Plankton community and copepod production in a temperate coastal lagoon: What is changing in a short temporal scale?

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ABSTRACT

Coastal lagoons are often exposed to intense short-term environmental changes and strong anthropogenic pressures influencing zooplanktonic communities and production. However, most works focus on long-term temporal scales using monthly or seasonal sampling strategies. The present study analysed the structure of the mesozooplanktonic assemblages, the production (egg production rates) and physiological condition (RNA:DNA ratio) of the copepod Acartia clausi in a temperate coastal lagoon (Ria Formosa) during the summer, using an intensive sampling approach. Salinity was the main factor affecting the short-term variability of mesozooplankton composition, followed by tidal phase (ebb tides) and semilunar cycle (spring tides). There was a positive relationship between the abundance of Appendicularia and the cladoceran Penilia avirostris with the toxic dinoflagellate Gymnodinium catenatum, suggesting no deleterious effects. The egg production rate of A. clausi was influenced by salinity and ammonia concentration, with a positive correlation between the egg productivity and the macronutrient, showing a possible adaptation of this calanoid species. The RNA:DNA index was positively related to egg production rate, suggesting that it is a good proxy for the reproductive output of copepods, even in short-term periods. This study shows that different timescales need to be included in regular monitoring of planktonic assemblages in coastal lagoons in order to understand the influence of environmental and anthropogenic variables on marine organisms.

1. Introduction

Coastal lagoons are shallow nutrient-rich ecosystems, being a typically unstable environment threatened by climate changes and usually under intense anthropogenic pressures (Barbosa and Chícharo, 2011). Planktonic organisms respond rapidly to modifications in the environment, therefore are considered good indicators of environmental change in the ecosystems. However, it is difficult to infer the specific links between environmental variability and plankton dynamics in coastal lagoons. Tidal dynamics is usually strong and the major mechanical energy input in coastal lagoons, forcing water circulation through turbulent mixing and driving the physical, chemical and biological interactions inside these ecosystems (Schelske and Odum, 1962). Besides changes in tides, daily variability in wind stress on the water surface and freshwater input generally provide part of the

mechanical energy necessary for the structuring of coastal lagoons (Barbosa, 2010).

The dynamics of planktonic communities in transitional systems highlights the unsteadiness of their spatial and temporal features. Most studies in these areas are developed under a monthly sampling strategy (e.g. Primo et al., 2009; Vieira et al., 2015), missing the short-term changes in the structure and production in those ecosystems, related to circatidal, circadian and circalunidian periodicities (Last et al., 2009). Although there is a significant number of studies on zooplankton communities in coastal lagoons worldwide, the patterns of planktonic production and the trophic interactions of their assemblages are poorly known (e.g. Repelin, 1985; Heerkloss et al., 1991; Sprung, 1994; Marques, 2005).

Planktonic communities can be quite distinct between coastal lagoons located in the same geographic area, depending on their

interactions with the sea, local hydrodynamics and the influence of other physical parameters. These communities have a relevant role in sustaining the functioning and productivity of these areas, serving as a breeding and feeding ground for many species of fish and birds, and supporting a wide range of human activities, such as fisheries, shellfish farming and tourism (Barbosa, 2010). There are several examples of planktivorous fishes using these coastal lagoons as nursery areas (e.g. anchovies, sardines and some sparids), or living here as residents (e.g. atherinids) (Chícharo et al., 2012). The planktonic production of these areas also supports the important production of bivalves (Chícharo and Chícharo, 2001). At the same time, these planktonic assemblages can also be a threat to the ecosystem services of coastal lagoons, when noxious blooms occur, including toxic microalgae that threaten higher trophic levels, zooplankton and bivalves (Cerejo and Dias, 2007), or harmful jellies that quickly consume early phases of fishes in nursery areas (Pereira et al., 2014). Therefore, the planktonic dynamics and production of these ecosystems need to be investigated in a significantly higher temporal resolution than the one usually used to study them.

Copepoda is the dominant group of mesozooplanktonic communities (Mauchline, 1998) and represents the major link between microbial food webs and higher trophic levels (Kiørboe, 1997). Among Copepoda, the genus *Acartia* is one of the most ubiquitous and abundant inhabiting coastal lagoons and estuarine systems (Azeiteiro et al., 2005; Leandro et al., 2007), thus, it is extremely important to estimate its secondary production. The most common technique used to estimate secondary production in laboratory and field studies is the egg production rate method (EPR) (Runge and Roff, 2000), that indicates the current nutritional status of wild caught copepods as well as their adaptations to environmental variability over a short period of time (~24 h). However, in the last decades, biochemical approaches such as the determination of RNA:DNA ratio have been developed to assess the physiological condition of copepods (Chícharo and Chícharo, 2008), as an indicator of growth (Elser et al., 2000; Wagner et al., 2001), nutritional condition (Wagner et al., 1998; Vehmaa et al., 2012), dormant condition (Kobari et al., 2013) and egg viability (Hogfors et al., 2011). Additionally, the RNA:DNA ratio has been shown to be correlated with female egg production (Nakata et al., 1994; Saiz et al., 1998; Gorokhova, 2003; Cruz et al., 2017), making it a potential alternative method for the estimation of production, with several advantages over the traditional techniques such as sensitivity, precision and repeatability.

In this study, we aim to analyse the short-term variability of the mesozooplanktonic assemblage structure in a temperate coastal lagoon (Ria Formosa) during the peak production period that occurs during summer, which is typical of the unimodal annual cycle of planktonic production that occurs in the temperate coastal lagoons (Barbosa and Chícharo, 2011). For that, we investigate the correlation between the mesozooplankton dynamics and the environmental conditions (abiotic and biotic parameters). Particularly, the production (EPR and female's secondary production) and physiological condition (RNA:DNA ratio) of *Acartia clausi* are assessed in relation to the main hydrological parameters and phytoplanktonic prey availability.

2. Methods

2.1. Study area

Ria Formosa is a highly productive system of great ecological and economic importance recognized by the Ramsar Convention and Natura 2000, being a National Park since 1987. It is a mesotidal coastal lagoon system located in the south of Portugal with approximately 55 km length and a maximum 6 km width, with a total wetland of circa 110 km², five sandy barrier islands and being permanently connected with the Atlantic Ocean by six inlets (Fig. 1). The tidal range varies between 0.5 m at neap tides and 3.5 m during spring tides, causing semidiurnal and fortnightly tidal amplitudes variations (Falcão and

Vale, 1990). It is a considerably shallow system with average depths of 2 m, reaching an average of 6 m at the main navigable channels (Newton and Mudge, 2003). The freshwater inputs are scarce except for few months in autumn and winter when rainfall occurs, leading to low run-offs. The salinity of the water ranges on average from 32 to 36.5 and the hydrodynamic circulation is dominated by the tidal cycle (Newton and Mudge, 2003). The climate is predominantly Mediterranean, characterized by hot dry summers and warm wet winters.

2.2. Sampling

Sampling occurred in a fixed station (37°00′16.91″N - 7°59′14.13″W) in the Ria Formosa lagoon system from July 28th to September 3rd, 2009 (Fig. 1). Mesozooplankton samples were collected with a WP2 conical net with 0.13 m² mouth opening and 200 μm mesh size, horizontally towed just below the sea surface, for 5 min at approximately 2 knots, equipped with a HydroBios flow meter. Every day and for 19 days, two hauls were taken in the morning around 11h00, and in the afternoon around 16h00. At the end of each haul, the cod end content was preserved in 4% buffered formalin for posterior determination of zooplankton composition and abundance. Environmental variables such as temperature, salinity, pH and dissolved oxygen were measured with a hand-held meter (VWR Symphony SP90M5). Precipitation data during the sampling period was obtained from the *Sistema Nacional Informação Recursos Hídricos* (http://snirh.pt/). Surface water samples were taken for the determination of nutrients concentration (nitrates, nitrites, ammonia, phosphates, and silica) and for identification and quantification of the major microplankton taxa. The dissolved inorganic macronutrients concentrations were determined according to a spectrophotometric method using Spectroquant cell test photometric kits (Merck Millipore) and the Spectroquant Nova 60 photometer (Merck Millipore). Samples of microplankton were preserved with acid Lugol's solution and subsamples of 50 ml were concentrated by gravimetric sedimentation by the Utermöhl technique (Hasle, 1978) and observed using a Zeiss IM35 inverted microscope with phase contrast and bright field illumination. The carbon content of the main microplankton groups were calculated based on the equations given in Smayda (1978), using the cells measurements performed by Garrido et al. (2008). Zooplankton samples were fractioned with a Folsom plankton splitter, and at least 500 organisms were counted and identified under a Leica S8 APO stereoscopic microscope. The abundance of taxa was expressed as the number of individuals per cubic meter (ind m^{-3}).

To obtain the total biomass, the displacement volume was calculated and converted to dry weight using the following equation (Wiebe, 1988):

$$\text{Log}_{10}(DV) = -1.842 + 0.865\,\text{Log}_{10}(DW)$$

where *DV* is the displacement volume (mL) and *DW* is dry weight (mg).

2.3. Egg production experiments

Egg production rates (EPR) experiments were performed for 12 days within the sampling period during high tide. To catch females of the free-spawning copepod *Acartia clausi* an additional tow was conducted with the same conical net, but with a lower velocity to avoid copepods disturbance. The cod-end content was gently transferred to a clean insulated container and diluted with surface seawater for transport to the laboratory. Then, females were gently sorted with the help of a glass pipette and placed in 500 ml glass goblets (experimental units), already filled with natural water sieved through a 50 μm mesh to remove any copepod eggs and metazoan zooplankton prior to the experiments. Six experimental units (500 ml goblets) were used at each experimental day, and 5 to 8 undamaged and actively swimming females were placed in each. The experiment lasted for 24 h, after which all the females were removed by sieving the water through a 200 μm mesh and placed in liquid nitrogen for posterior RNA:DNA ratio analysis. Additionally, all
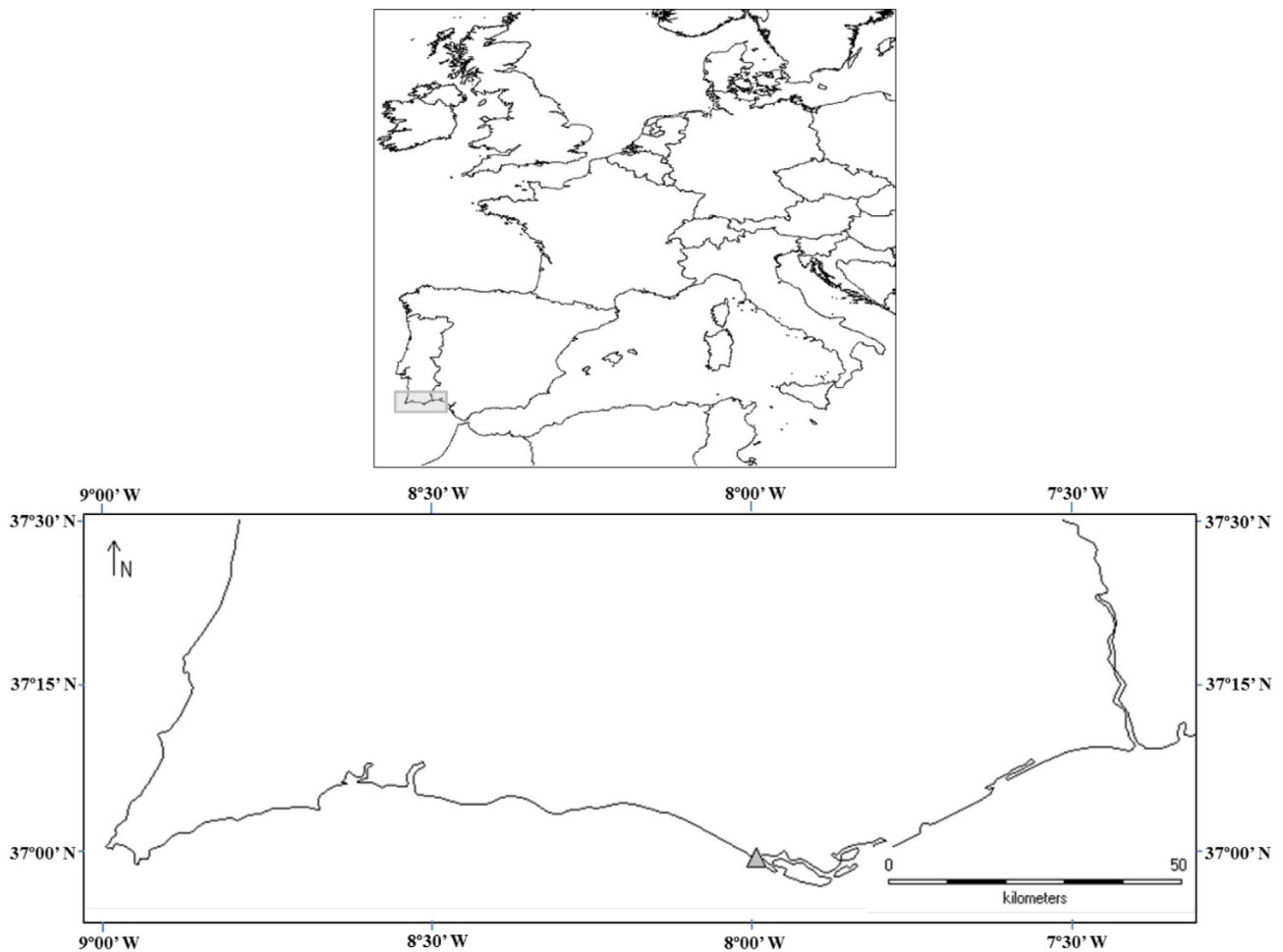
Fig. 1. Map of the studied area with the fixed sampling station located in the western part of Ria Formosa Coastal lagoon (light grey triangle).

the existing eggs and nauplii were counted to determine the egg production rate.

The Carbon-specific egg production rates (*SEP*) of *Acartia clausi* was calculated according to the equation:

$$SEP = EPR \times \frac{We}{Wf}$$

where *EPR* is the number of eggs female^{-1} day^{-1}, *We* is the egg carbon content and *Wf* is the female carbon biomass. Egg carbon content was assumed to be 0.04 µg C egg^{-1} (Kiørboe and Sabatini, 1995). Female carbon weights were estimated from prosome lengths (PL) using the equation log(Wf) = 3.055*log(PL)$^{-8.444}$ (Ayukai, 1987). The biomass was calculated by multiplying the female carbon weight by the abundance of females. Female copepod production was then calculated by multiplying the female biomass by SEP.

2.4. RNA:DNA ratio analysis

RNA and DNA of adult females of *Acartia clausi* used in the EPR experiments were estimated with the microplate fluorescent assay (MFA) of Ikeda et al. (2007). The MFA assay is a modification of the sequential fluorometric method of Bentle et al. (1981) in which DNA and RNA in a single sample are determined sequentially by the addition of DNase and RNase. The sequential fluorometric method was modified to the MFA with 96-well microtiter plates and the DNase step was eliminated using a sarcosyl extraction technique (Wagner et al., 1998).

The wet weight (WW) of a batch of 5 to 30 females was determined to obtain at least 0.5 mg of WW. The organisms were homogenized by sonication (3 pulses of 50 A during 1 min) with cold sarcosyl extraction

buffer. The volume of extraction buffer was 100 µl (0.5%). Afterwards, all the samples were shaken for 30 min at room temperature on a vortex mixer equipped with a multiple-vial head. Next, they were centrifuged (12,000 × *g*; 0–4 °C) for 15 min to separate insoluble copepods remains. The samples were subsequently diluted 1:10 with Tris buffer to reduce the sarcosyl concentration to 0.05%. In each run, duplicate 50 µl aliquots of supernatants of the samples and duplicates of 0, 0.6, 1.1, 1.7 and 2.3 µg ml^{-1} DNA standard solutions (λ-phagus 0.25 µg µl^{-1} from Roche) and 0, 3.6, 7.3, 10.9 and 14.6 µg ml^{-1} RNA standard solutions (16s–23s *E. coli* 4 µg µl^{-1} from Roche) were transferred to 96-well microplates (type nuclon black round bottom). The mean ratio of the slopes of the standard curves was 2.5 ± 0.2, which allows comparing the RNA/DNA ratio results determined by other protocols (Caldarone et al., 2006). The fluorescence was then scanned after addition of the fluorescent dye on a microplate reader (Biotek synergy HT model SIAFRTD) with 360 nm (excitation) and 590 nm (emission) (first scan-total fluorescence RNA and DNA). After the first reading, RNase solution (15 µl, 0.12 µg ml^{-1}) was added to each well and incubated at 37 °C for 30 min. The concentration of DNA was calculated directly by the standard curve. The concentration of RNA was determined indirectly by subtraction of DNA fluorescence (second scan) from total fluorescence (first scan).

2.5. Data analysis

The diversity of the mesozooplankton communities was determined as the number of species (species richness) and the Shannon-Wiener index, using PRIMER-6 software (Clarke and Gorley, 2006).

After the exploratory analysis of the mesozooplankton data using linear models, the data distributions were checked. Generalized Additive Mixed Models (GAMMs) were used to evaluate the potential contribution of selected independent environmental variables (water temperature, salinity, dissolved oxygen, food availability, nutrients, tidal phase and tidal coefficient) in explaining the variability of the dependent variables (abundance of the main mesozooplankton taxa). GAMMs are a flexible class of statistical predictive models which allow nonlinear relationships between a set of predictors and a dependent variable, using data not collected according to a balanced design and dealing with heterogeneity or temporal correlation in the counts. The models were fitted in the open source software R 2.15.3 (R Development Core Team, 2013), using the gamm function from the mgcv library (Wood, 2006), with all smoothness parameters estimated using restricted maximum likelihood (REML). Smoothing splines were used to represent the nonlinear effect of the predictors. The tidal phase was used as a factor, attributing it to each sampling at each specific time. The dependent variable was modelled using Gaussian distribution functions with a logarithmic link. The temporal auto-correlation of the data was treated with an autoregressive model of order one (AR-1) (from the nlme library for R). From the full set of calculated models (considering different explanatory variables and lognormal distribution functions), we selected the best models, and thereby the explanatory variables most likely responsible for the variability of mesozooplankton abundances, based on Akaike Information Criterion (AIC; Sakamoto et al., 1986). AIC gives information about the degree of fit of a model with the number of variables, to find the most parsimonious model. The statistical significance of the terms in the model (based on the approximate p-values produced by GAMM) was also considered. All the dependent variables were log transformed prior the analysis.

Generalized Linear Models (GLM; Venables and Ripley, 2002) were used to analyse the variability of EPR and RNA:DNA ratio of *Acartia clausi*. For both response variables, a Gaussian GLM was used with an identity link. The following predictor variables were considered for EPR: temperature, salinity, dissolved oxygen, food availability and nutrients. To analyse the RNA:DNA ratio variability and possible relation to egg production rates, the same independent variables were used adding the EPR. Model predictors were selected and removed by backward elimination based on the AIC. Only those predictors which contributed significantly to the model were kept. All the model analyses were also performed using the open source software R 2.15.3 (R Development Core Team, 2013).

3. Results

3.1. Environmental conditions and food availability

Water temperature ranged from 20.8 and 27.8 °C and salinity from 34.3 to 37.6, the latter with lower values registered at the end of August and beginning of September (Fig. 2). Dissolved oxygen ranged from 4.2 to 8.7 mg L^{-1} and pH between 8 and 8.3 (see Table S1, Supplementary material). Ammonia showed a minimum value of 0.04 mg L^{-1} and a maximum of 0.68 mg L^{-1} (Fig. 2), nitrites with a minimum of 0.002 mg L^{-1} and a maximum of 0.056 mg L^{-1}, nitrates with values ranging from 0.03 to 0.24 mg L^{-1}, phosphate concentrations from 0.04 to 0.21 mg L^{-1}, and silica concentrations between 0.06 and 0.85 mg L^{-1} (see Table S1, Supplementary material). All the macronutrients increased the concentrations with time, showing maximum values at the end of August. There was no precipitation during all the sampling period.

Considering the carbon content of the main groups of microplankton (Fig. 3; Table 1), dinoflagellates were clearly dominant during this study, with densities ranging from 1.6 to 802.8 µg C L^{-1}, with higher values occurring at the end of the sampling period (see Table S2, Supplementary material). The most abundant taxa were *Protoperidinium* spp., *Protoperidinium quinquecorne*, *Gymnodinium* spp., *Gymnodinium*
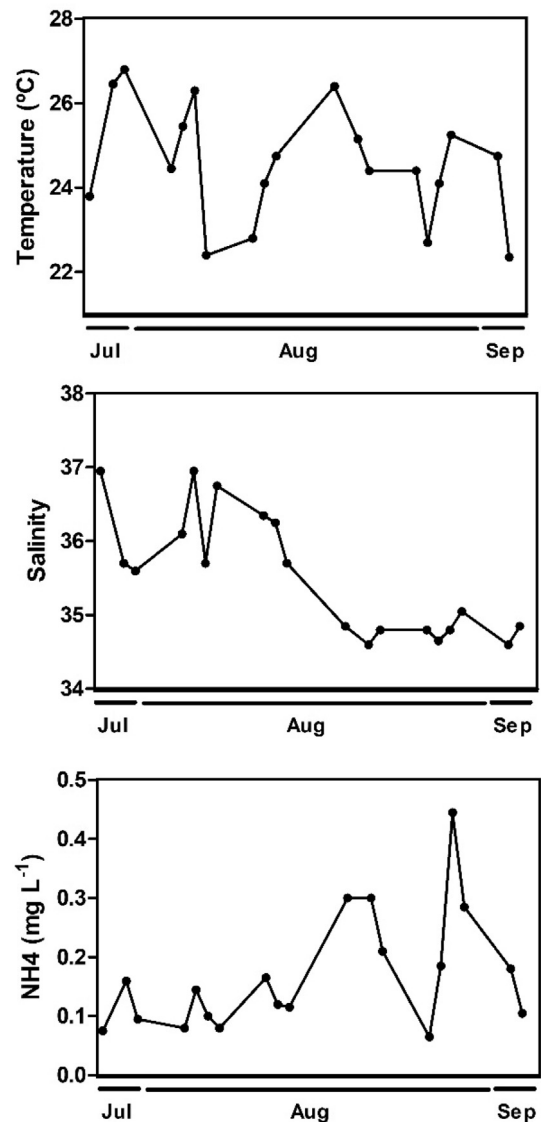

Fig. 2. Environmental parameters measured in Ria Formosa lagoon during the sampling days from July 28th to September 3rd, 2009, that influenced significantly the zooplankton community and production (Temperature °C, Salinity, NH4 – ammonia mg L^{-1}).

catenatum, *Scrippsiella trochoidea* and *Prorocentrum* spp. (Fig. 3; Table 1). The mean carbon content of ciliates oscillated throughout the sampling period, with a minimum value of 2.2 and a maximum of 72.4 µg C L^{-1}, while the diatoms were the least abundant group (minimum: 0.3 µg C L^{-1} maximum: 16.4 µg C L^{-1}) (Fig. 3). The most representative taxa of diatoms were *Rhizosolenia* spp. and *Leptocylindrus* spp. (Table 1).

3.2. Mesozooplankton biomass, diversity and abundance: environmental conditions influence

The biomass of mesozooplankton did not show any clear pattern with time and ranged from 3.3 to 208.8 mg m^{-3}. The Shannon-Wiener diversity index presented values between 1.1 and 2.3, while species richness varied between a minimum of 11 to a maximum of 24 taxa (see Table S3, Supplementary material), and these indices did not show any clear pattern with time, as well.

Total mesozooplankton abundance was on average 6220.9 ± 9225.8 ind m^{-3} ranging between 206.4 and 38970.0 ind m^{-3}, and higher values were registered at the end of August and beginning of September (Table 2; see Table S4, Supplementary material)).
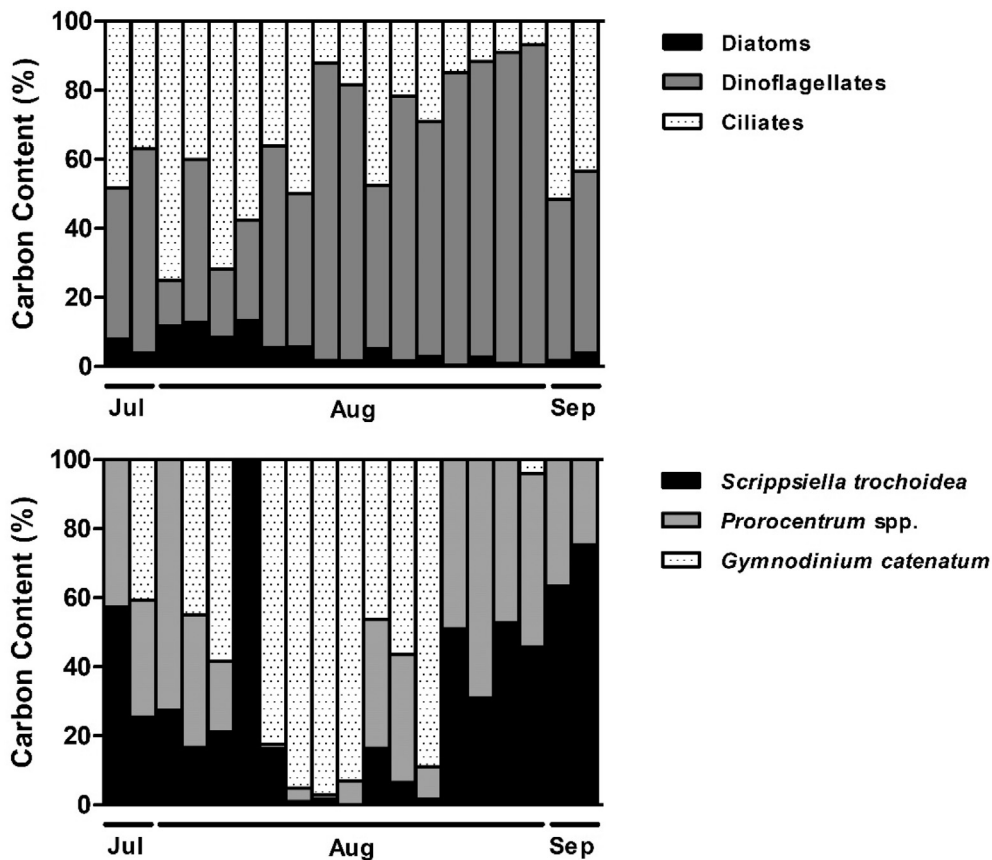
Fig. 3. Daily average of carbon content (µg C L^{-1}) of the main microplankton groups and dinoflagellates species identified in Ria Formosa from July 28th to September 3rd, 2009, that influenced significantly the zooplankton community and production. Data are represented as %.

Table 1
Mean carbon content (µg C L^{-1}) of the main microplankton taxa identified in Ria Formosa lagoon from July 28th (28J) to September 3rd (03S), 2009.

Taxa	Mean ± SD
Diatoms	3.8 ± 3.1
Rhizosolenia spp.	2.7 ± 2.4
Leptocilyndrus spp.	0.6 ± 2.5
Dinoflagellates	102.4 ± 157.6
Scrippsiella trochoidea	0.8 ± 1.4
Protoperidinium spp.	36.4 ± 134.7
Protoperidinium quinquecorne	38.0 ± 73.1
Gymnodinium spp.	8.5 ± 17.3
Gymnodinium catenatum	1.8 ± 4.3
Prorocentrum spp.	1.0 ± 1.4
Ciliates	30.6 ± 18.8

Table 2
Mean abundances (ind.m^{-3}) of mesozooplankton taxa identified in Ria Formosa lagoon from July 28th to September 3rd, 2009.

Taxa	Mean ± SD	Taxa	Mean ± SD
Hydromedusae	2.0 ± 5.9	*Corycaeus* spp.	5.1 ± 22.0
Siphonophora	0.3 ± 1.8	Harpacticoida	1103.8 ± 1614.1
Mollusca	1144.3 ± 2930.8	*Euterpina acutifrons*	158.6 ± 359.7
Gastropoda larvae	1131.7 ± 2934.5	*Clytemnestra* spp.	5.6 ± 19.9
Bivalvia larvae	12.6 ± 29.3	*Microsetella* spp.	0.1 ± 0.6
Polychaeta larvae	6.3 ± 14.1	Cirripedia	131.0 ± 187.1
Amphipoda	104.8 ± 139.2	Nauplii	105.9 ± 169.7
Isopoda	4.0 ± 9.8	Cyprids	25.1 ± 38.5
Cladocera	1242.4 ± 2824.4	Ostracoda	0.8 ± 3.4
Podon spp.	392.7 ± 971.5	Cumacea	0.2 ± 1.0
Evadne spp.	52.7 ± 119.1	Decapoda	36.8 ± 65.6
Penilia avirostris	797.1 ± 2122.1	Caridea	8.2 ± 33.2
Copepoda	3453.7 ± 4592.0	Thalassinidae	0.04 ± 0.2
Nauplii	125.3 ± 203.5	Anomura	17.8 ± 45.1
Paracalanus spp.	204.1 ± 354.3	Brachyura	10.7 ± 16.6
Clausocalanus spp.	0.6 ± 3.1	Chaetognatha	11.2 ± 26.0
Acartia clausi	394.0 ± 821.4	Doliolida	9.1 ± 48.9
Paracartia grani	6.6 ± 39.7	Appendicularia	51.1 ± 154.8
Temora longicornis	2.0 ± 7.9	Ascidiacea	22.5 ± 32.3
Temora stylifera	1.9 ± 8.2	Ichthyoplankton	0.5 ± 0.9
Centropages chierchiae	737.9 ± 2320.4	Eggs	0.1 ± 0.3
Pontellidae	44.6 ± 116.7	Larvae	0.4 ± 0.9
Oithona spp.	634.8 ± 1630.4	Total Zooplankton	6220.9 ± 9225.8
Oncaea spp.	28.8 ± 86.7		

A total of 41 taxa of mesozooplankton were identified during the sampling period (Table 2). Copepoda was the most abundant group with an average of 3453.7 ± 4592.0 ind m^{-3}, followed by Cladocera (1242.4 ± 2824.4 ind m^{-3}; Fig. 4), Mollusca larvae (1144.3 ± 2930.8 ind m^{-3}) and Cirripedia larvae (131.0 ± 187.1 ind m^{-3}; Fig. 4). Copepoda abundance showed higher peaks in the second half of the sampling period (Fig. 4), very similar to the total zooplankton abundances pattern, and the dominant species were the Calanoida *Centropages chierchiae* (21.4%), *Acartia clausi* (11.4%), *Paracalanus* spp. (5.9%), the Cyclopoida *Oithona* spp. (18.4%) and the Harpacticoida *Euterpina acutifrons* (4.6%) (Fig. 5; Table 2).

Salinity was the most important environmental factor that explained the distribution of most mesozooplankton taxa (Table 3). This parameter influenced the abundance of Copepoda, as well as of particular
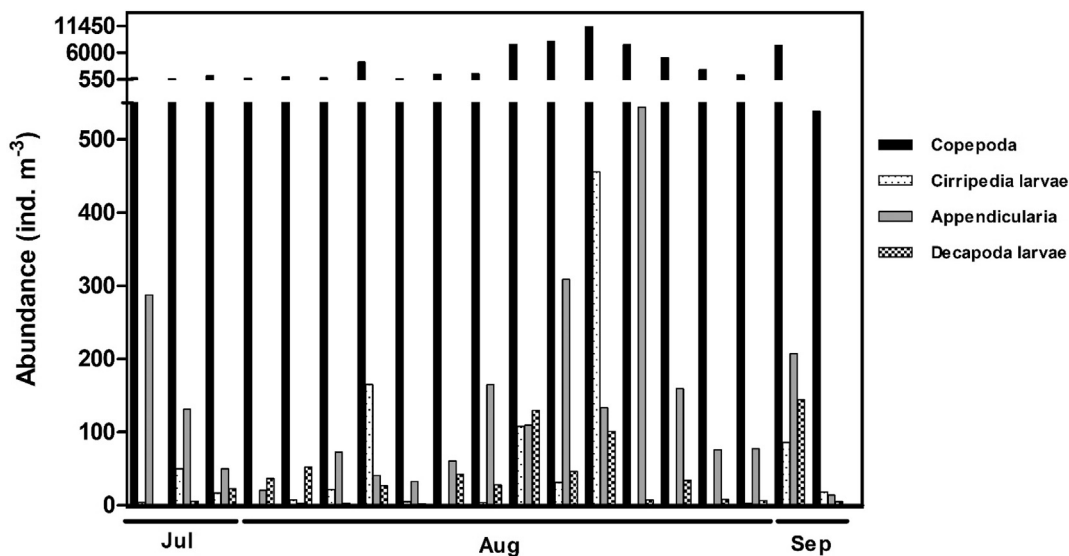
Fig. 4. Daily average abundances (ind.m^{-3}) of the main mesozooplankton taxa in Ria Formosa lagoon from July 28th to September 3rd, 2009, that were significantly influenced by the environmental conditions.
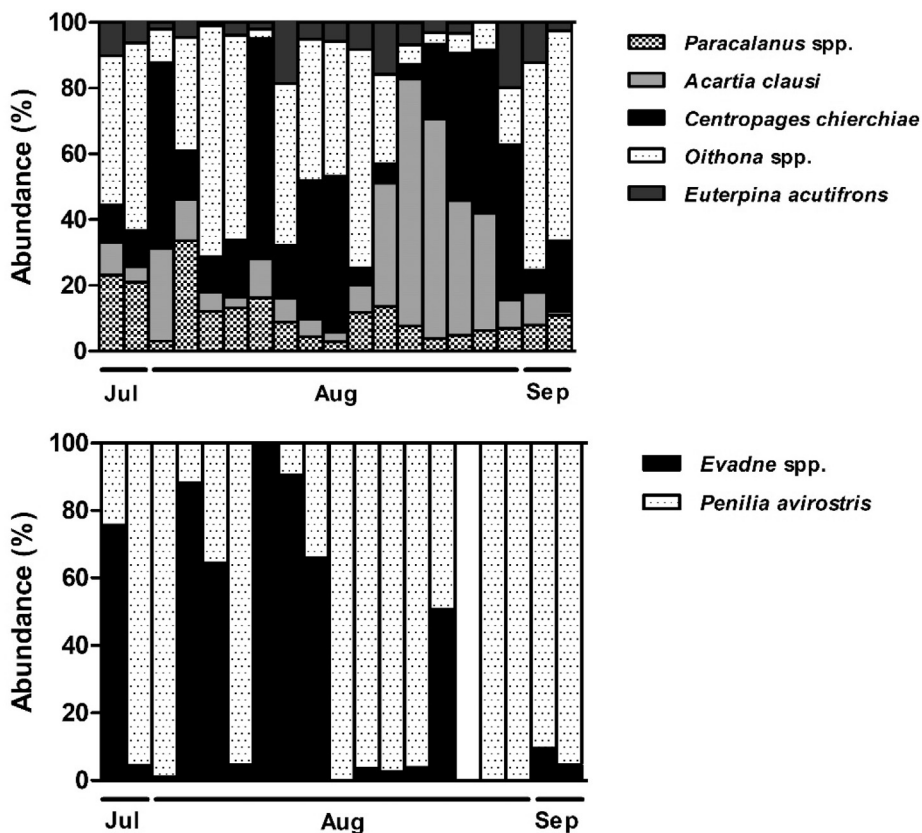

Fig. 5. Abundances (ind.m^{-3}) of the main mesozooplankton species in Ria Formosa lagoon from July 28th to September 3rd, 2009, that were significantly influenced by the environmental conditions. Data are represented as %.

species within this group, such as *Paracalanus* spp., *Acartia clausi*, *Centropages chierchiae*, *Oithona* spp. and *Euterpina acutifrons*, but also influenced Cirripedia larvae. The abundance of all taxa seemed to respond to an optimal salinity around 34.5–35 and then decreased towards higher salinities. The tidal phase explained also the abundances of Copepoda (*Paracalanus* spp., *A. clausi*, *C. chierchiae* and *Oithona* spp.), Decapoda larvae (Fig. 4) and the species richness being positively related to ebb tides, negatively related to low tides and in some cases also to flood tides (*A. clausi*, Decapoda larvae and species richness)

(Table 3). Tidal coefficient significantly influenced Copepoda (*Oithona* spp. and *E. acutifrons*) and Decapoda larvae, the three showing higher abundances when the tidal range increased, i.e. during spring tides (Table 3). Appendicularia (Fig. 4) registered higher abundances around 27 °C and was the only taxa influenced by the increase in temperature (Table 3). The abundance of the toxic dinoflagellate *Gymnodinium catenatum* was positively related to the abundance of Appendicularia and the cladoceran *Penilia avirostris* (Table 3), while two other dinoflagellates, *Scrippsiella trochoidea* and *Prorocentrum* spp., were

Table 3
Results of Generalized Additive Mixed Models (GAMMs), species richness (SR) and abundance of mesozooplankton taxa in ind. m^{-3} (Copep – Copepoda; *Parac.* – *Paracalanus* spp.; *Acart.* – *Acartia clausi*; *Centrop.* – *Centropages chierchiae*; *Oith* – *Oithona* spp.; *Euterp.* – *Euterpina acutifrons*; *Evad.* – *Evadne* spp.; *Penil.* – *Penilia avirostris*; Append. – Appendicularia; Decap. – Decapoda larvae; Cirrip. – Cirripedia larvae) with indication of significant explanatory variables: tidal phase as a factorial parameter (F – Flood; H – High; L – Low) and smooth terms (T – temperature °C; S – salinity; TC – tidal coefficient m; Diat – diatoms µg C L^{-1}; Scripp – *Scrippsiella trochoidea* µg C L^{-1}; Proroc - *Prorocentrum* spp. µg C L^{-1}; Gymnc– *Gymnodinium catenatum* µg C L^{-1}. AIC is the Akaike Information Criterion. (Signif. codes: '***' $p < .001$ '**' $p < .01$ '*' $p < .05$).

Taxa	SR	Copep.	Parac	Acart	Centrop	Oith	Euterp	Evad	Penil	Append	Decap	Cirrip
Parametric coefficients												
Tide E	18.7***	28.5***	9.0 ***	13.6***	6.8***	12.9***					6.9***	
Tide F	−3.5**	−1.4	−0.8	−3.7***	−1.4	−1.4					−3.1**	
Tide H	−0.2	−2.0	0.4	−1.4	0.1	0.2					−1.5	
Tide L	−2.7*	−3.0**	−3.1**	−5.0***	−2.4*	−5.7***					−3.5**	
Smooth terms												
s(T)										6.1**		
s(S)		7.7***	3.7*	5.2**	2.9*	5.0*	3.6*					5.3*
s(TC)		16.1***				14.6***	12.6**				6.6*	
s(Diat)								5.2**				
s(Scripp)								8.0***				
s(Proroc)									3.1*			
s(Gymnc)									4.4*	15.9***		
AIC		142.9	147.5	142.9	172.0	140.7	160.3	164.0	192.7	154.7	150.9	139.8

significantly related to the variability in the abundance of the cladocerans *Evadne* spp. and *P. avirostris*, respectively, presenting higher abundances when the carbon content of the two dinoflagellate genus increased (Table 3). Higher biomass of total Diatoms influenced *Evadne* spp. abundance.

3.3. *Acartia clausi* EPR, RNA:DNA ratio and secondary production

The abundance of *Acartia clausi* females varied throughout the studied period, with maximum values of 662.5 ind. m^{-3}, a minimum of 7.3 ind. m^{-3}, and an average of 128.6 ± 178.4 ind. m^{-3} (Fig. 6a). The higher abundances were found at the end of August and beginning of September. EPR of *Acartia clausi* varied between 2 ± 0.6 and 12.5 ± 2.2 eggs female^{-1} day^{-1}, with an average of 7.5 ± 2.9 eggs female^{-1} day^{-1} (Fig. 6b). RNA:DNA ratio ranged from 0.1 and 3.6, with an average of 1.8 ± 1.3 and higher values registered during the second half of August and beginning of September (Fig. 6c). The secondary production of females reflected their biomass and varied between 1.73 and 207.8 µg C m^{-3} day^{-1} and an average summer production of 42.6 ± 59 µg C m^{-3} day^{-1}. Higher values were found at the end of the sampling period (Fig. 6d).

Total EPR of *Acartia clausi* was statistically significant and positively related to salinity and ammonium (Table 4). The RNA:DNA ratio was significantly and positively related to EPR, and negatively related to temperature (Table 4).

4. Discussion

4.1. Mesozooplankton community: influence of environmental conditions

Salinity has been considered an important factor influencing zooplankton community structure, especially in estuarine and coastal environments that are subject of constant changes (Gunter, 1961; Day et al., 1989; Greenwald and Hurlbert, 1993). In the present study, salinity was one of the main factors affecting the abundances and variability of several taxa, including Copepoda (*Paracalanus* spp., *Acartia clausi*, *Centropages chierchiae*, *Oithona* spp. and *Euterpina acutifrons*) and Cirripedia larvae. The optimal salinity that favoured higher abundances of all the referred taxa was around 34.5–35, above which the abundances started to decrease. This is in accordance to a previous study in the northern Adriatic Sea with salinities ranging up to ~38, where a negative correlation was found between the abundance of

calanoid species and salinity, with species such as *Acartia clausi* and *Centropages kroyeri* preferring lower salinities (Bojanić Varezić et al., 2015). Regarding the Cirripedia, several studies have shown that salinity is also one of the main factors influencing their early life stages (Anil and Kurian, 1996; Nasrolahi et al., 2016). In fact, the lower values of the copepod species and Cirripedia larvae abundances observed at the beginning of the present study indicate that the salinity values (> 36) inside the Ria Formosa lagoon were above their optimal metabolic conditions. There was no precipitation during the sampling period and the scarce and small rivers that provide freshwater inputs in this area are usually dry during summer months (Newton and Mudge, 2003). Therefore, salinity was considerably high during most of the sampling consequence of shallow depth and high temperatures leading to increase evaporation, as well as by the inflow of seawater through the tidal cycle. During the last days of the sampling period, salinity values were slightly lower (~34–35), which can be explained by the Submarine Groundwater Discharge (SGD). This discharge can be defined as any and all flow of water on continental margins from the seabed to the coastal ocean, regardless of the fluid composition or driving force (Burnett et al., 2003) and usually leads to an augment of nutrients concentrations in the area, that may increase the occurrence of algal blooms (Baptista, 1993). In fact, there was an increase of the majority of nutrients at the end of August and beginning of September, and the occurrence of harmful algal blooms, such as *Dynophysis acuta* and *Gymnodinium catenatum*, during August 2009 (Brito et al., 2012), supporting the idea of an SGD. The occurrence of SGD in the studied area was also reported by Leote et al. (2008) and Ibánhez et al. (2013), suggesting it as an important nutrient source to Ria Formosa.

As expected, the tidal cycle influenced the abundance of several zooplanktonic taxa in the lagoon. Neritic copepod species such as *Paracalanus parvus*, *Acartia clausi*, *Centropages chierchiae* and *Oithona* spp., as well as species richness showed a high correlation with the ebb tidal phase. A previous work has shown a tendency for higher copepod abundances towards the outer lagoon, particularly of neritic calanoids and cyclopoids (Marques, 2005). The lower abundances at the inner lagoon can be explained by the fact that they may be avoiding a deeper transport into it, moving to lower depths during flood tide and ascending to upper water column on ebb flows. In fact, the present sampling station is in an intermediate place between the inlet and the inner lagoon, and these copepods can use this strategy to prevent from penetrating into the lagoon and rapidly return to the open sea. Ebb currents are generally faster than flood ones in the main inner channels
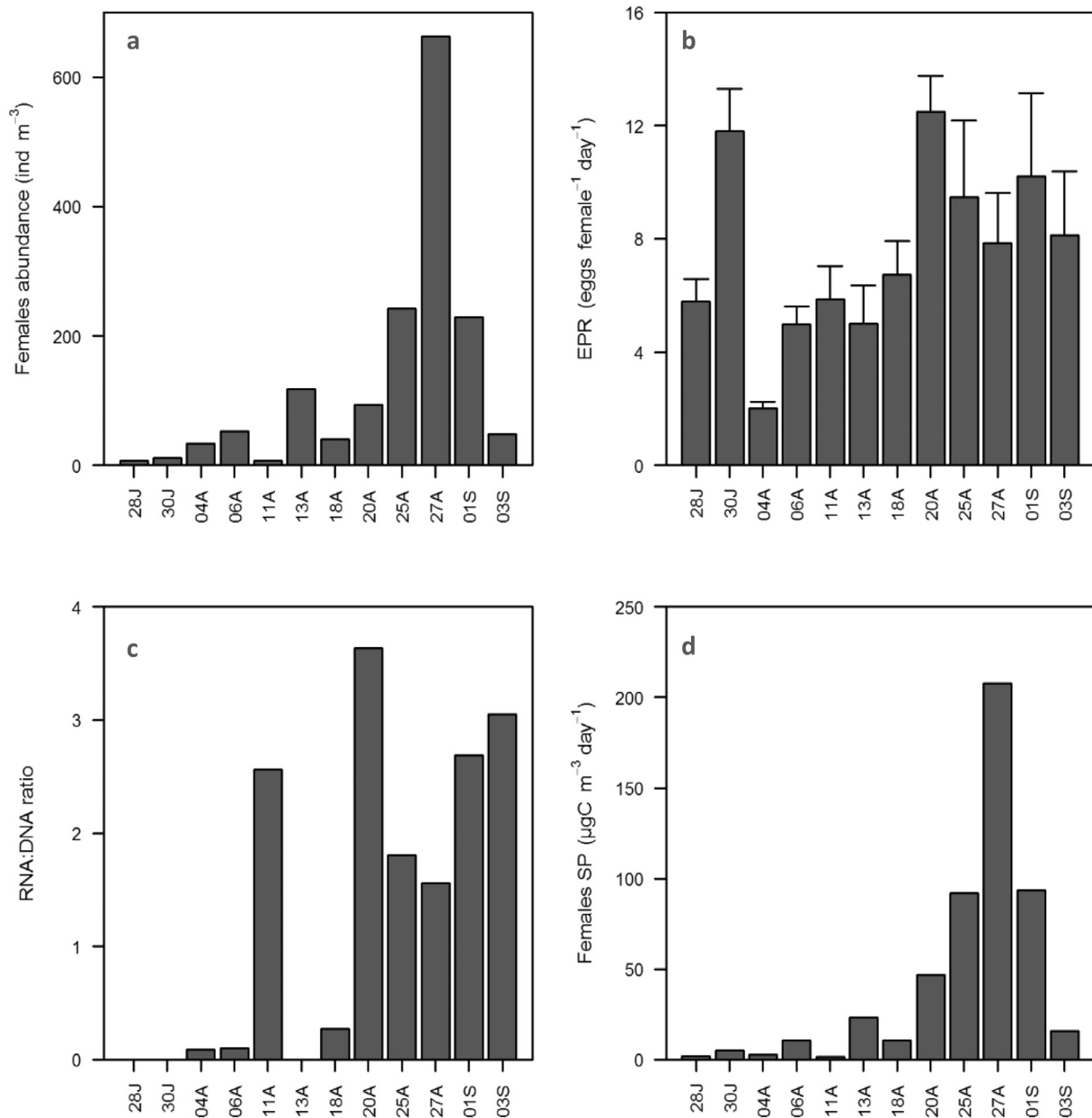
Fig. 6. *Acartia clausi* females abundance (ind.m^{-3}) (a), egg production rates (eggs female^{-1} day^{-1}) (b), RNA:DNA ratio (c) and females secondary production (μg C m^{-3} day^{-1}) (d) in Ria Formosa lagoon during twelve sampling days from 28th July (28J) to 3rd September (03S), 2009.

(Pacheco et al., 2010), favouring this behaviour. Decapod larvae abundance was also correlated to the ebb flow, which is in accordance with previous works showing that these early life stages selectively use tidal currents to disperse into the open sea and return to the lagoon or estuary during later larval stages to settle (Brookins and Epifanio, 1985; Morgan and Christy, 1997). The tidal coefficient also explained the variability of Copepoda (*Oithona* spp. and *Euterpina acutifrons*) and Decapoda larvae. The abundances of these taxa were higher when there was an increase of the tidal coefficients (spring tides). During spring tides, the studied area is mainly dominated by ebb tides presenting higher mean velocities when compared to flood tides (Pacheco et al., 2010), which can be related to higher copepod abundances occurring during these periods. Also, many decapods release their larvae to the environment on a semilunar cycle frequency when the tidal coefficient is higher (spring tides) (Forward Jr, 1987; Flores et al., 2007), the same pattern showed by decapods during the present study.

During the summer period of the studied area, temperature affected

the abundance of Appendicularia (*Oikopleura* spp.) species. Previous studies on appendicularians showed that temperature is one of the main factors influencing population dynamics (Troedsson et al., 2002; Troedsson et al., 2013). Short generation times and high maximum rate of lifetime reproductive fitness influenced by the increase of temperature and food concentrations together with a low number of predators would induce a higher abundance of Appendicularia (Deibel and Lowen, 2012). However, during the studied period, the presence of potential predators such as copepods (López-Urrutia et al., 2004) in high densities must have limited Appendicularia abundance, despite the increase when the temperature raised. Additionally, there was a positive correlation between the abundances of *Oikopleura* spp. and *Gymnodinium catenatum*, despite the toxicity produced by the latter. This result is in accordance with the study conducted by Badylak and Phlips (2008) during a harmful algal bloom event where *Oikopleura dioica* abundances mimicked the toxic dinoflagellate *Pyrodinium bahamense* abundances pattern, while two copepod species abundances, *Oithona*

Table 4
Coefficients and significance (*p*-value) of each of the explanatory variables of the Generalized Linear Models (GLMs - Gaussian) describing the variation of the egg production rates (EPR) and the RNA:DNA ratios of *Acartia clausi*. NH_4 represents ammonia. Levels of significance are represented as ***$p < .0001$, **$p < .001$, *$p < .01$ and n.i. represents variables not included in the final model after backward stepwise regression. AIC is the Akaike Information Criterion, LogLik is the log-likelihood of the fitted model, DF is the degrees of freedom.

Coefficients	Independent variables	EPR	RNA:DNA
Gaussian (identity)	EPR	–	0.35**
	Temperature	n.i.	−0.54*
	Salinity	−2.07*	n.i.
	NH_4	19.40*	–
	Diatoms	n.i.	n.i.
	Dinoflagellates	n.i.	n.i.
	Ciliates	n.i.	n.i.
	AIC	54.69	19.7
	LogLik	−23.35	9.27
	DF	4	4

colcarva and *Acartia tonsa* declined. The cladoceran *Penilia avirostris* also showed a positive correlation with *G. catenatum*, revealing that this species is not affected by the presence of the toxic dinoflagellate. In fact, other planktonic organisms such as *Acartia clausi* can ingest the toxic dinoflagellate *G. catenatum* with no apparent adverse effects in the feeding and egg production rates (Palomares-García et al., 2006). Observations by Schultz and Kiørboe (2009) suggested that both *Pseudocalanus elongatus* and *Temora longicornis* could feed at high rates on *Karenia mikimotoi* for 24 h without any apparent deleterious effects. *A. clausi* was also shown to ingest more toxic cells of *Alexandrium minutum* as its concentration increased, although with this diet hatching success and nauplii production decreased (Frangópulos et al., 2000). In summary, present results add more evidence to the fact that some organisms are not affected by the presence of toxic microalgae and must have developed defences against their toxicity.

In the present study, the cladocerans *Penilia avirostris* and *Evadne* spp. were correlated with the dinoflagellates *Prorocentrum* spp. and *Scrippsiella trochoidea*, respectively. Atienza et al. (2006) have found that the cladoceran *Penilia avirostris* ingests a broad spectrum of prey types including dinoflagellates and shows a behaviourally driven plasticity in prey selection. The fact that dinoflagellates were the dominant group of microplankton during this study may explain the positive correlation between these species. Due to the morphological characteristics of *Evadne spinifera*, Nival and Ravera (1979) suggested that this species can feed on organisms between 20 and 170 μm of total length, and probably can catch and hold animal prey or large algae. Moreover, Katechakis and Stibor (2004) studying feeding selectivity of cladoceran species, showed higher grazing coefficients of the congeneric *Evadne nordmanni* for the size classes 125, 175 and 205 μm, corresponding to an active selection of large diatoms. This supports the correlation found in the present study between *Evadne* spp. and diatoms, suggesting that this phytoplankton group is an important prey influencing the population dynamics of this species.

4.2. Acartia clausi production and RNA:DNA ratios: influence of environmental conditions

The main factors shaping the variability of egg production rates of *Acartia clausi* in a short temporal scale were salinity and ammonium. Previous studies reported that the main factors influencing *Acartia* reproduction are food availability (e.g. Uye, 1981; Kimmerer et al., 2005) and temperature (e.g. Uye, 1981; Castro-Longoria, 2003). Although it is well known that food is an important factor for the reproduction of *A.*

clausi (Uye, 1981; Pagano et al., 2004), it was not the case in the present study as this factor was not selected by the model that explain EPR variability (Table 4). This can be attributed to the fact that food was not a limiting factor during this season, when a relatively high abundance of dinoflagellates occurred, which are an essential food source for the reproduction of *A. clausi* (Band-Schmidt et al., 2008). Furthermore, most of the previous studies have been conducted in a higher temporal scale than the present one or in laboratory experimental conditions, therefore lacking a higher temporal resolution. As stated before, there was a salinity decreased (34–35) and macronutrients increased at the end of August and beginning of September, which corresponded to higher productivity of this calanoid species. Castro-Longoria (2003) recorded the highest *A. clausi* EPR at a salinity of 35, in an experiment using a salinity range between 15 and 35. Moreover, *A. clausi* egg production in a Northern African lagoon was negatively correlated to salinity, where it decreased with values over 35 occurring during the summer (Annabi-Trabelsi et al., 2012). In fact, the metabolism of this species in terms of respiration rates was lowest at a salinity of 35, meaning that it is well adapted to these conditions (Gaudy et al., 2000). The decrease of reproduction in salinities > 35 mean that salinity could be a limiting factor for *A. clausi* even when it encounters suitable food conditions. The other factor influencing the reproduction was ammonia, showing an increment in egg production rates when this nutrient increased. Only a few previous studies have observed the same pattern despite the toxicity of this nutrient. Moraitou-Apostolopoulou and Verriopoulos (1981) have found that *A. clausi* inhabiting polluted areas always laid a high number of eggs. Additionally, Buttino (1994) showed that chronic exposure to ammonia produced a significant increment in egg production rates of the same species during the experimental period of exposure. It seems that *A. clausi* when exposed to stressful environments may have a reproductive strategy of increasing the egg production or even that the population has developed an adaptation to higher levels of pollutants.

The secondary production of *A. clausi* females was also higher at the end of the sampling period, peaking in the last days of August, and reflected mostly the curve of female's biomass rather than the egg production rates, which presented a lower variability throughout the sampling period. Although the egg production rate method includes only the adult fraction, underestimating the total secondary production, it has several advantages such as shorter periods of incubation, such as replicability and accuracy of biomass and fecundity measurements, and simplification in the identification (Poulet et al., 1995). Leandro et al. (2014) estimated the secondary production of *A. clausi* juvenile fraction in the coastal lagoon of Ria de Aveiro, NW Portugal, in a monthly basis, but using the growth rate approach with a temperature dependent growth model. They obtained higher values during September (23.9 μg C m^{-3} day^{-1}) which are comparable to what was found in the present study during the same season (average: 42.6 ± 59 μg C m^{-3} day^{-1}). Moreover, the summer production of *A. clausi* females in the adjacent coastal waters of Guadiana river estuary was higher than in Ria Formosa but also comparable to what was found in the present study (average values of 98.8 ± 84.3 μg C m^{-3} day^{-1}; Cruz et al., 2017). These results suggest that there is a potential considerable flow of energy and matter through the planktonic food web, especially to the early stages of the important fisheries resources (e.g. *Sardina pilchardus*, *Engraulis encrasicolus*, Sparidae) (Chícharo et al., 2012) that use Ria Formosa lagoon system as a nursery habitat.

RNA:DNA index of *A. clausi* adult females was positively related to the EPR and negatively to temperature, during the short sampling period. Previous studies, using different approaches such as laboratory experiments (*Acartia grani*: Saiz et al., 1998; *Acartia bifilosa*: Gorokhova, 2003) or in situ experiments (*Paracalanus* sp.: Nakata et al., 1994; *Calanus sinicus*: Ning et al., 2013; *Acartia* spp.: Cruz et al., 2017) have already shown this significant relationship between the two growth indices. This result is adding more evidence for the replacement of a more laborious and time-consuming method such as the egg production

rate determination with the RNA:DNA ratio methodology.

Temperature has a strong effect on biochemical reactions such as the protein synthesis, i.e. higher temperatures will increase the metabolic rates without increasing the RNA concentration (Chícharo and Chícharo, 2008). Nakata et al. (1994) found significant differences between the RNA:DNA ratio between sampling stations with different sea surface temperatures, and higher values of the index occurred when the temperature was lower. Saiz et al. (1998) also found a temperature dependency relationship between the EPR of *A. grani* and the RNA:DNA index. On the other hand, some studies did not find any correlation between temperature and the ecophysiological index (Gorockova, 2003; Ning et al., 2013; Cruz et al., 2017). The fact that there was no food limitation during the sampling period probably induced a more evident influence of temperature on the biochemical ratio. Also, even though the determination of RNA:DNA ratio occurred over a short temporal period, there was a high range of temperatures tested (between 23.1 and 27 °C). Previous studies indicate that with a broader temperature range (> 2 °C) there is a need to include both temperature and RNA:DNA ratio terms when modelling fish larvae growth rates (Buckley et al., 1999). Regarding the copepods, particularly the genus *Acartia*, there are still some incongruences among studies in relation to the best predictors to achieve a good model of EPR estimation. Therefore, further in situ and laboratory experiments should be accomplished using different time scales, relating the EPR, RNA:DNA ratio, temperature and food availability.

5. Conclusions

In the present study, salinity (particularly lower values ~34.5–35) was the main factor inducing higher abundances of several zooplanktonic taxa and production of the calanoid copepod *Acartia clausi*. The expected occurrence of warmer and drier summers in the future due to climate change may promote higher salinity values in Ria Formosa lagoon system (Williams, 2001) affecting the mesozooplankton abundance and production, which will probably disturb the entire food web. Episodic events such as Submarine Groundwater Discharges will certainly be helpful to reverse a high salinity scenario.

Neritic copepods and decapod larvae seem to be favoured by ebb flows, using these currents to be transported to the open sea. Copepods seem to avoid the inner parts of the lagoon, while decapods use them to complete their larval development with the dispersion of newly hatched larvae into the ocean. To a lesser extent, semilunar cycle (tidal coefficient), temperature and food availability were also important in shaping zooplanktonic communities. The blooms of toxic dinoflagellates such as *Gymnodinium catenatum*, that are common in Ria Formosa lagoon during summer, do not have any deleterious effects in some taxa abundance (e.g., Apendicularia and *Penilia avirostris*).

Besides salinity, the macronutrient ammonia was present in low concentrations in the water, which is generally recorded in many eutrophic areas, and induced higher fecundity of *A. clausi* females, suggesting that this copepod is well adapted to these conditions. RNA:DNA ratio was proved to be a good proxy of egg production rate. However, further experiments should be conducted, in different time-scales, to understand how different variables influence these biological indexes and to achieve a good model to simplify the estimation of secondary production.

Ria Formosa coastal lagoon system presented a high variability in the plankton community (microplankton and mesozooplankton), as well as in the environmental factors that are constantly shaping species abundances, composition or production. It is unquestionable that long-term temporal scales studies using monthly or seasonal sampling strategies are important to continue monitoring plankton communities. However, the use of a more intensive sampling during the most productive seasons will be helpful to understand how sudden environmental changes can influence the community structure and production of these organisms, that are reflected in the interannual variability often difficult to explain.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seares.2020.101858.

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