1 ONTOGENETIC CHANGES IN THE ELEMENTAL COMPOSITION AND
2 STOICHIOMETRY OF MARINE COPEPODS WITH DIFFERENT LIFE HISTORY
3 STRATEGIES

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ABSTRACT

We describe the ontogenetic variation in elemental and stoichiometric composition of two copepod species with very contrasted life history patterns, the calanoid *Paracartia grani* and the cyclopoid *Oithona davisae*. The first species is a broadcasting, highly productive copepod, whereas the latter is an egg-carrying copepod, much less productive. We reared cultures of both species under conditions of excess food and analysed their C, N and P composition, and their molar ratios, along development. Both species differed on their specific P content, whereas the specific C and N content were similar. As expected, the specific P content of *P. grani* were higher, resulting in lower C:P and N:P ratios. Furthermore, we compared our elemental composition data with previously reported stage-specific (nauplii and adult female) maximum growth rates of these two species. We found that for *O. davisae* the ontogenetic variation in specific P content agreed with the reported differences in growth rate along development; however, in the case of *P. grani*, in which juvenile and adult growth rates are similar, the variations in specific P content along development did not reflect the growth rate pattern.

INTRODUCTION

Biological stoichiometry studies the balance of energy and multiple chemical elements in living systems (Elser *et al.*, 2000). The elemental composition and stoichiometry, i.e. the relationship between these structural chemical components (defined as elemental ratios) of an organism are the result of the balance between their availability in the environment and their organismic demands (Sternner and Elser, 2002).

The stoichiometric approach applied to zooplankton has been very productive, in particular in relation to food quality limitation and the implications for different life history strategies (Sternner and Elser, 2002, and references therein). Most development in this field, however, has occurred in freshwater systems, focused on cladocerans and copepods (Sternner and Elser, 2002). While freshwater zooplankton C:N ratios appear rather stable, their P content may experience large variations (Andersen and Hessen, 1991), affecting, therefore, the C:P and N:P ratios. These differences on P body content in freshwater zooplankton are considered to reflect the variations in the organism's content of RNA (rich in P), which is associated to growth (growth-rate hypothesis; Elser *et al.*, 1996; Sternner and Elser, 2002). Some of the dissimilarities in P content among taxa and species, however, can also be constitutional. For instance, Andersen and Hessen (1991) reported that rapidly-growing groups like *Daphnia* typically exhibit higher P content than freshwater copepods. For organisms consisting of multiple developmental stages and that may show different life history traits through development, however, such coupling between P content and growth might not be straightforward and should be addressed at the stage level. Thus, for instance, Carrillo *et al.* (2001) and Villar-Argaiz *et al.* (2002) found that during the seasonal development of a population of the freshwater copepod *Mixodiaptomus laciniatus*, the concept of strict homeostasis was questionable and the copepods experienced considerable interstage variability (and to some extent, at the intrastage level as well); moreover, these authors observed that the changes in body stoichiometry along the development of this copepod species reflected the different nutrient demands associated with the differences among stages in life strategy (e.g. nauplii vs. late copepodites or adults).

In the marine environment, the application of the stoichiometric approach to zooplankton and in particular to copepods has been comparatively lesser. With the exception of high latitudes in which copepods accumulate large lipid storages to endure the unfavourable season, most marine copepods are known to keep also rather low and stable C:N ratios, and it is essentially the P content that may experience large variations

(Båmstedt, 1986; Gismervik, 1997; Koski, 1999). Besides this high latitude adaptation, it is not known, however, how other life history traits like foraging behaviour and reproductive strategy may affect marine copepod stoichiometry. The ontogenetic changes in the elemental composition of marine copepods have also been seldom studied. The work of Meunier *et al.* (2016) has described the pattern of variation along the development in the elemental ratios of the marine copepod *Acartia tonsa*. These authors found, like previously reported for freshwater copepods (Carrillo *et al.*, 2001), a negative relationship between somatic growth rate and N:P ratios, with the naupliar stages presenting higher growth rates and lower N:P values than the copepodite stages. However, the recent study of Mathews *et al.* (2018) with the small calanoid *Parvocalanus crassirostris*, which compared growth and stoichiometry of nauplii and copepodites, did not show consistent correspondence with C:P ratios.

Organisms adopt different life history strategies to optimize their fitness to the environment. In the marine pelagic environment, it is well established that most calanoid copepods are free spawners, while cyclopoids carry their eggs in sacs (Kiørboe and Sabatini, 1994; Kiørboe and Sabatini, 1995). The differences in life history between marine calanoid and cyclopoid copepods are accompanied by other traits (e.g. feeding rates, metabolic activity, total reproductive output, lifespan, egg mortality) that determine their capability to cope with environmental variability and recruit populations (Almeda *et al.*, 2011; Paffenhöfer, 1993; Saiz *et al.*, 2017). Both groups of copepods also differ in the growth pattern exhibited along ontogeny. Thus, maximum growth rates of juveniles and adults of broadcasting species (expressed as somatic growth and egg production, respectively) are, under non-limiting resources, rather similar (Berggreen *et al.*, 1988; Fryd *et al.*, 1991; Hirst and Bunker, 2003; Sekiguchi *et al.*, 1980); contrarily, in egg-carrying species copepods maximum adult growth rates are much lower than the juvenile ones (Kiørboe and Sabatini, 1994; Sabatini and Kiørboe, 1994). The effect of these different life strategies on copepod elemental composition and stoichiometry, in particular on P content, have not been studied.

Here, we aim to assess the ontogenetic changes in elemental composition and stoichiometry of two copepod species, *Paracartia (Acartia) grani* and *Oithona davisae*, representative of these very distinct life history strategies. *P. grani* is a medium-sized calanoid copepod (adult female cephalothorax length of ca. 1000 μm) inhabiting coastal waters and estuaries in the eastern Atlantic Ocean and the Mediterranean Sea. This species is in many aspects very similar to the closely-related and most widely

distributed and studied *Acartia tonsa* (Saiz *et al.*, 1992). *P. grani* is a broadcast spawning copepod with high reproductive output (Calbet and Alcaraz, 1996; Saiz *et al.*, 1998), omnivorous and capable of creating feeding currents and achieve high daily rations (Olivares *et al.*, 2019; Saiz *et al.*, 2015) and high growth rates (Calbet and Alcaraz, 1997). Contrarily, *O. davisae* is a small cyclopoid copepod (adult female cephalothorax length of ca. 330 μm) originally from the Pacific that has expanded its distribution as invasive species in many areas (western Pacific: Ferrari and Orsi, 1984; Mediterranean Sea: Saiz *et al.*, 2003; North Sea: Cornils and Wend-Heckmann, 2015; Black Sea: Temnykh and Nishida, 2012). *O. davisae* is a strict ambush feeder, also omnivorous, depending on relatively large and motile prey to feed (Saiz *et al.*, 2014). Copepods from the genus *Oithona* spawn the eggs in a sac that is kept attached to the female until hatching. These distinct life-history strategies result in clear differences in the physiological performance of both copepods. Thus, *O. davisae* exhibits maximum growth rates (Almeda *et al.*, 2011; Almeda *et al.*, 2010b; Zamora-Terol and Saiz, 2013) comparatively much lower than those typical of calanoid copepods (e.g. Berggreen *et al.*, 1988; Calbet and Alcaraz, 1997). Our hypothesis is that these marked differences in life history traits between both species (i.e. feeding rates, somatic growth and reproductive output) will be reflected in the body P content and stoichiometric ratios along their development.

METHOD

Study species

Copepods originate from our cultures at the Institut de Ciències del Mar. *Paracartia grani* was isolated from waters near Barcelona (Spain), and has been kept at 19°C in the laboratory for >10 years (Saiz *et al.*, 2015); it is reared on a diet of the cryptophyte *Rhodomonas salina* grown on f/2 medium. *Oithona davisae* was also collected in Mediterranean waters and has been kept in continuous culture in our lab since 2000 (Saiz *et al.*, 2003); it is grown at 19°C on a diet based on the heterotrophic dinoflagellate *Oxyrrhis marina* (which in turn was fed *R. salina*). Typical elemental composition of both prey, obtained from determinations in different occasions on exponentially growing batch cultures, are: *R. salina* (size: 7.4 ± 0.24 SE μm ESD): 36 ± 4.2 SE pg C cell⁻¹, 8 ± 0.8 SE pg N cell⁻¹ and 1.0 ± 0.08 SE pg P cell⁻¹, and *Ox. marina* (size: 20 ± 0.3 SE μm ESD): 522 ± 86 SE pg C cell⁻¹, 107 ± 10 SE pg N cell⁻¹ and 22 ± 0.2 SE

pg P cell⁻¹ (unpublished data; different culture volumes were filtered on pre-combusted GF/F and analysed using the same analytical methods described below for copepods).

Copepod cultures

The copepod cohorts were generated following the methods described in Henriksen *et al.* (2007). The rearing tanks were located in a temperature controlled walk-in room (19°C) with a 10h:14h D:N light cycle. Shortly, for *Oithona davisae* we removed ovigerous females from stock cultures with a submerged 130- μ m sieve and carefully transferred them to 15-L transparent polycarbonate round containers filled with 0.1- μ m filtered seawater, where the eggs hatched. For *Paracartia grani*, the bottom of the stock culture tanks was cleaned and adults were allowed to spawn eggs for 24 h; we siphoned out the eggs from the bottom of the tank and transferred them to the 15-L polycarbonate containers filled with 0.1- μ m filtered seawater for hatching. After hatching, the copepod cohorts were routinely fed suspensions of exponentially growing cultures of either *Rhodomonas salina* (*P. grani*) or *Oxyrrhis marina* (*O. davisae*). Prior to providing fresh food, the rearing tanks were sampled, the remaining prey suspensions checked with a Coulter Multisizer III particle counter, and the desired nominal concentrations adjusted using our prey stock cultures. The prey concentrations used for rearing the copepods were at satiation or close to satiation levels; i.e., >19000 cells mL⁻¹ *R. salina* for juveniles and adults of *P. grani* (Olivares *et al.*, 2019), and ca. 800 cell mL⁻¹ of *Ox. marina* for *O. davisae* juveniles and adults (Almeda *et al.*, 2010a; Zamora-Terol and Saiz, 2013). Typically, food was provided 5 times a week, with extra amounts for weekends. Nevertheless, to avoid any potential bias due to “weekend” effects, samples for copepod elemental composition analysis were never withdrawn at the beginning of the week.

Chemical and biological analyses

Through the rearing of the cohorts we took samples on a daily basis to obtain specimens at different development stages to measure their size and elemental composition. We reared several cohorts for each species in order to complete the whole range of developmental stages because the amounts of individuals, in particular the youngest stages, required for the C, N, P and size analyses, in occasions, expended most of the available individuals per cohort and a new one had to be generated.

The water samples were carefully filtered through submerged sieves (of varying mesh size, depending of the targeted development stage), the copepods transferred to 0.1- μ m filtered seawater, then narcotized with MS-222 (Saiz *et al.*, 2015) and their abundance estimated under the stereomicroscope. For the very small stages we then took four aliquots of different volumes (starting with 200-250 mL for the earliest naupliar stages, accounting ca. 3500-6000 individuals, and progressively decreasing as copepods were larger), and filtered them onto pre-combusted 25-mm Whatman GF/C filters (450°C, 5 h); two of these filters were devoted to C and N analysis, whereas the remaining two for P analysis (see below). When larger specimens were present, narcotized copepods were individually picked and transferred in groups of different amounts according to size (from several hundred specimens down to 15 individuals for adult female *Paracartia grani*) onto the four replicated pre-combusted filters (two for C and N, and two for P). We took particular care to pipette out any exuviae if present prior to filtering or pipetting the individuals onto the filters. Additional aliquots were preserved in formalin to verify the abundance and to measure the body size. In the case of *Oithona davisae*, eggs sacs of ovigerous females were detached from the body after gently stirring with a vortex mixer, and then both egg sacs (groups of 500-600 egg sacs) and non-ovigerous females (groups of 200 individuals) were separately picked out in groups and processed for C, N and P analysis as described above. In addition, we preserved some detached sacs in formalin to be dissected to determine clutch size and later calculate the C, N and P content on a per egg basis. For *Paracartia grani*, which spawns free eggs, eggs were siphoned from the bottom of the culture tanks onto a 60 μ m submerged sieve. Posteriorly, the sieve was sprayed thoroughly with filtered seawater to remove debris and faecal pellets, and eggs were aliquoted in groups of ca. 10500 eggs to be later processed for C, N and P analysis. Samples for C and N analysis were posteriorly dried for 48 h at 60°C and kept in a desiccator until analysis with a Flash EA1112 (Thermo Finnigan) CHNS analyser. Filters for particulate P were frozen at -80°C immediately after preparation; later, the filters were subjected to acid persulfate digestion and then analysed as dissolved inorganic P with a Seal Analytical AA3 (Bran+Luebbe) analyser (Isari *et al.*, 2013).

We took pictures of the formalin preserved specimens with a digital camera mounted on an inverted microscope. Total body lengths (nauplii) and cephalothorax lengths (copepodites and adults) were measured from the pictures with the ImageJ software; in the case of eggs, the contour of the eggs was drawn and either an average

diameter (*Paracartia grani* eggs) or major and minor diameters (*Oithona davisae* eggs) were estimated. We also estimated the body volume of copepods (*BV*) in order to standardize their C, N and P content. To do that, the contours of the naupliar body and the copepodite cephalothorax in the pictures were drawn in Image J, and major and minor axes of the fitted ellipse automatically obtained. Then, we used the equation:

$$BV = \frac{\pi}{6} \times (L \times H \times W),$$

where *L* and *W* correspond to respectively the fitted major and minor axis obtained from the contour measurement with ImageJ. *H* (height) was derived from *W* using the flattening factors (*H/W*) reported for *Oithona nana* (0.80; Viñas *et al.*, 2010) and *Acartia tonsa* (0.88; Fernandez Araoz, 1991; Hoffmeyer and Torres, 2001).

Statistical analyses

The power scaling of elemental composition with body size was determined by simple linear regression after log transformation. Because the P analyses were conducted in independent filters of those for C and N analysis, the standard errors of C:P and N:P molar elemental ratios were calculated by error propagation (Sternner and Elser, 2002), using the equation:

$$SE_{A:B} = \frac{\bar{A}}{\bar{B}} \times \sqrt{\left(\frac{SE_A}{\bar{A}}\right)^2 + \left(\frac{SE_B}{\bar{B}}\right)^2},$$

where $\bar{A}$ and $\bar{B}$ are the arithmetic means of the variables *A* and *B*, *SE_A* and *SE_B* are the respective standard errors, and *SE_{A:B}* is the standard error of the ratio $\bar{A}:\bar{B}$.

The relationships between molar elemental ratios and body size were also analysed by simple linear regression. Differences between regression slopes were tested by ANCOVA. Either *t*-test or ANOVA were conducted when necessary. Statistics were conducted with Prism 7 (GraphPad Software, Inc.).

RESULTS

Elemental content of the eggs

Eggs of *Paracartia grani* were spherical, with average diameter of $82.7 \pm 0.28 \mu\text{m}$, and their elemental content averaged $38.2 \pm 3.11\text{SE ng C}$, $8.1 \pm 0.70\text{SE ng N}$ and $2.6 \pm 0.21\text{SE ng P}$ per egg. The eggs of *Oithona davisae* were more flattened, likely because of being packed within a sac, with mean size $51.8 \pm 0.35\text{SE} \times 43.1 \pm 0.30\text{SE} \mu\text{m}$ and elemental content $6.4 \pm 0.42\text{SE ng C}$, $1.4 \pm 0.07\text{SE ng N}$ and $0.28 \pm 0.007\text{SE ng P}$ per egg. Molar

ratios for *P. grani* and *O. davisae* eggs were, respectively: C:N=5.5±0.03SE and 5.3±0.15SE, C:P = 38.0±4.38SE and 58.8±4.16SE, and N:P=6.9±0.82 SE and 11.1±0.59SE.

Ontogenetic changes in body length and elemental content

The body length of *Paracartia grani* ranged from ca. 100 µm for the earliest nauplii to ca. 380 µm for the first copepodite stage and increased up to a mean size of 1016 µm for the adult female (Fig. 1a). The male of *P. grani* was 12% smaller than the female. The C content of *P. grani* ranged from 0.02 µg C for the earlier stages to ca. 5 µg C for adult females (Fig. 1a). The N and P elemental content ranged from 4.3 ng N and 1.3 ng P for the earliest stages to mean values of ca. 1.2 µg N and 0.18 µg P for adult females. The elemental content (C, N and P) in *P. grani* scaled to body length as a power function (Fig. 1a; Table I). The scaling factor (slope) was similar for C and N (respectively, 2.48±0.086SE and 2.50±0.085SE), but decreased for P (2.18±0.042; ANCOVA: $F_{2,42}=5.98$, $p=0.005$).

Body length of *Oithona davisae* ranged from 87 µm for the earliest nauplii to ca. 200 µm for the earliest copepodites up to mean body lengths of ca. 340 µm for adult males and females, which had similar sizes (Fig. 1b). The C content ranged from 5 ng C for the earliest nauplii to ca. 0.31 µg C for (non-ovigerous) adult females, whereas the N and P content ranged from 1 ng N and 0.3 ng P for the earliest nauplii to 70 ng N and 11 ng P for the adult females (Fig. 1b). Similar to *Paracartia grani*, the relationships between body length and elemental content followed a power function (Fig. 1b; Table I). The slopes of the power relationship between body size and C and N content appeared slightly higher than those for P, but the differences were not statistically significant (ANCOVA: $F_{2,42}=1.28$, $p=0.29$).

Ontogenetic changes in body volume and specific elemental content

The body volume of both copepod species related to body length by a power function with a scaling factor close to 3 (Figs. 2a, 3a). The scaling factor for *Paracartia grani* (2.52±0.052SE) was slightly lower than that for *Oithona davisae* (2.80±0.062SE), likely due to the comparatively wider shape of the latter species, i.e. higher dorsal width/body length ratio (e.g. width/length ratio for adult females: *P. grani* = 0.29±0.001SE; *O. davisae* = 0.47±0.005SE).

The ontogenetic changes in the specific elemental content of *Paracartia grani*, normalized per unit of body volume, are shown in Fig. 2. The variation in the specific C and N content did not show a statistically significant trend with size (stage) (Figs. 2b,c; Table I), and averaged $86.3 \pm 3.81 \text{ SE } \mu\text{g C mm}^{-3}$ and $19.6 \pm 0.86 \text{ SE } \mu\text{g N mm}^{-3}$, respectively. On the other hand, the specific P content declined significantly with body size and ranged between 2.8 and $7.2 \mu\text{g P mm}^{-3}$ (Fig. 2d; Table I), with an average value of $4.6 \pm 0.33 \text{ SE } \mu\text{g P mm}^{-3}$.

Similarly, the specific C and N content of *Oithona davisae* were also independent of body size (Figs. 3b,c; Table I), and averaged respectively $78.6 \pm 4.81 \text{ SE } \mu\text{g C mm}^{-3}$ and $18.7 \pm 1.18 \text{ SE } \mu\text{g N mm}^{-3}$. The specific P content, however, declined from ca. $4.8 \mu\text{g P mm}^{-3}$ in early nauplii to ca. $2.8 \mu\text{g P mm}^{-3}$ in adults (Fig. 3d; Table I), with an average value of $3.8 \pm 0.24 \text{ SE } \mu\text{g N mm}^{-3}$ (Fig. 3d).

The average specific C and N content did not differ between both species (two-tailed *t*-tests; specific C content: $t=1.27$, $\text{df}=29$, $p=0.22$; specific N content: $t=0.63$, $\text{df}=29$, $p=0.53$). However, *Oithona davisae* had overall lower specific P content (19%; two-tailed *t*-test: $t=2.12$, $\text{df}=29$, $p=0.042$). This difference in P content between species was mainly driven by the differences at the early stages of development, whereas adults of both species had similar content per unit body volume (see Figs. 2d and 3d).

Changes in elemental ratios through development

C:N ratios for *Paracartia grani* were independent of size, with a mean C:N molar ratio of $5.1 \pm 0.06 \text{ SE}$ (Fig. 4a; Table I). The N:P and C:P molar ratios in *P. grani* averaged $10.0 \pm 0.82 \text{ SE}$ and $51.4 \pm 4.03 \text{ SE}$, respectively; both ratios significantly increased with size (Fig. 4a,b; Table I).

Similar to *Paracartia grani*, C:N molar ratios of *Oithona davisae* did not change along the development (Fig. 4c; Table I); mean C:N molar ratio for *O. davisae* was $4.9 \pm 0.10 \text{ SE}$ (Fig. 4c). Both N:P and C:P molar ratios of *O. davisae* were positively related to body size (Fig. 4c,d; Table I). These positive relationships were fundamentally driven by the high N:P and C:P ratios of late copepodites and adults (body sizes $>300 \mu\text{m}$; mean N:P ratio: $14.2 \pm 1.14 \text{ SE}$; mean C:P ratio: $69.0 \pm 4.71 \text{ SE}$); if we exclude these stages from the analysis (i.e. only take into consideration body sizes $<300 \mu\text{m}$), both N:P and C:P ratios were independent of body size (N:P ratio: $F_{1,9}=0.0095$, $p=0.92$; C:P ratio: $F_{1,9}=0.053$, $p=0.82$), with mean values of $10.1 \pm 0.46 \text{ SE}$ and $48.7 \pm 2.20 \text{ SE}$, respectively.

DISCUSSION

In this study we have compared the changes in elemental composition and molar stoichiometric ratios along the ontogeny of two copepod species with very contrasting life history traits, such as feeding rates, reproductive strategy and fecundity rates (Almeda *et al.*, 2011; Calbet and Alcaraz, 1996; Zamora-Terol and Saiz, 2013), and their ageing patterns and total life reproductive output (Saiz *et al.*, 2017; Saiz *et al.*, 2015). We expected these differences would reflect in their elemental content and C:N:P ratios.

Elemental contents of Paracartia grani and Oithona davisae

As typically reported for other copepods (Berggreen *et al.*, 1988; Durbin and Durbin, 1978; Sabatini and Kiørboe, 1994), the elemental content of both studied species scaled potentially to the body length. In our study, the power factors were similar for C and N and differed slightly between species (ca. 2.5 for *Paracartia grani*; ca. 2.7 for *Oithona davisae*); conversely, the power factors for P were overall lower than those for C and N, and also differed slightly between species (ca. 2.2 for *P. grani*; ca. 2.5 for *O. davisae*). These patterns, however, were based on linear dimensions (body length), and did not consider differences in body mass or volume between species (due to different body morphology). We, therefore, standardized the elemental content to body volume of both species along development. The C and N content per unit of body volume remained relatively constant and independent of stage (size) and species. We haven't found any other direct estimates of C and N content per unit body volume for copepods, except the estimate for C reported by Uye and Sano (1998) for adult *O. davisae* (which was 83.4 $\mu\text{g C mm}^{-3}$, very close to our estimate for this species, 90.0 $\mu\text{g C mm}^{-3}$) and those for mixed zooplankton (mainly copepods) reported by Alcaraz *et al.* (2003) in their Table 2, which overall are within the range of variation, but somewhat lower to the ones we found. The body volumes in Alcaraz *et al.* (2003) were probably overestimated because height and width of individuals were assumed to be isometric, therefore explaining the slight difference in comparison to ours.

In order to allow a comparison of our data with those studies where copepod elemental composition is normalized with dry mass instead of body volume, we have also derived the dry mass in our samples from volume. To do that, we have first

converted body volume to wet mass assuming a copepod wet mass density $\rho=1.4 \text{ g cm}^{-3}$, a reasonable value for both *Oithona* and *Acartia* species (range: 1.03-1.05 g cm^{-3} ; Hubareva and Svetlichny, 2016; Svetlichny *et al.*, 2017), and then assumed a dry weight:wet weight conversion factor of 0.2 for medium latitude copepods (Båmstedt, 1986, his Table 1.13). Thus, for $\rho=1.04$, the elemental composition of both species as % of dry mass is:

Oithona davisae: %C=41±2.5, %N=10±0.6, %P=2.0±0.12

Paracartia grani: %C=44.9±2.0, %N=10±0.4, %P=2.4±0.17

Using ρ values of either 1.03 or 1.05 instead, the differences in dry-mass normalized values were negligible (up to approximately 1 unit for %C, and only 0.3 or less units of variation for %N and %P), indicating that these estimates are quite robust to variations in ρ .

Our copepod elemental contents expressed as % of dry mass are close to the ones reported for C and N in the review by Bamsted (1986) and Kiørboe (2013). Regarding %P, Bamsted (1986) mentioned that the few data available at the time seldom exceeded 1% dry mass. Uye and Matsuda (1988) reported the P content (as % dry mass) for 11 copepod species of the Inland Sea of Japan to range between 0.70 and 1.76%, with an average of 1.6% for adult females of *Acartia*. Walve and Larsson (1999) reported average elemental contents of 48%C, 12%N and 1.6%P for copepodite stages of *Acartia* in the Baltic Sea, phosphorus contents being more variable (range 1.1-2.0). Our P estimates, in the order of 2-2.4%, are reasonable since our copepods were maintained in the laboratory feeding ad libitum, while most (if not all) of the above-mentioned reports are on field copepods, under different environmental conditions. Regarding other studies where ontogenetic variation (from nauplii to adults) in elemental composition and stoichiometry are dealt with, neither the work Meunier et al.

(2016) with *Acartia tonsa* nor the one by Matherws et al (2018) with *Parvocalanus crassirostris* provided elemental content per unit dry mass, and they present the data only as stoichiometric ratios.

Stoichiometric composition and life history traits of Paracartia grani and Oithona davisae

We found that *P. grani* and *O. davisae* C:N molar ratios were overall similar and fell within the range of values reported for marine copepods (Båmstedt, 1986). In fact, heterotrophs are in general much more homeostatic than autotrophs, and variations in stoichiometric ratios are more constrained (Sternner and Elser, 2002). Thus, the low variation in specific C and N content we found explains the uniformity of C:N ratios along ontogeny in both species. Typically, copepod C:N ratios are not expected to change much through ontogeny, except in high latitudes where large amounts of lipids to undergo the unfavourable season might be stored, resulting in higher C:N ratios given the lower relative content of N in lipids (Båmstedt, 1986). Low and medium latitude copepods like *Paracartia grani* and *Oithona davisae* may store minor quantities of lipids as tiny oil droplets under high food supply (pers. obs.), but these amounts are certainly fairly small and would have little influence on C:N ratios. In agreement with our study, Meunier *et al.* (2016) also reported rather constant C:N ratios (ca. 5), comparable to ours, along the development of the calanoid copepod *Acartia tonsa*.

We found that in both copepod species the P content per unit of body volume was inversely related to size, denoting that through development older stages had a lower relative content of P. This decrease in P content reflected on the variations in C:P and N:P molar ratios, in particular on the late copepodite stages and adults. In freshwater zooplankton (and particularly copepods) it has been described that most stoichiometric variation in body composition usually occurs in the P content (Sternner and Elser, 2002, and references therein). These differences in P body content are commonly attributed to changes in the amount of nucleic acids, particularly RNA, since other molecules rich in P are considered to be at very low concentrations in zooplankton (Sternner and Elser, 2002). The growth rate hypothesis (Elser *et al.*, 1996) states that given the link between RNA content and growth rate, the high RNA content of fast-growing animals will result in a larger allocation of P in the body and therefore lower C:P and N:P ratios (Sternner and Elser, 2002). In freshwater zooplankton the growth rate hypothesis has been

supported by comparisons between groups with distinct life history strategies and taxon-specific growth rates (e.g. copepods vs. *Daphnia*, or *K*- vs *r*-strategists; Sterner and Elser, 2002) and along the development of high-mountain lake copepods, in which the slower growth rates in older developmental stages were associated with a decrease in their relative content of P (Carrillo *et al.*, 2001; Villar-Argaiz *et al.*, 2002). In marine copepods, several studies have showed that RNA content varies positively with growth (either somatic or egg production; (Gorokhova, 2003; Saiz *et al.*, 1998; Wagner *et al.*, 2001; Yebra *et al.*, 2011), but unfortunately these studies typically do not report the possible changes in copepod stoichiometry.

In our study, we did not conduct any growth experiments coupled with our stoichiometric analyses, and did not aim to test the growth rate hypothesis. Nevertheless, given the contrasting life history traits between *Paracartia grani* and *Oithona davisae* and the previous knowledge on the biology and physiology of these two species, we anticipated that the known differences in growth between both species might reflect on their stoichiometric ratios. For the sake of the following discussion, we provide in Table II and Fig. 5 a summary of several life history traits reported in the literature for *Paracartia grani* and *Oithona davisae*. Thus, the much lower prey encounter rates (i.e., lower maximum clearance rates), result of the strict ambush feeding behaviour of *Oithona davisae*, and the much lower clutch size, associated to the egg carrying reproductive strategy exhibited by this species, set the constraints for the large differences in productivity between both copepod groups, both on a daily basis and on a per lifespan basis. These differences in strategy also reflect at the ontogenetic level. Figure 5 shows the maximum somatic (nauplii) growth rates and maximum weight-specific egg production rates reported in previous works carried out in our laboratory with both species, under the same food type and satiating conditions (*P. grani*: Calbet and Alcaraz, 1996; Calbet and Alcaraz, 1997; *O. davisae*: Almeda *et al.*, 2010b; Zamora-Terol and Saiz, 2013). Maximum growth rates of *P. grani* were overall more than twice those of *O. davisae* (Fig. 5; two-way ANOVA, $p < 0.001$). Growth patterns along ontogeny, however, contrasted between species. Juvenile growth rates of *P. grani* did not differ from weight-specific egg production rates (Fig. 5; Sidak's multiple comparisons test, $p = 0.21$); contrarily, maximum naupliar growth rates of *O. davisae* were 86% higher than those of females (Fig. 5; Sidak's multiple comparisons test, $p < 0.001$). The similarity in maximum growth rates of juvenile (somatic) and adult females (egg production) of *P. grani* agrees well with previous assessments in other

small coastal calanoid copepods, which under non-limiting food conditions typically show similar growth rates along ontogeny (Berggreen *et al.*, 1988; Fryd *et al.*, 1991; Sekiguchi *et al.*, 1980). Contrarily, maximum female weight-specific growth rates of *O. davisae* seem to be severely constrained in comparison to the naupliar ones, likely because its egg-carrying reproductive strategy (Kiørboe and Sabatini, 1994; Saiz *et al.*, 2017).

The interspecific differences in specific P content we found conform well to the expected differences in maximum growth rates between both species. Likewise, the ontogenetic data on *Oithona davisae* appear to agree with the postulates of the growth rate hypothesis, since the variations in specific P content closely match the growth rate differences reported along development for this copepod species. However, the lack of expected differences between juvenile and adult growth rates in *Paracartia grani* does not correspond with the large variation in specific P content along development.

Very few studies have really addressed the relationship between growth rates and stoichiometry in marine copepods. To our knowledge only Meunier *et al.* (2016) have provided clear evidence of an increase in N:P and C:P ratios associated with a decline in growth along development in marine copepods. It is worth noticing, however, that the ontogenetic decline in somatic growth observed in their culture of the calanoid *Acartia tonsa* might suggest a certain degree of food limitation by the older stages, since, as mentioned before, many studies have reported that *A. tonsa* and other similar small calanoids show similar growth rates along ontogeny when reared under excess food (e.g. (Berggreen *et al.*, 1988; Hirst and Bunker, 2003). In the more recent work of Mathews *et al.* (2018) on the marine calanoid *Parvocalanus crassirostris*, however, at 25°C under nutrient replete food, naupliar growth rates were approximately 2.5 times higher than the copepodite ones, but the naupliar C:P ratios, instead of lower, were ca. 1.6 times higher than those of copepodites, indicating lower P content in the nauplii (their Fig. 2).

It is not surprising that the variations in P content in copepods might not be always strongly linked to growth. That could be the case, for instance, when food is not limiting (Frost *et al.*, 2005). Moreover changes in P content might also reflect changes in different biochemical compounds besides nucleic acids or different physiological processes (Tang and Dam, 1999). For instance, Vrede *et al.* (1999) found that in the freshwater copepod *Eudiaptomus gracilis* the fraction of total P content associated to nucleic acids decrease from copepodite stages IV and V (ca. 0.7) to the adult stage (ca.

0.5). In the case of the adult copepod, a large fraction of the P analysed in the female body were the consequence of the P-rich oocytes (egg N:P ratios range 7-11, see Results) in mature ovaries waiting until the next spawning event, and therefore being the result of biosynthetic activity. Thus, *P. grani* feeding on *Rhodomonas salina* at satiating conditions can spawn at rates of approximately 54 eggs ind⁻¹ d⁻¹ (Calbet and Alcaraz, 1996; Calbet and Alcaraz, 1997), these eggs accounting for 77% of the total female P content. On the other hand, for *O. davisae*, much less productive, the future eggs in the ovaries may roughly account for only 20% in the female P content (excluding egg sacs, outside the body). Finally, it is worth noticing that Carrillo *et al.* (2001) and Villar-Argaiz *et al.* (2002), in their studies of the population of the freshwater copepod *Mixodiaptomus laciniatus* inhabiting a high-altitude small lake, already highlighted the substantial differences in copepod body stoichiometry associated to differences in life strategy and nutrient demands along ontogeny (nauplii vs. copepodites), and also reported considerable intra-stage variations in P content, instead of strict homeostasis (for instance linked to moulting, Carrillo *et al.*, 2001). The process of development and moulting for juveniles (adult copepods do not moult), with the formation of new structures and appendages in each new instar, together with the metamorphosis to the copepodite stage certainly involve high levels of metabolic activity (and therefore P requirements) that might not be necessarily equivalent to increase in somatic growth. Thus, the intraspecific variations in specific elemental content do not only reflect changes in growth rates but are also the result of different constraints and elemental requirements linked to the particularities of the life history (e.g. free spawner vs. egg carrier). In our study, using cultivated marine copepod species reared under satiation conditions, the influence of these life history traits in stoichiometry were evident, and set the basis to understand the differences in P requirements and the possible impact of P limitation on the productivity of both species in their natural habitat.

CONCLUSIONS

Our study has shown that two marine copepod species, the calanoid *Paracartia grani* and the cyclopoid *Oithona davisae*, with very different life history strategies, have similar specific C and N content, but differ on their specific P content. The higher specific P content (and lower C:P and N:P ratios) of *Paracartia grani* agree with the overall higher levels of metabolic activity and growth rates exhibited by this species.

Our study also suggests, however, that when elemental composition is considered at the intraspecific level (ontogeny), the variations in P content might not always correspond with ontogenetic differences in growth. In particular, the variations in specific P content along the development of *P. grani* appeared uncoupled to the reported maximal juvenile and adult growth rates.

Definitively, more data on other species are needed to envisage whether the pattern observed in *P. grani* is a particular one or is actually the rule that applies for broadcaster copepods. Further experimentation, coupling growth rates with stoichiometry and biochemistry, in particular under conditions of nutrient limitation, are necessary to obtain a better understanding of the underpinning mechanisms linking elemental composition and growth rate in marine copepods (Tang and Dam, 1999).

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513 **DATA ARCHIVING**

514 After publication the data will be deposited in a digital repository.

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TABLE LEGENDS

Table I. Regression statistics for the linear fits shown in Figs. 1, 2 and 3.

Elemental content as μg per individual. Specific elemental content as μg per mm^3 . BL: body length; *: log transformed data; ns: not significant.

Table II. Compilation of published data on different life history traits of

Paracartia grani and *Oithona davisae*. Unless otherwise specified, data

shown correspond to reported mean values from laboratory studies carried

out at 18-19°C in *ad libitum* conditions, using the cryptophyte *Rhodomonas*

salina as food for *Paracartia grani*, and the heterotrophic dinoflagellate

Oxyrrhis marina as food for *Oithona davisae*. n.a.: not available

1 **Table I.**

2

	<i>Paracartia grani</i>	<i>Oithona davisae</i>
C contents vs BL*	$F_{1,14}=831, r^2=0.98, p<0.001$	$F_{1,14}=566, r^2=0.98, p<0.001$
N contents vs BL*	$F_{1,14}=873, r^2=0.98, p<0.001$	$F_{1,14}=685, r^2=0.98, p<0.001$
P contents vs BL*	$F_{1,14}=2666, r^2=0.99; p<0.001$	$F_{1,14}=833, r^2=0.98, p<0.001$
specific C contents vs BL	$F_{1,14}=0.098, p=0.76$ (ns)	$F_{1,13}=1.38, p=0.26$ (ns)
specific N contents vs BL	$F_{1,14}=0.0015, p=0.97$ (ns)	$F_{1,13}=1.39, p=0.26$ (ns)
specific P contents vs BL	$F_{1,14}=60.4, r^2=0.81, p<0.001$	$F_{1,13}=18.5, r^2=0.59, p<0.001$
C:N molar ratio vs BL	$F_{1,14}=3.4, p=0.09$ (ns)	$F_{1,14}=0.037, p=0.85$ (ns)
N:P molar ratio vs BL	$F_{1,14}=44.3, r^2=0.76, p<0.001$	$F_{1,14}=5.53, r^2=0.28, p=0.034$
C:P molar ratio vs BL	$F_{1,14}=35.3, r^2=0.72, p<0.001$	$F_{1,14}=6.54, r^2=0.32, p=0.023$

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Table II.

Life history trait	<i>Paracartia grani</i>	<i>Oithona davisae</i>	Reference
Adult body size	Male: 893 μm Female: 1016 μm	Male: 344 μm Female: 350 μm	This study
Adult body mass	Male: 3.6 $\mu\text{g C}$ Female: 4.9 $\mu\text{g C}$	Male: 0.24 $\mu\text{g C}$ Female: 0.31 $\mu\text{g C}$	This study
Mean life expectancy	Male: n.a. Female: 24 d	Male: 14 d Female: 28 d	Saiz et al. 2015, Saiz et al. 2017
Tolerance to starvation (median survival)	Male: 6 d Female: 6 d	Male: 8 d Female: 15 d	Saiz et al. 2017, Saiz, unpub. data
Respiration rate (in filtered seawater)	Female: 2.2 nmols O_2 $\text{ind}^{-1} \text{h}^{-1}$	Female: 0.19 nmols O_2 $\text{ind}^{-1} \text{h}^{-1}$	Isari et al. 2015, Saiz et al. 2015
Feeding strategy	Suspension feeding (may switch to ambush feeding on large motile prey)	Strict ambush feeding	Henriksen et al. 2007, Saiz et al. 2014, Olivares et al. 2019, unpub. obs.
Maximum clearance rate (on <i>Oxyrrhis marina</i> , ca. 17-18 μm ESD)	Female: 88 $\text{mL ind}^{-1} \text{d}^{-1}$	Female: 4.6 $\text{mL ind}^{-1} \text{d}^{-1}$	Calbet et al. 2007, Saiz et al. 2014
Reproductive strategy	Broadcasting	Egg carrying	Saiz et al. 2015, Saiz et al. 2017
Egg size (diameter)	83 μm	47 μm	This study
Egg mass	0.038 $\mu\text{g C}$	0.006 $\mu\text{g C}$	This study
Clutch size	50 eggs (per day)	20 eggs (both sacs)	Zamora-Terol and Saiz 2013
Egg production rate	50 eggs $\text{ind}^{-1} \text{d}^{-1}$	6 eggs $\text{ind}^{-1} \text{d}^{-1}$	Zamora-Terol and Saiz 2013, Calbet and Alcaraz 1996, Calbet and Alcaraz 1997
Life span total reproductive output	2500 eggs	110 eggs	Saiz et al. 2015, Saiz et al. 2017

FIGURE LEGENDS

Fig. 1. Elemental content (C, N and P) as a function of body length of *Paracartia grani* (a) and *Oithona davisae* (b). Arrows highlight the transition from naupliar to copepodite stage.

Fig. 2. *Paracartia grani*. Relationship between ontogenetic changes in body length and, respectively, body volume (a), specific C content (b), specific N content (c), and specific P content (d).

Fig. 3. *Oithona davisae*. Relationship between ontogenetic changes in body length and, respectively, body volume (a), specific C content (b), specific N content (c), and specific P content (d).

Fig. 4. Relationships between ontogenetic changes in body length and, respectively, C:N and N:P molar ratios of *Paracartia grani* (a) and *Oithona davisae* (c), and C:P molar ratios of *Paracartia grani* (b) and *Oithona davisae* (d).

Fig. 5. Maximum growth rates of nauplii (somatic) and adult females (egg production) of *Paracartia grani* and *Oithona davisae*. Data for *P. grani* from Calbet and Alcaraz (1996) and Calbet and Alcaraz (1997). Data for *O. davisae* from Almeda *et al.* (2010b) and Zamora-Terol and Saiz (2013). Error bars are 95% confidence intervals. See text for more details.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5