

### Lipid allocation in late-stage barnacle larvae from subtropical and temperate waters

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19	RUNNING PAGE HEAD: Larval physiology of two chthamalids
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#### 1 ABSTRACT

2 The transition of planktonic late-stage barnacle larvae to a benthic life requires enough energy to power settlement and metamorphosis, and may be compromised by food limitation during early 3 4 ontogeny. We carried out a comparative study to better understand the larval physiology of 5 space-monopolizing barnacles exposed to contrasting regimes of primary productivity: 6 *Chthamalus bisinuatus* under a meso-oligotrophic regime on the southeastern coast of Brazil, 7 and Chthamalus montagui under a highly-productive regime on the south coast of the British Isles. We used an index based on lipid composition, the triacylglycerol (TAG) to phospholipid 8 9 (PL) ratio, to characterize lipid allocation (energy/structure) in the tissues of cyprid larvae and 10 anticipated depleted TAG reserves in cyprids from less productive waters. Despite the 11 considerably different levels of primary productivity between subtropical  $(1.31 \pm 0.4 \text{ mg Chl})$ 12  $a/m^3$ ) and temperate waters (3.09 ± 1.2 mg Chl- $a/m^3$ ), TAG/PL and settlement success were comparable for C. bisinuatus and C. montagui. Lipid allocation of daily cohorts was also 13 comparable for both chthamalids, with cyprids equally storing TAG reserves ( $\geq 50$  % of total 14 lipid content). This points to an energetic threshold below which nauplii cannot develop to a 15 cyprid and/or selection for lipid accumulation under poor trophic conditions. We highlight the 16 17 challenges of directly relating estimates of primary productivity with food supply and larval physiological status, as lower Chl-a concentrations do not necessarily indicate food limitation for 18 19 barnacle nauplii. We propose a conceptual model to clarify the process of lipid allocation 20 (energetic to structural lipids) in the tissues of cyprid larvae.

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22 KEYWORDS: Supply-side Ecology, Settlement dynamics, Larval physiology, Lipids,

23 Energetics

#### 1. INTRODUCTION

2 The recruitment dynamics of marine benthic organisms have been extensively studied for over seven decades (e.g. Barnes 1956, Connell 1961, 1985, Crisp 1976, Hawkins & Hartnoll 3 4 1982, Roughgarden et al. 1985, Rodriguez et al. 1993, Jenkins et al. 1999, Barbosa et al. 2016, 5 Giménez et al. 2017). Variations in these dynamics have been related to spatial (e.g. Burrows et 6 al. 2010) and temporal (e.g. Jenkins et al. 2008, Kasten et al. 2019a) patterns of pelagic food supply as indicated by chlorophyll-a concentration in coastal waters (Hawkins & Hartnoll 1982, 7 Menge 1997, Leslie et al. 2005, Mazzuco et al. 2015). For instance, Burrows et al. (2010) 8 9 reported that barnacles reached larger sizes in areas of high chlorophyll-a concentration, 10 suggesting that food-limited growth, rather than differential survival caused this trend. High 11 phytoplankton concentrations have also been suggested to increase recruitment by increasing the 12 survival of larvae and recruits, by producing better fed, larger individuals (Menge et al. 1997). Moreover, more evidence has emerged that plasticity in larval condition can regulate post-13 14 settlement events, independent of larval abundance (Jenkins 2005, Tremblay et al. 2007, Allen & Marshall 2010, Giménez 2010). Across a variety of taxa, juvenile performance can be 15 compromised by poor physiological condition of preceding larval stages (e.g. in the gastropod 16 17 Nucella ostrina, Moran & Emlet 2001; barnacle Semibalanus balanoides, Jarrett 2003; bryozoan Watersipora subtorquata, Marshall & Keough 2004; tubeworm Hydroides diramphus, Allen & 18 19 Marshall 2010; crab Carcinus maenas, Giménez 2010). 20 Many factors can affect larval condition, including: (1) quality and quantity of pelagic food sources (Burrows et al. 2010, Toupoint et al. 2012), (2) pelagic larval duration (Pechenik et al. 21 22 1993) and/or (3) maternal investment (Freuchet et al. 2015, Kasten et al. 2019b). Such effects 23 appear to vary strongly under different environmental conditions (Marshall & Keough 2004).

1 Temporal changes in food availability are generally considered to be greater at higher latitudes as 2 a result of seasonal changes in physical variables affecting primary production (Lawrence 1976). Low-latitude systems lack the large phytoplanktonic blooms found at higher latitudes (Ciotti et 3 4 al. 2010). They are typically oligotrophic year-round and depleted of high-quality dietary fatty 5 acids (Colombo et al. 2017, Leal et al. 2018, 2019), shown to be critical for the development of 6 invertebrate larvae (Pernet et al. 2004, Tremblay et al. 2007). Latitudinal changes in egg size (i.e. an increase in egg size with a decrease of temperature) were addressed by classic works by 7 Thorson (1950), who attributed developmental trends to different food availability in different 8 9 latitudes, and by Rass (1941), who attributed egg size trends to temperature-dependent changes 10 in yolk accumulation patterns (see Laptikhovsky 2006, for a new look at Thorson's and Rass's 11 rules). Further, a decreasing latitudinal trend in lipid storage has also been reported for 12 zooplankton groups, with those found in polar systems accumulating the largest lipid reserves 13 (e.g. planktonic crustaceans, such as the amphipods *Themisto libellula* and *T. abyssorum*, the Antarctic krill Euphausia superba and the copepod Calanus propinguus; Lee et al. 2006). In 14 coastal waters, such generalizations are difficult as phytoplankton blooms (that lead to a build-up 15 of storage lipids in planktonic larvae) may result not only from upwelling of nutrient-rich waters, 16 17 but also from local wind forcing and coastal circulation (e.g. scattered events of high productivity in subtropical shores that raise baseline oligotrophic conditions to eutrophic ones 18 19 during short periods, 1 to 2 days; Kasten & Flores 2013) plus nutrient-rich estuarine plumes 20 (Gorman et al. 2019). On intertidal shores, enhanced quality of settling larval stages of benthic species may be 21

critical to allow for a rapid metamorphosis and early juvenile survival (e.g. subtropical
 chthamalid barnacles with larger lipid reserves grow faster for the first days in the benthos; Leal

1 et al. 2020). Triacylglycerol (TAG) is often the major storage lipid and the primary energetic 2 source of lecithotrophic meroplanktonic larvae (e.g. acorn barnacle Semibalanus balanoides, Holland & Walker 1975; king crab Lithodes santolla, Kattner et al. 2003). Besides its energetic 3 supply role, TAG functions as a source of essential fatty acids for membrane integrity, regulating 4 5 the physiological metabolism of many crustacean species, especially the synthetic process of 6 moulting hormones (O'Connor & Gilbert 1968). In non-feeding larvae, depleting TAG reserves can affect pre-attachment exploratory behaviour, so that larvae become less discriminating in 7 8 their 'choice' of settlement substrate (Miron et al. 2000, Marshall & Keough 2003, Tremblay et 9 al. 2007), and may ultimately lead to 'desperate' settlement, that is, metamorphosis occurs in 10 response to a nonspecific cue or even spontaneously (Knight-Jones 1953, Crisp 1955, Lucas et 11 al. 1979). Further, competent larvae with higher energy reserves have been shown to settle in 12 better quality microhabitats (Marshall & Keough 2003), grow faster for the first days in the benthos (Thiyagarajan et al. 2003, Leal et al. 2020), and have greater chances of post-settlement 13 14 survival (Thiyagarajan et al. 2005). A deeper understanding of supply-driven population dynamics (e.g. Underwood & Fairweather 1989, Minchinton & Scheibling 1991, Bertness et al. 15 1992, Delany et al. 2003) in the intertidal realm would therefore benefit from studies exploring 16 17 larval lipidomics of foundation benthic species.

In the present study, we compared the larval physiology, supply, and settlement of two closely related space-monopolizing barnacles of the genus *Chthamalus*: the subtropical *C*. *bisinuatus* and temperate *C. montagui*, inhabiting shores exposed to relatively low and high coastal primary productivity, respectively. We specifically focused on lipid allocation in latestage barnacle larvae, the settlement stage. During their planktonic phase, barnacles pass through 6 naupliar stages before moulting into a cyprid. The nauplius stage I is non-feeding, surviving for

a few days on glycoprotein and lipid yolk reserves before moulting to stage II (Rainbow & 1 2 Walker, 1977). Stages II to VI are planktotrophic, feeding on phytoplankton, with nauplii increasing in size at each moult, and building up lipid reserves needed to support the non-feeding 3 cyprid, specialized for substrate exploration and settlement. We assessed lipid allocation in 4 5 cyprid larvae by using a ratio of energetic to structural lipids (triacylglycerol to phospholipid; 6 TAG/PL ratio), while also measuring other larval traits, including size, lipid droplet cover, and total lipid content. Given the generally poorer primary productivity at subtropical shores, we 7 anticipated lower TAG/PL and settlement success of C. bisinuatus compared to C. montagui 8 9 cyprids, which (naupliar) development occurs in more productive waters. A conceptual model clarifying the process of lipid allocation and energy storage in the tissues of cyprid larvae has 10 11 been developed to place our work into a wider context.

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- 13

#### 2. MATERIAL AND METHODS

#### 14 2.1. Study sites and species

Late-stage (cyprid) larvae of two chthamalid barnacle species were sampled from two 15 different latitudes (subtropical and temperate) in the Atlantic: Chthamalus bisinuatus, on the 16 17 southeastern coast of Brazil in the São Sebastião Channel, and Chthamalus montagui, on the south coast of the British Isles, in Plymouth Sound in the English Channel (see Figure 1). These 18 19 species are considered ecological equivalents as they occupy the same ecological niche (the 20 upper midlittoral zone) forming a conspicuous Chthamalus zone. Chthamalid cover was mostly close to saturation at all sampled sites (80–100%). Comparable shores were sampled at two sites 21 22 per region (separated by 3-6 km), during March 2015 in São Sebastião and August 2015 in 23 Plymouth Sound. These dates correspond to late summer/early autumn months in either region.

1 Samples were first processed at the Center for Marine Biology of the University of São Paulo (CEBIMar/USP, São Paulo, Brazil) and at the Marine Biological Association of the United 2 Kingdom (MBA, Plymouth, British Isles), and later analysed at the Institute of Ocean Sciences 3 4 of Rimouski (ISMER/UQAR; Rimouski, Canada). In Brazil, the rocky shores of Baleeiro Head (23°49'41"S, 45°25'22"W) and Feiticeira Beach 5 6 (23°50'38"S, 45°24'28"W) were sampled. Facing the São Sebastião Channel (SSC, northern coast of São Paulo State), these shores are characterized by a steep rocky habitat with a nearly 7 8 saturated (close to 100% cover) Chthamalus zone in the mid-high littoral (e.g. Kasten and Flores 9 2013; Barbosa et al. 2016). Natural populations of C. bisinuatus along the São Paulo coastline 10 may exhibit a very clear fortnightly rhythm of larval release (Bueno et al. 2010). The SSC is 11 principally a meso-oligotrophic system, with a seasonal chlorophyll-a pattern reaching maximum 12 concentrations in winter  $(0.36 \pm 0.12 \text{ mg/m}^3; \text{Ciotti et al. 2010})$ . During late summer, organic detritus dominate nearshore suspended matter composition over the SSC (Leal et al. 2018). 13 In the English Channel, the rocky shores of Mount Batten (50°21'28"N, 4°7'41"W) and 14 Plymouth Hoe (50°21'47"N, 4°8'17"W) were sampled. These shores are located in Plymouth 15 Sound (PS), an area characterized by dense populations of chthamalid barnacles (e.g. Southward 16 17 1991, Jenkins 2005). Of these, C. montagui is more abundant in embayed areas where there are more particles suspended in the water column (Burrows et al. 1999). Here C. montagui breeds 18 19 between the beginning of May and the end of September, with asynchronous multiple broods 20 within a defined breeding season (Burrows et al. 1992). Settlement patterns of chthamalids over PS are largely dictated by larval choice rather than late-stage larval supply (Jenkins 2005). The 21 22 English Channel, as is typical of temperate systems, has two yearly productivity peaks (spring 23 bloom of diatoms followed by summer dominance of flagellates; Rodriguez et al. 2000). In

1	contrast with other cold-temperate acorn barnacle species such as Semibalanus balanoides, in
2	which release of single larval brood is synchronized to match the predictable spring
3	phytoplankton bloom of diatoms (e.g. Barnes 1956, Crisp & Spencer 1958, Hawkins & Hartnoll
4	1982), flagellates appear to be the type of phytoplankton which best promotes successful
5	development of the planktonic larvae of Chthamalus (Moyse 1963; Burrows et al. 1992).
6	Chlorophyll-a concentration in the study area (measured at the Western Channel Observatory
7	Station L4; https://www.westernchannelobservatory.org.uk/) is at its lowest during winter, and
8	higher during the characteristic spring and autumn phytoplankton blooms (reaching nearly 3
9	mg/m <sup>3</sup> from August through September; Skákala et al. 2018).
10	
11	2.2. Field sampling
12	Sampling was conducted consecutively over a 4-day period in Brazil (03-March to 06-
13	March-2015) and in the British Isles (19-Aug to 22-Aug-2015). Two replicate plankton tows (10
14	m apart; mesh 160 $\mu$ m; 50-100 m tow length at sub-surface) were collected daily at each of the 2
15	sites in the São Sebastião Channel and Plymouth Sound, 100-200 metres offshore, to estimate
16	larval supply (cyprids/m <sup>3</sup> ) and larval morpho-physiological traits. A minimum of 20 cyprids per
17	tow sample were collected for morphometric measures (see below 2.4) and lipid analysis (see
18	below 2.5). Whilst C. bisinuatus was the single chthamalid barnacle species occurring on the
19	subtropical shores sampled, the temperate C. montagui co-occurred with a congener, C. stellatus.
20	Morphological differences between the larvae of C. stellatus and C. montagui have been
21	previously described, with C. montagui being consistently smaller than its congener (over 1500
22	chthamalid cyprids sampled in the PS area; Jenkins 2005). The cyprid carapace length was
23	therefore used to distinguish between both temperate Chthamalus spp. (Power et al. 1999). All

1	temperate cyprids analysed were well below 540 $\mu$ m (see results), the reported length threshold
2	for C. montagui (Powers et al. 1999, Jenkins 2005). Daily settlement rates (individuals/plate/day)
3	were determined by random deployment of 10 settlement plates ( $10cm \times 10cm$ ; covered with
4	rough tape 3M <sup>TM</sup> Safety-Walk) over the 4-day sampling period at each site. Plates were replaced
5	every 24 hours and surroundings were cleared for potential predators. Settlement success was
6	estimated by calculating settlement/supply ratios, and the size of the recently metamorphosed
7	juveniles (rostro-carinal diameter) was measured (see below 2.4).
8	
9	2.3. Remote estimation of primary productivity
10	The trophic conditions at each sampling region were characterized using remote estimates of
11	chlorophyll-a concentration (Chl-a; mg/m <sup>3</sup> ), a commonly used proxy of food supply (similarly to
12	Mazzuco et al. 2015, Kasten et al. 2019a). Remote sensing data for surface Chl-a was acquired
13	from the MODIS-Aqua sensor through the OceanColor database
14	(https://oceancolor.gsfc.nasa.gov/l3/). Level-3 mapped images of 4 km resolution were processed
15	using the software SeaDAS (Version 7.5.3). Local estimates were averaged for one specific cell
16	$(4 \text{ km} \times 4 \text{ km})$ for each region, as sites within regions were not distant enough to warrant site-
17	specific cells. Daily averages were extracted for 5 dates where remote sensing data was available
18	for the São Sebastião Channel (23°56'15" S, 45°26'15" W; dates: 23-02-15, 25-02-15, 27-02-15,
19	03-03-15, 04-03-15) and Plymouth Sound (50°16'15" N, 4°11'15" W; dates: 06-08-15, 08-08-15,
20	17-08-15, 18-08-15, 23-08-15). The extracted dates spanned the pelagic larval development
21	(from nauplius to cyprid) of both species (12 days for C. bisinuatus, Barbosa et al. 2016; 14-17
22	days for C. montagui, Burrows et al. 1999).
23	

#### 1 2.4. Morphometric analysis

2 We focused on cyprid traits that are known to affect post-settlement performance (Thiyagarajan et al. 2003, Jarret 2003, Tremblay et al. 2007, Leal et al. 2020): (1) size (carapace 3 length; µm) and (2) lipid droplet cover (droplets area/body area; %). The transparent carapace of 4 5 cyprid larvae makes it easy to visualize the conspicuous lipid droplets stored anteriorly, around 6 the midgut and at the bases of the thoracic limbs (Figure 2), that constitute the cyprid's main energetic reserves (Rainbow & Walker 1977, Tremblay et al. 2007). Recent evidence using C. 7 bisinuatus cyprids shows that the visual estimation of lipid droplet cover can be used as a proxy 8 9 of TAG content, given the strong correlation between visual lipid area and nile-red stained lipid 10 area under a fluorescence microscope (Guerra et al. *unpublished*). Based on this, size and lipid 11 droplet cover were measured for 170 C. bisinuatus cyprids, and 153 C. montagui cyprids. 12 Cyprids were photographed alive under a stereomicroscope (100  $\times$ ) in lateral view for later measurements through image analysis. Similarly, juveniles settled on the artificial plates (1-day 13 14 old) were photographed daily under a stereomicroscope to obtain measurements of rostro-carinal diameters (148 C. bisinuatus juveniles, and 442 C. montagui juveniles). All morphometric 15 analyses were done using the software Fiji, an open-source platform for scientific image analysis 16 17 (Schindelin et al. 2012).

18

19 2.5. Lipid analysis

To quantify the larval total lipid content (μg/larva) and lipid fractions (classes), cyprids
collected from nearshore waters were preserved at -80°C in dichloromethane-methanol vials
(2:1, v/v) for lipid analysis (2 samples of 20 cyprids per day, per site, comprising a total of 640
cyprids). Lipid extraction was done according to Folch et al. (1957) and classes determined using

1	an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan) developed in a four-
2	solvent system (Parrish 1999). Three main lipid classes were identified: Triacylglycerols (TAG),
3	phospholipids (PL) and acetone mobile polar lipids (AMPL). TAG are storage lipids that make
4	up most of the energetic reserves, PL are the main components of cell membranes, and AMPL
5	are a group constituted mainly from glycolipids, pigments, and degradation products of PL
6	(Parrish 1999). Storage lipids are generally associated with energy supply, while structural lipids
7	are important for the cell membrane stability (Fraser 1989). Most of the variability in individual
8	lipid content reflects changes in TAG levels because this class of lipids most closely reflects
9	changes in factors such as food supply and temperature, which are known to influence the
10	deposition or loss of lipids (Arts et al. 1993).
11	
12	2.5.1. The TAG/PL ratio
13	Given the size-dependency associated with TAG (Fraser 1989), the ratio of triacylglycerol to
14	phospholipid (TAG/PL ratio) was used to characterize lipid allocation in larval tissues, that is,
15	standardized TAG measures by a lipid class related to body size, PL. The ratio of storage to
16	membrane lipids has been found to be a good indicator of physiological condition in a number of
17	fish, bivalve and crustacean larvae (Fraser, 1989, Lochmann et al. 1995, Harding & Fraser 1999).
18	Håkanson (1993) defined fish larvae in poor condition to be those with a ratio less than 0.2. For
19	lobster larvae, Harding & Fraser (1999) considered a condition index less than 0.1 the 'point-of-
20	no-return'. For chthamalid barnacles, the threshold of TAG below which nauplii and subsequent
21	cyprid stages do not survive in the wild is yet to be estimated. However, through a laboratory
22	experiment, Freuchet et al. (2015) have shown that the ratio of storage to membrane lipids in $C$ .
23	bisinuatus nauplii (larval feeding stages) exposed to low food supply corresponded to 0.7, as

opposed to a ratio of 1.9 in nauplii fed high food supply. As TAG reserves in (non-feeding)
cyprid larvae are largely linked to those accumulated during the precedent naupliar stages, we
argue that the TAG/PL ratio can be used to distinguish cyprids with high endogenous TAG
reserves (ratio far greater than 1) that fuel larval metabolic activity, such as escaping from
predators and swimming (pertinent for substrate exploration behaviour; Miron et al. 2000,
Tremblay et al. 2007), from energy-depleted cyprids (ratio far below 1), maintaining basal
metabolism.

8

9 2.6. Statistical analysis

10 A nested Analysis of Variance (ANOVA) was used to determine if statistically significant differences in settlement/supply ratio (dependent variable) existed between Species/Regions (C. 11 12 bisinuatus in the São Sebastião Channel vs. C. montagui in the English Channel). A balanced design with Sites (Site 1, Site 2) nested within Region was used. With this approach, we tested 13 14 the hypothesis that larger settlement/supply ratios would be expected in areas with greater productivity (Plymouth Sound in the English Channel, see results) with regional differences 15 overwhelming any small-scale variation. Using the same design, differences in larval lipid 16 17 allocation (TAG/PL ratio) between C. bisinuatus and C. montagui were tested by running a nested ANOVA. Differences in frequency distributions of cyprid size, lipid droplet cover, and 18 19 juvenile size, between species were tested using two-sample independent Kolmogorov-Smirnov 20 tests, to understand whether selection pressures on morphological larval traits differed among subtropical and temperate environments. To investigate the relationship between morpho-21 22 physiological larval traits, a Pearson correlation matrix was computed for the variables cyprid 23 size, lipid droplet cover, total lipid content, and TAG/PL ratio. The R software (version 3.6.1; R

Core Team 2019) and packages ggplot2 (Wickham 2016) and ggpubr (Kassambara 2019) were
 used to conduct statistical analysis and as a graphical tool.

3

#### 4 **3. RESULTS**

6 Monthly estimates of pelagic primary production (Chl-*a*) for 2015 (Fig. 1) revealed that

7 sampling was carried out during a peak of Chl-*a* for both the São Sebastião Channel (SSC)

8 (March, 2.85 mg/m<sup>3</sup>) and Plymouth Sound (PS) (August, 5.17 mg/m<sup>3</sup>). Throughout the sampling

9 period, daily estimates averaged  $1.31 \pm 0.4 \text{ mg/m}^3$  for the SSC, contrasting with the higher Chl-a

10 concentration of PS,  $3.09 \pm 1.2 \text{ mg/m}^3$  (Fig. 3A). Larval supply (Fig. 3B) was around three times

11 higher at the temperate shores  $(158 \pm 46 \text{ ind/m}^3)$  compared to the subtropical ones  $(46 \pm 14)$ 

12 ind/m<sup>3</sup>). The same pattern held true for daily settlement rates (Fig. 3C), with *Chthamalus* 

13 *montagui* settling at higher rates (~11 settlers/plate/day) compared to its subtropical congener

14 Chthamalus bisinuatus (~4 settlers/plate/day). However, settlement/supply ratios (Fig. 3D) were

15 similar, with no differences being found between regions, nor between sites within regions (p >

16 0.05; Table 1). This indicates comparable rates of larval settlement per number of larvae in the

17 water column, that is, *C. bisinuatus* cyprids settled in the same proportion as those of *C*.

18 *montagui* despite developing in less productive waters. Within-region variability (coefficient of

19 variation; % CV) was, however, high for supply (SSC: 68 %; PS: 72 %) and settlement (SSC: 73

20 %; PS: 56 %) patterns, suggesting that the generality of these results must be interpreted with

22

21

23 3.2. Morpho-physiological larval traits

caution, in light of the variability observed.

1	The size-frequency distribution curves differed between species for the cyprid stage
2	(Kolmogorov-Smirnov K <sub>d 170, 153</sub> = 7.00, $p < 0.0001$ ; Fig. 4A) and juvenile stage (K <sub>d 148, 442</sub> =
3	7.50, $p < 0.0001$ ; Fig. 4C), with <i>C. montagui</i> being consistently larger than its subtropical
4	congener. Lipid droplet cover distributions also differed (K <sub>d 170, 153</sub> = 5.04, $p < 0.0001$ ; Fig. 4B),
5	with C. bisinuatus having greater covers than the temperate C. montagui. C. bisinuatus averaged
6	$224\pm28~\mu m$ in cyprids' carapace length, $24\pm8~\%$ in lipid droplet cover, and $226\pm23~\mu m$ in
7	juvenile size (Mean $\pm$ SD). <i>C. montagui</i> averaged 290 $\pm$ 70 $\mu$ m in cyprids' carapace length, 14 $\pm$
8	7 % in lipid droplet cover, and 283 $\pm$ 41 $\mu m$ in juvenile size.
9	A detailed description of the lipid classes found in the larval tissue of both chthamalids is
10	given in Table 2. Total lipid content averaged 50 µg/cyprid for C. bisinuatus, and 48.5 µg/cyprid
11	for C. montagui. Triacylglycerols (TAG) and phospholipids (PL) were the major lipid classes of
12	both species, constituting more than 90% of the total larval lipid content. The TAG/PL ratio was
13	similar for both species (1.2 for <i>C. bisinuatus</i> , and 1.3 for <i>C. montagui</i> ; $p > 0.05$ , Table 1),
14	indicating that larvae similarly allocated lipids to energy storage and cellular structure, and that
15	larvae had previously accumulated TAG reserves during their naupliar development. There was,
16	however, considerable intra-specific variation (% CV) in this condition index (30 % in $C$ .
17	bisinuatus and 32 % in C. montagui), which might explain the nearly significant effect of site in
18	the ANOVA comparisons ( $p = 0.05$ ; Table 1).
19	The relationship between the TAG/PL ratio and other larval traits shows that cyprids with
20	similar physiological status may vary in size (Fig. 5A), lipid droplet cover (Fig. 5B), and total
21	lipid content (Fig. 5C); yet, some patterns emerged. The highest ratios (TAG/PL $\ge$ 1.5) found in
22	C. montagui cyprids comprised small-sized cohorts (< 300 $\mu$ m) with lipid droplets covering a
23	greater area of the body ( $\geq$ 20 % cover). This was confirmed by the positive relationship between

1 TAG/PL and lipid droplet cover (p = 0.01, r = 0.90; Table 3). The lipidic costs associated with 2 larger sizes can be further evidenced by the (nearly significant) negative relationship between 3 size and total lipid content (p = 0.05, r = -0.74; Table 3). No significant relationships were 4 detected among *C. bisinuatus* larval traits (Table 3). *C. bisinuatus* cyprids had a similar size-5 range ( $200 - 245 \mu m$ ) regardless of TAG/PL ratio, but similar to their temperate counterparts, the 6 highest ratios were found in cyprids that had a greater lipid droplet cover ( $\geq 20 \%$  cover).

7

### 8 4. DISCUSSION

9 In this study, we show that similar lipid allocation in late-stage barnacle larvae can occur in 10 Chthamalus species exposed to contrasting trophic regimes, contrary to the expected depletion of 11 TAG reserves in cyprid larvae from less productive waters. This points to an energetic threshold 12 below which nauplii cannot develop to a cyprid and/or selection for lipid accumulation under poor trophic conditions. The equivalence in physiological status between larvae given by the 13 TAG/PL ratio was reflected in our finding of similar supply/settlement ratios in Chthamalus 14 bisinuatus and Chthamalus montagui. We highlight the challenges of directly relating estimates 15 of primary productivity with food supply and larval physiology, as lower Chl-a concentrations 16 17 do not necessarily indicate food limitation for barnacle nauplii. We also propose a conceptual model for the integration of morpho-physiological traits when assessing lipid allocation 18 19 (energetic to structural lipids) in the tissues of cyprid larvae.

20

21 4.1. The ratio of storage to membrane lipids: TAG/PL

As reported in studies with other benthic invertebrates (Delaunay et al. 1992, Abad et al. 1995), triacylglycerols (TAG) and phospholipids (PL) were the main lipid classes found in

1	cyprids of both Chthamalus species here tested. TAG alone accounted for 50 % of the total lipid
2	content in C. bisinuatus, and 53 % in C. montagui. These values are similar to those reported by
3	Holland and Walker (1975) in balanid cyprids, where neutral lipids comprised 66% of the total
4	lipid fraction. The need for TAG accumulation in pre-metamorphic stages is easily understood,
5	given the reliance on endogenous energy during metamorphosis (Lucas et al. 1979, Thiyagarajan
6	et al. 2003). Moreover, higher TAG accumulation reduces sinking rates, which has been
7	suggested to reduce energy costs of swimming cyprids to maintain optimal depth (Burrows et al.
8	1999) and allow for a more prolonged search for a suitable substrate (Lucas et al. 1979).
9	Total lipids and TAG are predictors of settlement success, post-metamorphic growth, and
10	survival in other species (e.g. Balanus amphitrite; Miron et al., 2000; Thiyagarajan et al. 2005,
11	Tremblay et al. 2007), and therefore an excellent reference index for larval quality. TAG adjusts
12	quickly to changes in food supply and temperature (Lochmann et al. 1995, Freites et al. 2002),
13	whereas PL provides structural-type functions (e.g. membrane stability) that are maintained
14	fairly constant (Fraser 1989) and correlate with the size of larvae, as demonstrated in fish and
15	crustaceans. We argue that the use of a lipid-based condition index (such as the TAG/PL ratio) is
16	useful to estimate the physiological condition of barnacle cyprids, by informing how larvae
17	allocate lipids in their tissues to energy storage and/or cellular structure (Fig. 6). We found
18	similar ratios for both chthamalid barnacle species, with a TAG/PL ratio above 1 (1.2 $C$ .
19	bisinuatus, 1.3 C. montagui) indicating that larvae had accumulated energetic (TAG) reserves
20	during their previous naupliar feeding stages and/or through maternal allocation. Estimates of the
21	TAG/PL ratio at the point-of-no-return (i.e. of non-viable larvae in the wild) are still lacking for
22	chthamalid cyprids and are necessary to make well-grounded inferences on larval physiological
23	status using lipid-based condition indices. We argue that analysing several morpho-physiological

1 traits together leads to a better understanding of larval condition (Fig. 6). For instance, focusing 2 on size-metrics alone would render large C. montagui cyprids the ones with highest quality, which appears not to be the case. Subtropical C. bisinuatus cyprids with 200 µm carapace length 3 4 had higher TAG reserves than C. montagui with 400 µm or more. We hypothesize that the larger 5 the cyprid, the bigger the structural and energetical lipidic demands it has. For a balanid barnacle 6 (Balanus glandula), Hentschel and Emlet (2000) found that a large nauplius with a high feeding rate and reduced time to cyprid moult might store the same amount of lipid as a smaller nauplius 7 8 with a slower feeding rate and a longer naupliar duration, demonstrating the plasticity in size and 9 age at metamorphosis, and suggested it to be an adaptation to environmental variability. As 10 suggested for other barnacle groups (balanids, Giménez et al. 2017, Hentschel and Emlet 2000), 11 the physiological condition of both chthamalids here described likely reflects local selection for 12 larval phenotypes that match the requirements for juvenile survival (see 4.3. discussion below).

13

#### 14 4.2. Effects of food supply

Our findings show that lower Chl-a concentrations do not necessarily indicate food limitation 15 for barnacle nauplii (see below 4.3.2), and do not provide support for the general assumption of 16 17 latitudinal gradients in larval lipid storage in this group of barnacles. Cyprid larvae of both 18 chthamalids had similar lipid profiles and contents, despite being exposed to contrasting trophic 19 regimes during their development. This might have been caused by differing (1) competition for 20 phytoplankton, (2) nutritional quality of food resources, and/or (3) physiology linked to differential lipid retention (see below 4.3.1). Starvation appears to be the biggest threat for 21 22 pelagic crustacean larvae, being driven by high metabolic demands (Morgan 1995) and food 23 limitation (Bashevkin & Morgan 2020). In nearshore waters, herbivorous zooplankton are,

1 however, rarely limited by food (Strathmann 1996), and barnacle nauplii are unlikely to be 2 strongly affected by competition (Bashevkin & Morgan 2020). To the authors knowledge, limited information is available either on particle size ingested by barnacle nauplii, or on 3 4 selective feeding behaviour, and for chthamalids, the only evidence was published by Stone 5 (1989), under laboratory conditions. This gap in the scientific literature was also recently pointed 6 by Bernal and Anil (2019). The authors found that picoplankton ( $\leq 2.0 \,\mu$ m), particularly the 7 picocyanobacteria Synechococcus, occupy a key position in the naupliar diet of Amphibalanus 8 amphitrite. Unlike larger phytoplankton (nano and micro), picocyanobacteria and other 9 picophytoplankton are better adapted to low-nutrient conditions, contributing significantly to the 10 bulk of primary production in oligotrophic waters (Bemal & Anil 2019). Even species adapted to 11 more productive regimes, such as the nauplii of *Chthamalus montagui* and *Balanus perforatus*, 12 have been grown successfully when fed on small cells (< 5 µm) of Micromonas pusilla and 13 Isochrysis galbana (Stone 1989). Vargas et al. (2006) also reports that nauplii of two barnacle 14 species, Jehlius cirratus and Notobalanus flosculus from central Chile, primarily graze on picophytoplankton and small nanophytoplankton ( $\leq 5 \mu m$ ) when incubated in natural sea water. 15 While low amounts of phytoplanktonic food resources are present over the SSC, detrital 16 17 pathways (e.g. phytodetritus, bacteria) prevail in these subtropical waters (Leal et al. 2018). It is possible therefore that naupliar stages of barnacles over the SSC are adapted to explore other 18 19 sources of food in their diet to build their energetic (TAG) reserves. Recent studies on the dietary 20 fatty acid composition of cyprid larvae inhabiting different latitudes (Chthamalus spp. and Semibalanus sp.) show that lipid reserves can be built from different food sources, not simply 21 22 microalgae (Leal et al. in prep). In C. bisinuatus for instance, a large proportion of energetic 23 reserves contain trophic tracers for terrestrial detritus and macrophytes (Leal et al. *in prep*).

1 Triacylglycerols, the main constituent of larval reserves, are tri-esters consisting of a glycerol 2 bound to three fatty acids, so that there are many different types of TAG, with the main division existing between saturated (e.g. 16:0 palmitic acid, 18:0 stearic acid) and unsaturated types (e.g. 3  $\omega$ -3 and  $\omega$ -6 essential fatty acids). As the fatty acid composition of lipid classes was not 4 5 characterized in this study, it is not possible to ascertain what type of fatty acids were the 6 building blocks of the TAG reserves for each species. It is possible that in temperate waters, microalgae-derived fatty acids (unsaturated) make up most of the food available for C. montagui 7 8 nauplii, whereas in subtropical waters, detrital matter (saturated) makes up most of the diet of C. 9 bisinuatus nauplii. That would mean that, although both species have similar TAG contents, their 10 fatty acid composition is probably different. We therefore urge further research to reconcile the 11 trophic ecology (e.g. feeding behaviour, feeding rates, dietary fatty acid composition) of 12 chthamalid barnacles.

Shifts in food during the ultimate naupliar instar (VI) particularly affect the cyprid's lipid 13 14 accumulation, stored in (neutral) lipid droplets (Hentschel & Emlet 2000). Considering the duration of pelagic larval development from nauplius to cyprid of both Chthamalus species - 12 15 days for C. bisinuatus (Barbosa et al. 2016), and 14-17 days for C. montagui (Burrows et al. 16 17 1999) – cyprid cohorts sampled might have been exposed to different food supply that reflect a lag in their TAG/PL ratio. For example, scattered events of high primary productivity during 18 19 short periods (1 to 2 days) have been reported for the SSC (Kasten & Flores 2013). Such 20 variability likely leads to differential condition of cyprid cohorts, as recently reported for C. bisinuatus cyprids (reaching values as high as 52%; Leal et al. 2020). This suggests that selection 21 22 for cohorts with high TAG reserves may occur in subtropical shores. The physiological condition 23 of cyprids is also largely determined by maternal allocation (Kasten et al., 2019b). In subtropical

1 C. bisinuatus, carry-over effects of maternal allocation seem to have the same effects as pelagic 2 inputs to larvae in the determination of cyprid size (c.a. 13% to 14% size increase; Kasten et al. 3 2019b), which may compensate for the low food supply in these subtropical shores. 4 5 4.3. Implications 6 4.3.1. Physiological thresholds in cyprid larvae 7 We suggest that there may be a threshold above which greater sizes are achieved in detriment 8 of lipid reserves, a cost that is likely too high for species that are exposed to low food supply. 9 Lipid accumulation has been suggested to be an adaptive strategy for species inhabiting habitat-10 limited shores (e.g. maternal provisioning essential at crowded shores, Kasten et al. 2019b). It 11 has also been suggested for *Semibalanus balanoides* larvae along the coast of West Scotland by 12 Giménez et al. (2017), who observed a counter-gradient pattern in larval reserves and Chl-a and suggested an adaptive role of increased reserves in buffering juveniles from low food 13 14 availability. They argued that ultimately, local adaptation will drive larval phenotypes to match the requirements for juvenile survival. In the case of C. bisinuatus, it is possible that TAG 15 reserves (stored as lipid droplets) for settlement and metamorphosis may offset the detrimental 16 17 effects of food scarcity during the critical first days in the benthos, where juveniles must grow as fast as possible (*C. bisinuatus* cyprids with more stored reserves lead to juveniles that grow 18 19 faster; Leal et al., 2020). In cold-temperate shores, peaks of C. montagui settlement follow major 20 phytoplanktonic blooms. Thus, food is likely not limiting at the onset of juvenile feeding, and 21 larvae may invest on size to counterbalance the slower growth rates imposed by low 22 temperatures. The impact of the observed physiological traits on metamorphic performance will 23 also depend on the post-metamorphic environment. Post-settlement processes (e.g. predation,

desiccation, microhabitat availability) can accentuate the patterns set pre-settlement or during
 settlement (Bohn et al. 2013a, b), making a comprehensive understanding of larval physiology in
 supply-side ecology critical.

A caveat must be given to comparing one species each in two locations, albeit with local 4 5 replication using a nested design, if wanting to draw wider conclusions about latitudinal patterns. 6 Ideally, more than one species should be used in each locality, with several localities in both 7 sides of the ocean. Unfortunately, there is only one chthamalid barnacle species in southeast Brazil. In Europe, C. stellatus has a more offshore larval distribution and morphology as well as 8 9 longer larval life with greater dispersal (Burrows et al. 1999, Pannacciulli et al. 1997) and is 10 more prevalent on exposed headlands (Crisp, Southward & Southward 1981, Jenkins 2005). The 11 field-based results from our comparative study, however, plus the final proposed conceptual 12 model, contribute to a better understanding of the physiology of settling barnacle larvae of closely-related Chthamalus species, and motivate further research on the effects of larval 13 14 energetics in shaping settlement of this important group of habitat-forming species.

15

16 4.3.2. The challenge of remotely estimating food supply

We highlight the challenge of directly relating estimates of primary productivity (Chl-*a*) with food supply and larval physiological status. We observed similar TAG/PL for barnacle cyprids exposed to considerably different levels of Chl-*a* ( $1.31 \pm 0.4 \text{ mg/m}^3$  in the SSC, southeast Brazil, *vs.*  $3.09 \pm 1.2 \text{ mg/m}^3$  in PS, southwest British Isles), for a period spanning 2-weeks prior sampling. Other studies have not found correlations between Chl-*a* and larval traits (e.g. Giménez et al. 2017, Ewers-Saucedo & Pappalardo 2019). As suggested by Ciotti et al. (2010), the general spatial patterns for Chl-*a* exist in the surface ocean (see Appendix Fig. 1), resulting

1 from differences in nutrient and light availability for phytoplankton growth set by regional and 2 global physical processes, as well as other important but less understood processes such as grazing, sedimentation and advection rates for phytoplankton cells. However, they neither 3 4 translate directly into primary production nor exportation rates for phytoplankton carbon. 5 Mechanisms related specifically to food quality (e.g. phytoplankton species, organic matter 6 composition), cannot be directly associated to Chl-a patterns. Further, nearshore trophic 7 subsidies to rocky shore communities, e.g. surf-zone hydrodynamics as subsidies of phytoplankton (sensu Morgan et al. 2018) by increasing the nutrient uptake, light exposure, and 8 9 suspension of surf diatoms from the sediment into the water column, are often overlooked. The 10 high concentrations of phytoplankton (far higher concentrations of phytoplankton in surf-zones 11 than offshore; Shanks et al. 2016, 2017), particulate organic matter, and detritus, can sustain 12 short and highly productive food webs. Most importantly, together with land-derived organic subsidies (e.g. rainfall-driven inputs in shallow subtropical waters; Gorman et al. 2019), such 13 nutrient apport to nearshore marine communities fails to be detected by remote estimates of 14 primary productivity, compromising assumptions made on food supply for suspension feeders, 15 especially in areas with strong detrital-based nearshore food webs, typical of low-latitudes (e.g. 16 17 Leal et al. 2018, 2019).

18

#### 19 **5. CONCLUSION**

20 Quantifying the lipid class fractions (TAG, PL) that are present in the total lipid content of 21 larvae gives important information regarding the way lipid compounds are allocated (energy 22 storage/cellular structure). The similar TAG/PL ratio observed for two closely-related barnacle 23 species exposed to different levels of surface Chl-*a* highlights the challenges of directly relating

1	estimates of primary productivity with larval physiological status, and the need to better describe
2	trophic resources available for pelagic naupliar stages. We hope to motivate further research,
3	ideally with more replicate species, to unveil latitudinal patterns in lipid allocation in this
4	important group of barnacles.
5	
6	
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18	LITERATURE CITED
19	Abad M, Ruiz C, Martínez D, Mosquera G, Sánchez JL (1995) Seasonal variations of lipid
20	classes and fatty acids in flat oyster, Ostrea edulis, from San Cibran (Galicia Spain). Comp
21	Biochem Physiol 110:109-118.
22	Allen RM, Marshall DJ (2010) The larval legacy: cascading effects of recruit phenotype on post-
23	recruitment interactions. Oikos 119:1977-1983.

1	Arts MT, Robarts RD, Evans (1993) Energy reserve lipids of zooplanktonic crustaceans from an
2	oligotrophic saline lake in relation to food resources and temperature. Can J Fish Aquat Sci
3	50:2404-2420.
4	Barbosa AC, Gomes CC, Pereira GC, Bueno M, Flores AAV (2016) Local biological drivers, not
5	remote forcing, predict settlement rate to a subtropical barnacle population. Mar Ecol Prog Ser
6	543:201-208.

Barnes H (1956) *Balanus balanoides* (L.) in the Firth of Clyde: the development and annual
variation of the larval population, and the causative factors. J Anim Ecol 25:72-84.

9 Bashevkin SM, Morgan SG (2020) Predation and competition. Pages 360-382 in K. Anger, S.

10 Harzsch, M. Thiel, editors. The Natural History of the Crustacea: Developmental Biology and

11 Larval Ecology. Volume 7. Oxford University Press, New York.

12 Bemal S, Anil AC (2019) Picophytoplankton *Synechococcus* as food for nauplii of

13 Amphibalanus amphitrite and Artemia salina. Hydrobiologia 835:21-36.Bertness MD, Gaines

14 SD, Stephens EG, Yund PO (1992) Components of recruitment in populations of the acorn

15 barnacle Semibalanus balanoides (Linnaeus). J Exp Mar Biol Ecol 156:199-215.

16 Bohn K, Richardson CA, Jenkins SR (2013a) The importance of larval supply, larval habitat

17 selection and post-settlement mortality in determining intertidal adult abundance of the invasive

18 gastropod *Crepidula fornicata*. J Exp Mar Biol Ecol 440:132-140.

19 Bohn K, Richardson CA, Jenkins SR (2013b) Larval microhabitat associations of the non-native

20 gastropod Crepidula fornicata and effects on recruitment success in the intertidal zone. J Exp

21 Mar Biol Ecol 448:289-297.

22 Bueno M, Moser GAO, Tocci BRC, Flores AAV (2010) Retention-favorable timing of

23 propagule release in barnacles and periwinkles. Mar Ecol Prog Ser 414:155-165.

1	Burrows MT, Hawkins SJ, Southward AJ (1992) A comparison of reproduction in co-occurring
2	chthamalid barnacles, Chthamalus stellatus (Poli) and Chthamalus montagui Southward. J Exp
3	Mar Biol Ecol 160:229-249.Burrows MT, Hawkins SJ, Southward AJ (1999) Larval
4	development of the intertidal barnacles Chthamalus stellatus and Chthamalus montagui. J Mar
5	Biol Assoc UK 79:93-101.
6	Burrows MT, Jenkins SR, Robb L, Harvey R (2010) Spatial variation in size and density of adult
7	and post-settlement Semibalanus balanoides: effects of oceanographic and local conditions. Mar
8	Ecol Prog Ser 398:207-219.
9	Ciotti AM, Garcia CAE, Jorge DSF (2010) Temporal and meridional variability of Satellite-
10	estimates of surface chlorophyll concentration over the Brazilian continental shelf. Pan-Am J
11	Aquat Sci 5:236-253.
12	Colombo SM, Wacker A, Parrish CC, Kainz MJ, Arts MT (2017) A fundamental dichotomy in
13	long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial
14	ecosystems. Environ Rev 25:163-174.
15	Connell JH (1961) Effects of competition, predation by Thais lapillus, and other factors on
16	natural populations of the barnacle Balanus balanoides. Ecol Monogr 31:61-104.
17	Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement
18	mortality in rocky intertidal communities. J Exp Mar Biol Ecol 93:11-45.
19	Crisp DJ (1955) The behaviour of barnacle cyprids in relation to water movements over a
20	surface. J Exp Biol 32:569-590.
21	Crisp DJ (1976) Settlement responses in marine organisms. pp. 83-124. Butterworths, London.

1	Crisp DJ, Southward AJ, Southward EC (1981) On the distribution of the intertidal barnacles
2	Chthamalus stellatus, Chthamalus montagui and Euraphia depressa. J Mar Biol Assoc UK
3	61:359-380.
4	Crisp DJ, Spencer CP (1958) The control of the hatching process in barnacles. Proc R Soc B
5	149:278-299.
6	Delany J, Myers AA, McGrath D, O'Riordan RM, Power AM (2003) Role of post-settlement
7	mortality and 'supply-side' ecology in setting patterns of intertidal distribution in the chthamalid
8	barnacles Chthamalus montagui and C. stellatus. Mar Ecol Prog Ser 249:207-214.
9	Delaunay F, Marty Y, Moal J, Samain JF (1992) Growth and lipid class composition of Pecten
10	maximus (L.) larvae grown under hatchery conditions. J Exp Mar Biol Ecol 163:209-219.
11	Ewers-Saucedo C, Pappalardo P (2019) Testing adaptive hypotheses on the evolution of larval
12	life history in acorn and stalked barnacles. Ecol Evol 9:11434-11447.
13	Folch J, Lees M, Sloanestanley GH (1957) A simple method for the isolation and purification of
14	total lipids from animals tissues. J Biol Chem 226:497-509.
15	Fraser JA (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean
16	larvae. Can J Fish Aquat Sci 46:1868-1873.
17	Freites L, Fernández-Reiriz MJ, Labarta U (2002) Lipid classes of mussel seeds Mytilus
18	galloprovincialis of subtidal and rocky shore origin. Aquaculture 207:97-111.
19	Freuchet F, Tremblay R, Flores AA (2015) Interacting environmental stressors modulate
20	reproductive output and larval performance in a tropical intertidal barnacle. Mar Ecol Prog Ser
21	532:161-175.
22	Giménez L (2010) Relationships between habitat conditions, larval traits, and juvenile
23	performance in a marine invertebrate. Ecology 91:1401-1413.

1	Giménez L, Torres G, Pettersen A, Burrows MT, Estévez A, Jenkins SR (2017) Scale-dependent
2	natural variation in larval nutritional reserves in a marine invertebrate: implications for
3	recruitment and cross-ecosystem coupling. Mar Ecol Progr Ser 570:141-155.
4	Gorman D, Pucci M, Soares LS, Turra A, Schlacher TA (2019) Land–Ocean Connectivity
5	Through Subsidies of Terrestrially Derived Organic Matter to a Nearshore Marine
6	Consumer. Ecosystems 22:796-804.Håkanson, J.L., 1993. Nutritional condition and growth rate
7	of anchovy larvae (Engraulis mordax) in the California Current: two contrasting years. Mar Biol,
8	115:309-316.
9	Harding, G.C. and Fraser, A.J., 1999. Application of the triacylglycerol/sterol condition index to
10	the interpretation of larval lobster Homarus americanus distribution in close proximity to
11	Georges Bank, Gulf of Maine. Mar Ecol Progr Ser 186:239-254.
12	Hawkins SJ, Hartnoll RG (1982) Settlement patterns of Semibalanus balanoides (L.) in the Isle
13	of Man (1977-1981). J Exp Mar Biol Ecol, 62:271-283.
14	Hentschel BT, Emlet RB (2000) Metamorphosis of barnacle nauplii: effects of food variability
15	and a comparison with amphibian models. Ecology 81:3495-3508.
16	Holland DL, Walker G (1975) The biochemical composition of the cypris larva of the barnacle
17	Balanus balanoides L. ICES J Mar Sci 36:162-165.
18	Jarrett JN (2003) Seasonal variation in larval condition and postsettlement performance of the
19	barnacle Semibalanus balanoides. Ecology 84:384-390.
20	Jenkins SR (2005) Larval habitat selection, not larval supply, determines settlement patterns and
21	adult distribution in two chthamalid barnacles. J Anim Ecol 74:893-904.
22	Jenkins SR, Murua J, Burrows MT (2008) Temporal changes in the strength of density-
23	dependent mortality and growth in intertidal barnacles. J Anim Ecol 77:573-584.

1	Jenkins SR, Norton TA, Hawkins SJ (1999) Settlement and post-settlement interactions between
2	Semibalanus balanoides (L.)(Crustacea: Cirripedia) and three species of fucoid canopy algae. J
3	Exp Mar Biol Ecol 236:49-67.
4	Kassambara A (2019) ggpubr: 'ggplot2' Based Publication. Ready Plots. R package version 0.2.2.
5	Kasten P, Flores AAV (2013) Disruption of endogenous tidal rhythms of larval release linked to
6	food supply and heat stress in an intertidal barnacle. Mar Ecol Prog Ser 472:185-198.
7	Kasten P, Jenkins SR, Tremblay R, Flores AAV (2019b) Evidence for enhanced late-stage larval
8	quality, not survival, through maternal carry-over effects in a space monopolizing barnacle.
9	Hydrobiologia 830:277-286.
10	Kasten P, Tremblay R, Flores AA (2019a) Mismatched seasonal patterns of larval production
11	and quality in subtropical barnacle populations along a coastal trophic gradient. Estuar Coast
12	Shelf S 224:43-50.
13	Kattner G, Graeve M, Calcagno JA, Lovrich GA, Thatje S, Anger K (2003) Lipid, fatty acid and
14	protein utilization during lecithotrophic larval development of Lithodes santolla (Molina) and
15	Paralomis granulosa (Jacquinot). J Exp Mar Biol Ecol 292:61-74.
16	Laptikhovsky V (2006) Latitudinal and bathymetric trends in egg size variation: a new look at
17	Thorson's and Rass's rules. Mar Ecol 27:7-14.
18	Lawrence JM (1976) Patterns of lipid storage in post-metamorphic marine invertebrates.
19	American Zoologist 16:747-762.
20	Leal I, Bouchard É, Flores AAV, Tremblay R (2018) Trophic cues as possible triggers of mussel
21	larval settlement in southeastern Brazil. Aquat Living Resour 31:26.
22	Leal I, Flores AA, Collin R, Tremblay R (2019) Drifting in the Caribbean: Hints from the
23	intertidal bivalve Isognomon alatus. Estuar Coast Shelf S 227:106333.

- 1 Leal I, Flores AAV, Archambault P, Collin R, Tremblay R (2020) Response of tropical and
- 2 subtropical chthamalid barnacles to increasing substrate temperatures. J Exp Mar Biol Ecol
  3 524:151281.
- 4 Leal I. Flores AAV, Archambault P, Collin R, Sejr MK, Thyrring J, Scrosati RA, Tremblay R (in
- 5 *prep*) Physiological lipid remodeling as a function of latitude in late-stage barnacle larvae.
- 6 Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser
  7 307:273-306.
- Leslie H, Breck E, Chan F, et al (2005) Barnacle reproductive hotspots linked to nearshore ocean
  conditions. Proc Natl Acad Sci USA 102:10534-10539.
- 10 Lochmann SE, Maillet GL, Frank KT, Taggart CT (1995) Lipid class composition as a measure
- of nutritional condition in individual larval Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci
  52:1294-1306.
- 13 Lucas MI, Walker G, Holland DL, Crisp DJ (1979) An energy budget for the free-swimming and
- 14 metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar Biol 55:221-229.
- 15 Marshall DJ, Keough MJ (2003) Variation in the dispersal potential of non-feeding invertebrate
- 16 larvae: the desperate larva hypothesis and larval size. Mar Ecol Prog Ser 255:145-153.
- 17 Marshall DJ, Keough MJ (2004) Variable effects of larval size on post-metamorphic
- 18 performance in the field. Mar Ecol Prog Ser 279:73-80.
- 19 Mazzuco ACA, Christofoletti RA, Pineda J, Starczak VR, Ciotti AM (2015) Temporal variation
- 20 in intertidal community recruitment and its relationships to physical forcings, chlorophyll-a
- 21 concentration and sea surface temperature. Mar Biol 162:1705-1725.

1	Menge BA, Daley BA, Wheeler PA, Dahlhoff E, Sanford E, Strub PT (1997) Benthic-pelagic
2	links and rocky intertidal communities: bottom-up effects on top-down control?. Proc Natl Acad
3	Sci 94:14530-14535.
4	Minchinton TE, Scheibling, RE (1991) The influence of larval supply and settlement on the
5	population structure of barnacles. Ecology, 72:1867-1879.

- 6 Miron G, Walters LJ, Tremblay R, Bourget E (2000) Physiological condition and barnacle larval
- 7 behavior: a preliminary look at the relationship between TAG/DNA ratio and larval substratum
- 8 exploration in *Balanus amphitrite*. Mar Ecol Prog Ser 198:303-310.
- 9 Moran AL, Emlet RB (2001) Offspring size and performance in variable environments: field
- 10 studies on a marine snail. Ecology 82:1597-1612.
- 11 Morgan SG (1995) Life and death in the plankton: larval mortality and adaptation. Pages 279-
- 12 322 in L.R. McEdward, editor. Ecology of marine invertebrate larvae. CRC Press, Boca Raton,
- 13 Florida.
- 14 Morgan SG, Shanks AL, MacMahan JH, Reniers AJ, Feddersen F (2018) Planktonic subsidies to
- 15 surf-zone and intertidal communities. Annu Rev Mar Sci 10:345-369.
- 16 Moyse J (1963) A comparison of the value of various flagellates and diatoms as food for
- 17 barnacle larvae. ICES J Mar Sci 28:175-187.
- 18 Pannacciulli FG, Bishop JDD, Hawkins SJ (1997) Genetic structure of populations of two
- 19 species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. Mar
- 20 Biol 128:73-82.
- 21 Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples.
- 22 In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer, New York, pp 4–20.

1	Pechenik JA, Rittschof D, Schmidt AR (1993) Influence of delayed metamorphosis on survival
2	and growth of juvenile barnacles Balanus amphitrite. Mar Biol 115:287-294.
3	Pernet F, Tremblay R, Langdon C, Bourget E (2004) Effect of additions of dietary
4	triacylglycerol microspheres on growth, survival, and settlement of mussel (Mytilus sp.) larvae.
5	Mar Biol 144:693-703.
6	Power AM, Piyapattanakorn S, Ruth MO, Iyengar A, Myers AA, Hawkins SJ, Delany J,
7	McGrath D, Maclean N (1999) Verification of cyprid size as a tool in the identification of two
8	European species of Chthamalus barnacles using mtDNA-RFLP analysis. Mar Ecol Progr Ser
9	191:251-256.
10	R Core Team (2019). R: A language and environment for statistical computing. R Foundation for
11	Statistical Computing, Vienna, Austria.
12	Rainbow PS, Walker G (1977) The functional morphology of the alimentary tract of barnacles
13	(Cirripedia: Thoracica). J Exp Mar Biol Ecol 28:183-206.Rass TS (1941) Geographic
14	Parallelisms in Morphology and Development of Teleost Fish of Northern Seas. MOIP, Moscow.
15	Rodriguez F, Fernandez E, Head RN, Harbour DS, Bratbak G, Heldal M, Harris RP (2000)
16	Temporal variability of viruses, bacteria, phytoplankton and zooplankton in the western English
17	Channel off Plymouth. J Mar Biol Assoc UK 80:575-586.
18	Rodriguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. Mar
19	Ecol Prog Ser, 97:193-207.
20	Roughgarden J, Iwasa Y, Baxter C (1985) Demographic theory for an open marine population
21	with space-limited recruitment. Ecology 66:54-67.

1	Schindelin J.	Arganda-Carrer	as I, Frise E	E, Kaynig	V. Longair M.	, Pietzsch T, Prei	bisch S.
	,	0	,	, , ,	, ,	· · · · · · · · · · · · · · · · · · ·	,

- 2 Rueden C, Saalfeld S, Schmid B, Tinevez JY (2012) Fiji: an open-source platform for biological-
- 3 image analysis. Nature methods 9:676.
- 4 Shanks AL, Morgan SG, MacMahan J, Reniers AJ (2017) Alongshore variation in barnacle
- 5 populations is determined by surf zone hydrodynamics. Ecol Monogr 87:508-532.
- 6 Shanks AL, Morgan SG, MacMahan J, Reniers AJ, Kudela R, Jarvis M, Brown J, Fujimura A,
- 7 Ziccarelli L, Griesemer C (2016) Variation in the abundance of Pseudo-nitzschia and domoic
- 8 acid with surf zone type. Harmful Algae 55:172-178.
- 9 Skákala J, Ford D, Brewin RJ, McEwan R, Kay S, Taylor B, de Mora L, Ciavatta S (2018) The
- 10 assimilation of phytoplankton functional types for operational forecasting in the northwest
- 11 European shelf. J Geophys Res Oceans 123:5230-5247.
- 12 Southward AJ (1991) Forty years of changes in species composition and population density of
- 13 barnacles on a rocky shore near Plymouth. J Mar Biol Assoc UK 71:495-513.
- 14 Stone CJ (1989) A comparison of algal diets for Cirripede nauplii. J Exp Mar Biol Ecol 132: 17-

15 40.

- 16 Strathmann RR (1996) Are planktonic larvae of marine benthic invertebrates too scarce to
- 17 compete within species?. Oceanol Acta 19:399-407.
- 18 Thiyagarajan V, Harder T, Qiu JW, Qian PY (2003) Energy content at metamorphosis and
- 19 growth rate of the early juvenile barnacle *Balanus amphitrite*. Mar Biol 143:543-554.
- 20 Thiyagarajan V, Hung OS, Chiu JMY, Wu RSS, Qian PY (2005) Growth and survival of
- 21 juvenile barnacle *Balanus amphitrite*: interactive effects of cyprid energy reserve and habitat.
- 22 Mar Ecol Prog Ser 299:229-237.

- Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol Rev
   25:1-45.
- 3 Toupoint N, Gilmore-Solomon L, Bourque F, Myrand B, Pernet F, Olivier F, Tremblay R (2012)
- 4 Match/mismatch between the *Mytilus edulis* larval supply and seston quality: effect on
- 5 recruitment. Ecology 93:1922-1934.
- 6 Tremblay R, Olivier F, Bourget E, Rittschof D (2007) Physiological condition of Balanus
- 7 *amphitrite* cyprid larvae determines habitat selection success. Mar Ecol Prog Ser 340:1-8.
- 8 Underwood AJ, Fairweather PG (1989) Supply-side ecology and benthic marine assemblages.
- 9 Trends Ecol Evol 4:16-20.
- 10 Vargas CA, Manríquez PH, Navarrete SA (2006) Feeding by larvae of intertidal invertebrates:
- 11 assessing their position in pelagic food webs. Ecology 87:444-457.
- 12 Wickham H (2016) Ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

# 1 **TABLES**

2 **Table 1.** Nested analyses of variance (ANOVA) summary table for regional/species differences

3 (São Sebastião Channel/C. bisinuatus versus Plymouth Sound/C. montagui) in supply to

4 settlement patterns and in lipid allocation in cyprid tissues given by the TAG/PL ratio.

5	Settlement/Supply ratio				TA	G/PL	ratio				
6	Source	df	SS	MS	F	р	df	SS	MS	F	р
	Region	1	0.01	0.01	0.40	0.59	1	0.36	0.36	2.80	0.11
7	Site(Region)	2	0.05	0.03	0.37	0.71	2	0.91	0.47	3.57	0.05
8	Residuals	4	0.28	0.07			20	2.55	0.13		
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1	<b>Table 2.</b> Lipid class composition of the cyprids collected from the subtropical (C. bisinuatus)
2	and temperate (C. montagui) nearshore waters. Lipid classes ( $\% \pm$ SE), divided in TAG
3	(triacylglycerol), PL (phospholipids) and AMPL (acetone mobile polar lipids), constituted the
4	larval total lipid content ( $\mu$ g/larva $\pm$ SE). The TAG/PL Ratio, i.e. the ratio of storage to
5	membrane lipids, is provided for both species.

7	Lipid class (%)	Chthamalus bisinuatus	Chthamalus montagui
8	TAG	$49.8 \pm 1.8$	52.8 ± 2.1
0	PL	$41.3 \pm 1.4$	$41.6\pm1.9$
9	AMPL	$8.8\pm 0.9$	$5.6\pm0.4$
10	∑ Lipid content (µg/larva)	$50.0\pm1.9$	48.5 ± 2.3
11	TAG/PL Ratio	1.21	1.27
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**Table 3.** Pearson correlation coefficients for cyprid morpho-physiological traits. Each sample (n)

2 corresponds to the daily average of two replicate plankton tows, representing daily cohorts.

		Chthan	alus bisinua	itus	Chthamalus montagui			
Variable		Size	Droplet cover	Total lipid	Size	Droplet cover	Total lipid	
	r	-0.10	0.60	0.00	-0.55	0.90	0.82	
TAG/PL	p-value	0.81	0.11	0.99	0.20	0.01 **	0.01 **	
	n	8	8	8	7	7	8	
	r		0.08	-0.43		-0.64	-0.741	
Size	p-value		0.85	0.29		0.12	0.05.	
	n		8	8		7	7	
	r			0.04			0.84	
Droplet cover	p-value			0.93			0.02 *	
	n			8			7	

3 Significance denoted as '\*\*\*' 0.001; '\*\*' 0.01; '\*' 0.05; '.' 0.1.





Channel). Monthly estimates of surface chlorophyll-a concentration (Chl a; mg/m<sup>3</sup>), acquired from MODIS-Aqua NASA Ocean Color Figure 1 – Map displaying sampled locations: southwest of the British Isles (Plymouth Sound) and southeast Brazil (São Sebastião database using Level-3 mapped images of 4 km resolution, indicate the year-round (winter solstice to summer solstice) pelagic primary productivity found at each location during 2015. Shaded months represent the sampling period. 19 20 18





Figure 3 – Daily parameters found at each sampling location, (A) Chlorophyll-*a* concentration, i.e. estimate of primary productivity, (B) Supply, i.e. cyprid abundance, (C) Settlement rate, and (D) Settlement/Supply ratio, i.e. a measure of settlement success. Error bars correspond to mean  $\pm$  SE. SSC stands for São Sebastião Channel (*C. bisinuatus*) and PS for Plymouth Sound in the English Channel (*C. montagui*).



Figure 4 – Histograms showing the distributions with density curves for *C. bisinuatus*(subtropical) and *C. montagui* (temperate): cyprid larvae (A) carapace length and (B) lipid
droplet cover, and early (1-day old) juveniles (C) rostro-carinal diameter.



Figure 5 – Series of scatterplots with marginal boxplots showing the TAG/PL of daily cyprid
cohorts (daily average of two replicate plankton tows per site) plotted against other morphophysiological traits: (A) size, (B) lipid droplet cover, and (C) total lipid content.



9 Figure 6. Conceptual model for the integration of morpho-physiological traits when assessing 10 lipid allocation (energy, structure) in late-stage barnacle larvae. The triacylglycerol (TAG) to phospholipid (PL) ratio can be used as an index of physiological condition. For instance, larvae 11 with a TAG/PL = 1 (non-shaded area of the scheme) resemble in physiological condition, 12 13 although having different sizes and lipid reserves, illustrating that larger sizes incur higher 14 metabolic costs. Larvae with TAG/PL  $\gg$  1 accumulate more energetic (TAG) reserves as opposed to larvae showing TAG/PL  $\ll$  1. This model clarifies how cyprid physiological 15 16 condition can be estimated with visual traits, when size and lipid droplet cover are integrated together. 17

## 1 APPENDIX



Figure 1 – Surface chlorophyll-a concentration (Chl-a; mg/m<sup>3</sup>) map, produced using data from
MODIS-Aqua NASA Ocean Color database using Level-3 mapped images of 4 km resolution,
showing the latitudinal patterns of pelagic Chl-a during 2015. Sampled coasts indicated (São
Sebastião Channel, Brazil, and the English Channel, British Isles).