better estimate their association with the OMZ. However, our index likely captures the dominant patterns of variability in egg production, so allows us to make some comparisons between egg production and changes in the environment. Previous studies in the region have successfully used the abundance of eggs collected from 10 m depth as an index of variability in the full water-column egg stock (Hidalgo and Escribano, 2007, 2008), or have found similar abundances when sampling from 50 to 0 m (Escribano, 1998) and 90 to 0 m (Escribano and McLaren, 1999). In other study areas, the egg production of *A. tonsa* has been shown to occur above the pycnocline (Hansen et al., 2010, 2012; McLaren, 1966), probably to allow the eggs to spend more time in surface waters, avoiding exposure to colder temperatures and poorly oxygen waters (Hansen et al, 2012). The lack of higher reproduction at low DO levels may be a behavioral adaptation to improve the survival and development of eggs since HS is positively correlated with DO ($r = 0.76$; $p < 0.001$; Fig. 5b), besides of a potential capacity of females on suppress laying eggs under short-term unfavorable conditions, as has been reported for *Calanus* sp. (Runge and Roff, 2000). We do not know *C. chilensis* spawning depths, but other *Calanus* species, e.g., *C. finmarchicus*, have been observed to move to the surface to spawn (Miller et al., 1991).

Copepod eggs are negatively buoyant and tend to sink, with their sinking velocities depending on their size and density (Mauchline, 1998). Sinking velocities together with the embryonic development rate determine whether eggs sink into the OMZ before hatching. The sinking rate of *C. chilensis* eggs is unknown, but is likely to be on the

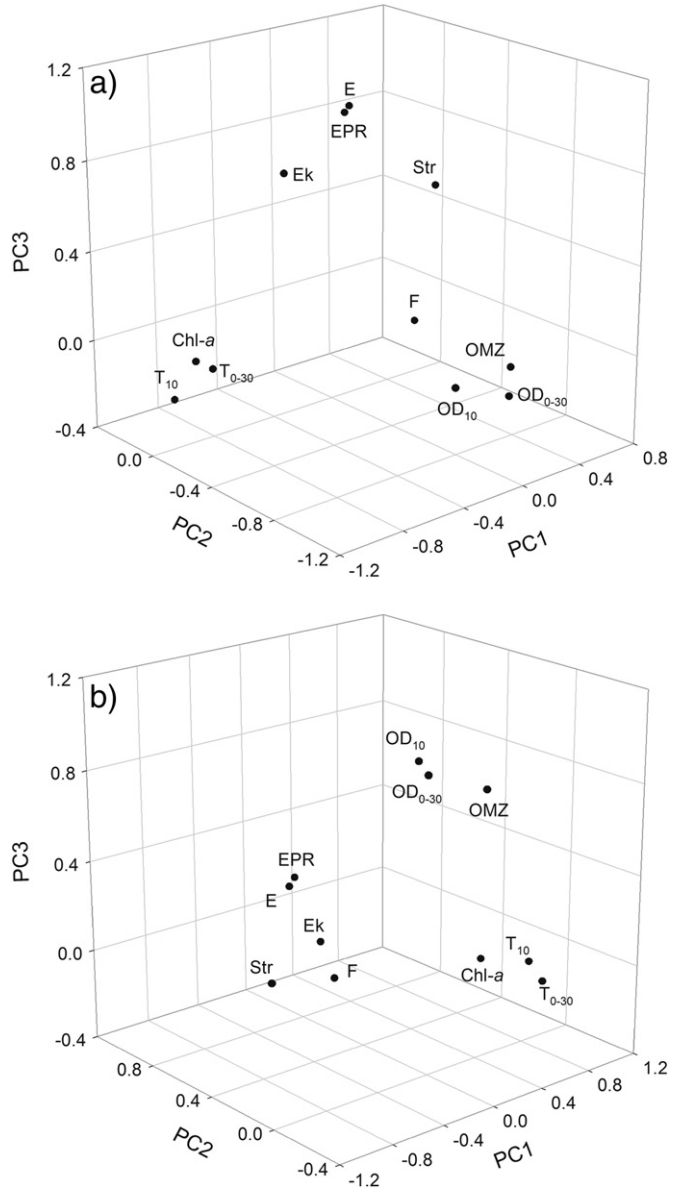

Fig. 7. Principal component analysis (PCA) applied to population parameters of a) *Calanus chilensis* and b) *Acartia tonsa*, along with oceanographic variables from the monthly time series at Mejillones Bay during 2010. The 1st three components were derived and plotted. Egg production rate (EPR), egg abundance (E), female abundance (F), temperature (T), dissolved oxygen (DO), upper boundary of the Oxygen Minimum Zone (~1 ml O_2 L^{-1}) (OMZ), Chlorophyll-*a* concentration at 10 m (Chl-*a*), Ekman transport as upwelling index (Ek) and, stratification index (Str). Subscript 10 corresponds to 10 m depth and 0–30 to the mean value between 0 and 30 m depth.

order of 25–35 m d^{-1} based on measurements of similarly sized eggs (diameter ~150 μm) produced by its congener, *C. finmarchicus* (Mauchline, 1998). *C. chilensis* hatches in 1.2 days at 15 °C (Escribano et al., 1998) and, our study shows that low DO is not limiting for the hatching of its eggs during spring/summer season, if *C. chilensis* eggs reach the OMZ they would be able to hatch and potentially had a successful development. Our results are supported by recent research off northern Peru which showed that *C. chilensis* stage V and adults were abundant in hypoxic waters at concentrations between 0.11 and 1.13 ml L^{-1} (equivalent to 5 μM and 50 μM, respectively); this is the only specie of Calanidae reported to be able to tolerate *in situ* hypoxia at different and non-resting developmental stages (Hirche et al., 2014). Population abundance of *C. chilensis* is linked to the OMZ depth and with upwelling (Hidalgo and Escribano, 2008), suggesting that

Table 3
Factor loadings from Principal Component Analysis (PCA) of oceanographic and biological measurements from a monthly sampling in Mejillones Bay in 2010. The 1st three principal components (1, 2, 3) and their explained variance (%) are shown. Factors used were egg abundance (Eggs), female abundance (Females), Egg Production Rate (EPR), mean 0–30 m temperature ($T_{0–30}$),10 m temperature (T_{10}), mean 0–30 m dissolved oxygen ($DO_{0–30}$), dissolved oxygen at 10 m depth (DO_{10}), depth of the upper limit of the Oxygen Minimum Zone (OMZ), Chlorophyll-*a* at 10 m depth (Chl-*a*), stratification index (Str) and upwelling index (Ek).

Variable component	*C. chilensis*			*A. tonsa*		
	1	2	3	1	2	3
Explained variance	29%	23%	21%	28%	24%	23%
Eggs	0.29	0.16	0.86	0.05	0.93	0.17
Females	0.55	−0.04	−0.13	−0.08	0.55	−0.12
EPR	0.25	0.14	0.84	0.04	0.89	0.22
$T_{0–30}$	−0.90	0.17	−0.26	0.96	−0.02	−0.15
T_{10}	−0.87	0.04	−0.06	0.90	0.03	−0.06
$DO_{0–30}$	0.21	−0.94	−0.14	−0.16	−0.11	0.96
DO_{10}	−0.15	−0.93	−0.02	0.14	0.17	0.91
OMZ	0.36	−0.82	−0.08	0.35	−0.09	0.82
Chl-*a*	−0.70	0.11	−0.15	0.73	0.21	−0.07
Str	0.63	−0.11	0.51	−0.78	0.36	0.04
Ek	−0.33	−0.02	0.70	0.02	0.70	−0.02

C. chilensis is better adapted to changes in DO concentrations compared with *A. tonsa* (Fig. 5a, b).

The sinking rate of *A. tonsa* eggs has been estimated between 13 and 24 m d^{-1} (Miller and Marcus, 1994). *A. tonsa* hatches between 2 and 3 days at the same temperature (McLaren et al., 1969) but, our experiments showed that the hatching success of *A. tonsa* is diminished at low DO levels, indicating that they would not hatch if they sink into the OMZ. However, in our experiments more than 40% of eggs hatch

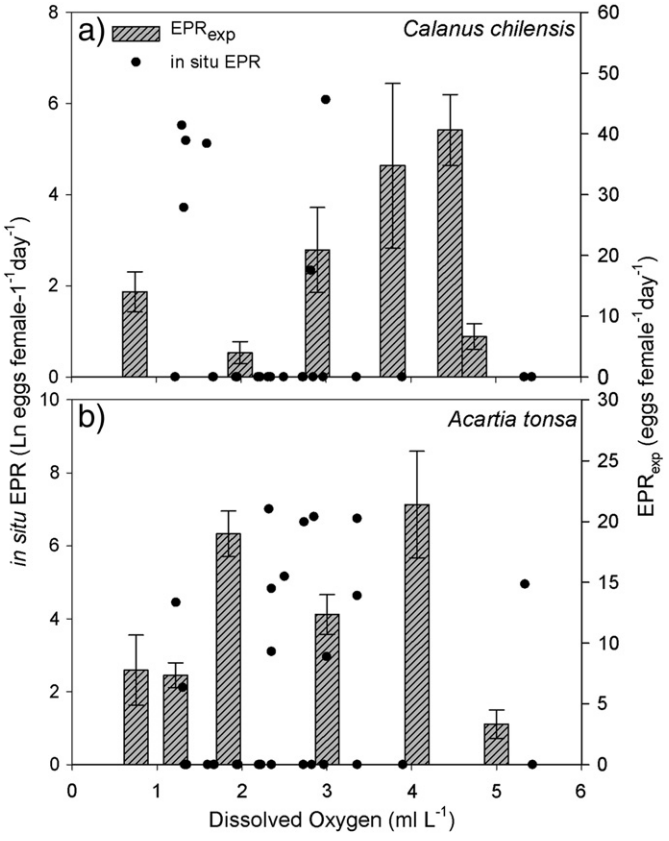

Fig. 8. *In situ* EPR (Ln egg female^{-1} d^{-1}) versus dissolved oxygen at 10 m depth (DO_{10}) compared with experimental egg production rate (EPR_{exp}) (egg female^{-1} d^{-1}) plotted versus experimental DO level: a) *C. chilensis* and b) *A. tonsa*.

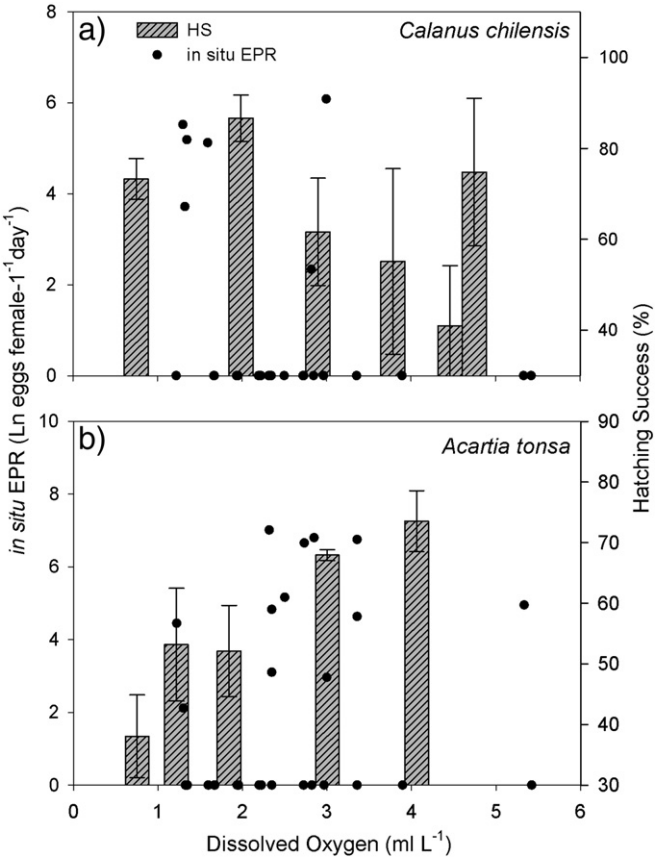

Fig. 9. *In situ* EPR (Ln egg female^{-1} d^{-1}) versus dissolved oxygen at 10 m depth (DO_{10}) overlayed on experimental hatching success (HS) (%) plotted versus experimental DO level for a) *C. chilensis* and b) *A. tonsa*.

within 24 h at 15 °C, suggesting a faster development (but not quantifiable) than previously registered for *A. tonsa*. According to this, the eggs of both species would reach the OMZ before hatching in Mejillones Bay, and during the spring and summer when surface waters are warmer, eggs could hatch before encountering low DO waters as has been suggested for *A. tonsa* in Chesapeake Bay (Roman et al., 1993). An increase in the stratification allows eggs to be kept in the oxygenated shallower waters above the OMZ where successful development can occur (Hidalgo and Escribano, 2008), below which the OMZ may act as an ecological barrier deficient in oxygen (BEDOX layer) (Donoso and Escribano, 2014).

Although different developmental stages might episodically encounter food limited conditions, food has been shown not to be a limiting factor for *C. chilensis* populations in northern Chile (Escribano, 1998; Torres and Escribano, 2003) whereas for *A. tonsa*, food limitation is unknown. In central-southern HCS, where upwelling is seasonal, variability in the production of *A. tonsa* is explained by temperature and food availability, indicating that food limitation can occur there (Vargas et al., 2006). In the semi-permanent upwelling off northern HCS, dominant copepods are persistent and abundant all year round (Hidalgo and Escribano, 2001). *C. chilensis* and *C. brachiatus* are examples of persistent species characterized by continuous cohorts and reproduction (Hidalgo and Escribano, 2008). Similarly, *A. tonsa* females were highly persistent in 2010 indicating that they also might not be food limited, and others factors such as low DO could be more relevant to the population dynamics of *A. tonsa*, mainly via decreased hatching success.

Experiments in systems that experience seasonal bottom hypoxia have shown that hypoxia increases mortality and reduces growth of *A. tonsa* and other copepods (Marcus et al., 2004; Richmond et al., 2006; Stalder and Marcus, 1997). This is consistent with our results (Fig. 5b, Table 2): *A. tonsa* populations from the Mejillones Bay and from coastal waters of Florida experienced *ca.* 50% mortality at concentrations of ~0.7 ml O_2 L^{-1} (Marcus et al., 2004; Table 2 in this study). On the other hand, congeners of *C. chilensis* show different responses. Females of *C. euxinus* from the Black sea are very tolerant to hypoxia with a survival of 75% at 0.47 ml O_2 $L^{-1}$and, a reduction in their respiration rates in low oxygen waters (Besiktepe et al., 2005) whereas females of *C. pacificus* from Puget Sound, Washington had high mortality (*c.a.* 90%) at DO levels below 1 ml O_2 L^{-1} (Keister and Grodzins, in prep.) perhaps because hypoxia in Puget Sound is less intense than in the Black Sea and the HCS (Besiktepe et al., 2005; Keister and Tuttle, 2013; Ulloa et al., 2012), guiding a local selective pressure to hypoxic conditions.

The mechanisms for adaptation to hypoxia in copepods are unknown. However, copepods that have the potential to perform DVM seem better adapted to stand strong vertical gradients in temperature and oxygen. For example, the large copepod (>4.5 mm, PL) *Eucalanus inermis* in Mejillones Bay have been described to move to deep water (>200) and probably in a lethargic mode staying within the OMZ (Hidalgo et al., 2005) or perhaps experiencing metabolic suppression as described for other organisms in OMZ systems (Siebel, 2011). By contrast, the small-sized copepod *A. tonsa* inhabit coastal waters with vertical distribution restricted to well oxygenated shallow waters above the OMZ (Escribano and Hidalgo, 2000; Escribano et al., 2009; Hidalgo et al., 2012, Hidalgo et al., 2010), whereas for *C. chilensis* (mid-sized copepod) recently has been reported to have a deeper distribution (Hirche et al., 2014) than *A. tonsa* in the HCS. *A. tonsa* seem less adapted to low oxygen waters than *C. chilensis*

The copepod community that inhabits Mejillones Bay is affected by the persistent upwelling occurring in the region (Escribano et al., 2012; Hidalgo and Escribano, 2008). Here, changes in temperature and stratification have been reported to be the main factors influencing their populations (Escribano et al., 1998, 2014), but DO could also be important (Fig. 7; Table 3), as Elliot et al (2012) suggest for the Gulf of Mexico. In the field, modulating factors do not act by themselves, individually, but are synergistic and diverse responses by the zooplankton community can be found. The two species in this study, *C. chilensis* and *A. tonsa*, respond differently to changes in DO, with *C. chilensis* seemingly better adapted to low oxygen than *A. tonsa*, an issue that could be balanced by the faster development of *A. tonsa*. The low DO concentrations associated with OMZ in the HCS could affect *A. tonsa* egg production and viability and be an important factor that modulates its population dynamics and structure. The negative correlations between species abundance, richness and diversity with OMZ depth (Hidalgo et al., 2010) suggest an important role of hypoxia in this ecosystem and also how the influence of the zooplankton respiration could decrease DO concentration helping to maintenance and increment the extent of the OMZ as an oxygen-deficient ecological barrier (Donoso and Escribano, 2014).

The upper depth of the OMZ in northern Chile determines the available habitat for copepods which reside in oxygenated surface waters. Deoxygenation of the surface layer could lead to an overall reduction in zooplankton biomass and to changes in species dominance through replacement of sensitive species with ones more tolerant to hypoxic conditions, or ones with faster development. Our results suggest that ecophysiological responses of dominant copepods of the HCS to environmental changes could be used to evaluate how global climate change may affect the pelagic realm in some of the most productive areas of the world, eastern boundary current systems.

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