



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

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ABSTRACT: 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 ontogeny. We carried out a comparative study to better understand the larval physiology of space-monopolizing barnacles exposed to contrasting regimes of primary productivity: *Chthamalus bisinuatus* under a meso-oligotrophic regime on the southeastern coast of Brazil, and *C. montagui* under a highly productive regime on the southwestern coast of the British Isles. We used an index based on lipid composition—the triacylglycerol (TAG) to phospholipid (PL) ratio—to characterize lipid allocation (energy/structure) in the tissues of cyprid larvae and anticipated depleted TAG reserves in cyprids from less productive waters. Despite the considerably different levels of primary productivity between subtropical ($1.31 \pm 0.4 \text{ mg chl } a \text{ m}^{-3}$) and temperate waters ($3.09 \pm 1.2 \text{ mg chl } a \text{ m}^{-3}$), TAG/PL ratio and settlement success were comparable for *C. bisinuatus* and *C. montagui*. Lipid allocation of daily cohorts was also comparable for both chthamalids, with cyprids equally storing TAG reserves ($\geq 50\%$ of total lipid content). This points to an energetic threshold below which nauplii cannot develop to a cyprid and/or selection for lipid accumulation under poor trophic conditions. We highlight the 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 barnacle nauplii. We propose a conceptual model to clarify the process of lipid allocation (energetic to structural lipids) in the tissues of cyprid larvae.

KEY WORDS: Supply-side ecology · Settlement dynamics · Larval physiology · Lipids · Energetics · *Chthamalus*

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1. INTRODUCTION

The recruitment dynamics of marine benthic organisms have been extensively studied for over 7 decades (e.g. Barnes 1956, Connell 1961, 1985, Crisp

1976, Hawkins & Hartnoll 1982, Roughgarden et al. 1985, Rodríguez et al. 1993, Jenkins et al. 1999, Barbosa et al. 2016, Giménez et al. 2017). Variations in these dynamics have been related to spatial (e.g. Burrows et al. 2010) and temporal (e.g. Jenkins et al.

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2008, Kasten et al. 2019a) patterns of pelagic food supply as indicated by chlorophyll *a* (chl *a*) concentration in coastal waters (Hawkins & Hartnoll 1982, Menge et al. 1997, Leslie et al. 2005, Mazzuco et al. 2015). For instance, Burrows et al. (2010) reported that barnacles reached larger sizes in areas of high chl *a* concentration, suggesting that food-limited growth, rather than differential survival, caused this trend. High phytoplankton concentrations have also been suggested to increase recruitment by increasing the survival of larvae and recruits, by producing better fed, larger individuals (Menge et al. 1997). Moreover, increasing evidence suggests that plasticity in larval condition can regulate post-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 compromised by poor physiological condition of preceding larval stages (e.g. in the gastropod *Nucella ostrina*, Moran & Emler 2001; barnacle *Semibalanus balanoides*, Jarrett 2003; bryozoan *Watersipora subtorquata*, Marshall & Keough 2004; tubeworm *Hydroides diramphus*, Allen & Marshall 2010; crab *Carcinus maenas*, Giménez 2010).

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. 1993) and/or (3) maternal investment (Freuchet et al. 2015, Kasten et al. 2019b). Such effects appear to vary strongly under different environmental conditions (Marshall & Keough 2004). Temporal changes in food availability are generally considered to be greater at higher latitudes as 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 al. 2010). They are typically oligotrophic year-round and depleted of high-quality dietary fatty acids (Colombo et al. 2017, Leal et al. 2018, 2019), shown to be critical for the development of 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 in temperature) were addressed in classic work by Thorson (1950), who attributed developmental trends to different food availability in different latitudes, and by Rass (1941), who attributed egg size trends to temperature-dependent changes in yolk accumulation patterns (see Laptikhovskiy 2006 for a new look at Thorson's and Rass's rules). Further, a latitudinal trend in lipid storage has also been reported for zooplankton groups, with those found in polar systems accumulating the largest lipid reserves (e.g. plank-

tonic crustaceans, such as the amphipods *Themisto libellula* and *T. abyssorum*, the Antarctic krill *Euphausia superba* and the copepod *Calanus propinquus*; Lee et al. 2006). In coastal waters, such generalizations are difficult, as phytoplankton blooms (that lead to a build-up of storage lipids in planktonic larvae) may result not only from upwelling of nutrient-rich waters, but also from local wind forcing and coastal circulation (e.g. scattered events of high productivity in subtropical shores that can raise baseline oligotrophic conditions to eutrophic ones during short periods, i.e. 1 to 2 d; Kasten & Flores 2013) plus riverine subsidies (e.g. terrestrially-derived organic matter) to shallow coastal waters (Gorman et al. 2019).

On intertidal shores, enhanced quality of settling larval stages of benthic species may be 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 et al. 2020). Triacylglycerol (TAG) is often the major storage lipid and the primary energetic 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 supply role, TAG functions as a source of essential fatty acids for membrane integrity, regulating the physiological metabolism of many crustacean species, especially the process of synthesizing 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 their 'choice' of settlement substrate (Miron et al. 2000, Marshall & Keough 2003, Tremblay et al. 2007) and may ultimately lead to 'desperate' settlement; that is, metamorphosis occurs in response to a nonspecific cue or even spontaneously (Knight-Jones 1953, Crisp 1955, Lucas et al. 1979). Further, competent larvae with higher energy reserves have been shown to settle in 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 survival (Thiyagarajan et al. 2005). A deeper understanding of supply-driven population dynamics (e.g. Underwood & Fairweather 1989, Minchinton & Scheibling 1991, Bertness et al. 1992, Delany et al. 2003) in the intertidal realm would therefore benefit from studies exploring larval lipidomics of foundation benthic species.

In the present study, we compared the larval physiology, supply and settlement of 2 closely related

space-monopolizing barnacles of the genus *Chthamalus*: the subtropical *C. bisinuatus* and the temperate *C. montagui*, inhabiting shores exposed to relatively low and high coastal primary productivity, respectively. We specifically focussed on lipid allocation in late-stage barnacle larvae: the settlement stage. During their planktonic phase, barnacles pass through 6 naupliar stages before moulting into a cyprid. Nauplius stage I is non-feeding, surviving for a few days on glycoprotein and lipid yolk reserves before moulting to stage II (Rainbow & 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 cyprid, which is specialized for substrate exploration and settlement. We assessed lipid allocation in cyprid larvae by using a ratio of energetic to structural lipids (TAG to phospholipid; 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 anticipated a lower TAG/PL ratio and settlement success of *C. bisinuatus* compared to *C. montagui* cyprids, whose (naupliar) development occurs in more productive waters. We developed a conceptual model clarifying the process of lipid allocation and energy storage in the tissues of cyprid larvae to place our work into a wider context.

2. MATERIALS AND METHODS

2.1. Study sites and species

Late-stage (cyprid) larvae of 2 chthamalid barnacle species were sampled from 2 different latitudes (subtropical and temperate) in the Atlantic: *Chthamalus bisinuatus*, on the southeastern coast of Brazil in the São Sebastião Channel (SSC), and *C. montagui*, on the southwestern coast of the British Isles, in Plymouth Sound (PS) in the English Channel (Fig. 1). These species are considered ecological equivalents as they occupy the same ecological niche (the 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 2 sites per region (separated by 3–6 km) during March 2015 in SSC and August 2015 in PS. These dates correspond to late summer/early autumn months in each region. Samples were first processed at the Center for Marine Biology of the University of São Paulo (São Paulo, Brazil) and at the Marine Biological Association of the United Kingdom (Plymouth, UK), and later analysed at the Institute of Ocean Sciences of Rimouski (Quebec, Canada).

In Brazil, the rocky shores of Baleeiro Head (23° 49' 41" S, 45° 25' 22" W) and Feiticeira Beach (23° 50' 38" S, 45° 24' 28" W) were sampled. Facing the SSC (northern coast of São Paulo State), these shores are char-

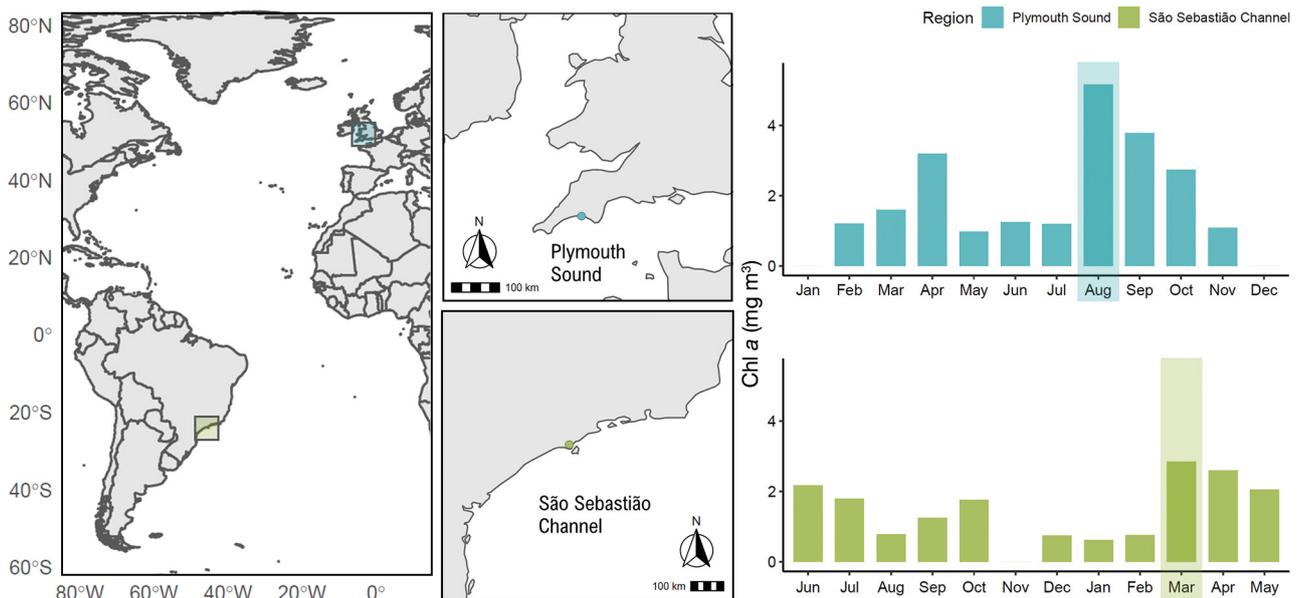


Fig. 1. Sampled locations: southwestern British Isles (Plymouth Sound) and southeastern Brazil (São Sebastião Channel). Monthly estimates of surface chlorophyll *a* (chl *a*) concentration (mg m⁻³), acquired from the MODIS-Aqua NASA Ocean Color database using Level-3 mapped images of 4 km resolution, indicate the year-round (winter solstice to summer solstice) pelagic primary productivity found at each location during 2015. Shaded months represent the sampling period

acterized by a steep rocky habitat with a nearly saturated (close to 100% cover) *Chthamalus* zone in the mid-high littoral (e.g. Kasten & Flores 2013, Barbosa et al. 2016). Natural populations of *C. bisinuatus* along the São Paulo coastline may exhibit a very clear fortnightly rhythm of larval release (Bueno et al. 2010). The SSC is principally a meso-oligotrophic system, with a seasonal chl *a* pattern reaching maximum concentrations in winter ($0.36 \pm 0.12 \text{ mg m}^{-3}$; Ciotti et al. 2010). During late summer, organic detritus dominates nearshore suspended matter composition over the SSC (Leal et al. 2018).

In the English Channel, the rocky shores of Mount Batten ($50^{\circ}21'28''\text{N}$, $4^{\circ}7'41''\text{W}$) and Plymouth Hoe ($50^{\circ}21'47''\text{N}$, $4^{\circ}8'17''\text{W}$) were sampled. These shores are located in PS, an area characterized by dense populations of chthamalid barnacles (e.g. Southward 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 between the beginning of May and the end of September, with asynchronous multiple broods 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 English Channel, as is typical of temperate systems, has 2 yearly productivity peaks (spring bloom of diatoms followed by summer dominance of flagellates; Rodríguez et al. 2000). In contrast with other cold-temperate acorn barnacle species such as *Semibalanus balanoides*, in which release of a single larval brood is synchronized to match the predictable spring phytoplankton bloom of diatoms (e.g. Barnes 1956, Crisp & Spencer 1958, Hawkins & Hartnoll 1982), the development of the planktonic larvae of *Chthamalus* appears to be promoted by flagellates (Moysé 1963, Burrows et al. 1992). Chl *a* concentration in the study area (measured at the Western Channel Observatory Station L4; <https://www.westernchannelobservatory.org.uk/>) is at its lowest during winter and highest during the characteristic spring and autumn phytoplankton blooms, reaching nearly 3 mg m^{-3} from August through September (Skákala et al. 2018).

2.2. Field sampling

Sampling was conducted over 4 consecutive days in Brazil (3–6 March 2015) and in the British Isles (19–22 August 2015). Two replicate plankton tows

(10 m apart; mesh $160 \mu\text{m}$; 50–100 m tow length at the sub-surface) were collected daily at each of the 2 sites in the SSC and PS, 100–200 m offshore, to estimate larval supply (cyprids m^{-3}) and larval morphophysiological traits. A minimum of 20 cyprids per tow sample were collected for morphometric measures (see Section 2.4) and lipid analysis (see Section 2.5). Whilst *C. bisinuatus* was the single chthamalid barnacle species occurring on the subtropical shores sampled, the temperate *C. montagui* co-occurred with a congener, *C. stellatus*. Morphological differences between the larvae of *C. stellatus* and *C. montagui* have been previously described, with *C. montagui* being consistently smaller than its congener among over 1500 chthamalid cyprids sampled in the PS area (Jenkins 2005). The cyprid carapace length was therefore used to distinguish between the 2 temperate *Chthamalus* spp. (Power et al. 1999). All temperate cyprids collected for this study were well below $540 \mu\text{m}$ (see Section 3), the reported length threshold for *C. montagui* (Power et al. 1999, Jenkins 2005). Daily settlement rates (ind. plate $^{-1}$ d $^{-1}$) were determined by random deployment of 10 settlement plates (10 cm \times 10 cm; covered with 3MTM Safety-Walk rough tape) over the 4 d sampling period at each site. Plates were replaced every 24 h, and surroundings were cleared for potential predators. Settlement success was estimated by calculating settlement to supply ratios, and the size of the recently metamorphosed juveniles (rostrum-carinal diameter) was measured (see Section 2.4).

2.3. Remote estimation of primary productivity

The trophic conditions in each sampling region were characterized using remote estimates of chl *a* concentration (mg m^{-3}), a commonly used proxy of food supply (cf. Mazzuco et al. 2015, Kasten et al. 2019a). Remote sensing data for surface chl *a* was acquired from the MODIS-Aqua sensor through the OceanColor database (<https://oceancolor.gsfc.nasa.gov/13/>). Level-3 mapped images of 4 km resolution were processed using the software SeaDAS (Version 7.5.3). Local estimates were averaged for 1 specific cell (4 \times 4 km) for each region, as sites within regions were not distant enough to warrant site-specific cells. Daily averages were extracted for 5 dates for which remote sensing data were available for the SSC ($23^{\circ}56'15''\text{S}$, $45^{\circ}26'15''\text{W}$; dates: 23, 25 and 27 February, 3 and 4 March 2015) and PS ($50^{\circ}16'15''\text{N}$, $4^{\circ}11'15''\text{W}$; dates: 6, 8, 17, 18 and 23 August 2015). The extracted dates spanned the pelagic larval

development (from nauplius to cyprid) of both species (12 d for *C. bisinuatus*, Barbosa et al. 2016; 14–17 d for *C. montagui*, Burrows et al. 1999).

2.4. Morphometric analysis

We focussed on cyprid traits that are known to affect post-settlement performance (Jarrett 2003, Thiyagarajan et al. 2003, Tremblay et al. 2007, Leal et al. 2020): (1) size (carapace length; μm) and (2) lipid droplet cover (droplet area/body area; %). The transparent carapace of cyprid larvae makes it easy to visualize the conspicuous lipid droplets stored anteriorly, around the midgut and at the bases of the thoracic limbs (Fig. 2), that constitute the cyprid's main energetic reserves (Rainbow & Walker 1977, Tremblay et al. 2007). Recent evidence using *C. bisinuatus* cyprids shows that the visual estimation of lipid droplet cover can be used as a proxy of TAG content, given the strong correlation between visual lipid area and Nile-red stained lipid area under a fluorescence microscope (P. Guerra et al. unpubl.). Based on this, size and lipid droplet cover were measured for 170 *C. bisinuatus* cyprids and 153 *C. montagui* cyprids. 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 d 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 analyses were done using the software Fiji, an open-source platform for scientific image analysis (Schindelin et al. 2012).



Fig. 2. Live *Chthamalus bisinuatus* cyprid larvae in lateral view. Conspicuous lipid droplets (the main larval energy storage) are visible in the anterior part of the carapace

2.5. Lipid analysis

To quantify the larval total lipid content ($\mu\text{g larva}^{-1}$) 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 were determined using an Iatroscan Mark-VI analyzer (Iatron Laboratories) developed in a 4-solvent system (Parrish 1999). Three main lipid classes were identified: TAGs, PLs and acetone mobile polar lipids (AMPLs). TAGs are storage lipids that make up most of the energetic reserves, PLs are the main components of cell membranes, and AMPLs are a group consisting mainly of glycolipids, pigments and degradation products of PLs (Parrish 1999). Storage lipids are generally associated with energy supply, while structural lipids are important for cell membrane stability (Fraser 1989). Most of the variability in individual lipid content reflects changes in TAG levels because this class of lipids most closely reflects changes in factors such as food supply and temperature, which are known to influence the deposition or loss of lipids (Arts et al. 1993).

Given the size-dependency associated with TAG (Fraser 1989), the TAG/PL ratio was used to characterize lipid allocation in larval tissues, that is, standardized TAG measures divided by a lipid class related to body size, PL. The ratio of storage to membrane lipids is a good indicator of physiological condition in a number of fish, bivalve and crustacean larvae (Fraser 1989, Lochmann et al. 1995, Harding & Fraser 1999). Håkanson (1993) defined fish larvae in poor condition to be those with a ratio <0.2 . For lobster larvae, Harding & Fraser (1999) considered a condition index <0.1 the 'point-of-no-return'. For chthamalid barnacles, the threshold of TAG below which nauplii and subsequent cyprid stages do not survive in the wild is yet to be estimated. However, through a laboratory experiment, Freuchet et al. (2015) showed that the ratio of storage to membrane lipids in *C. bisinuatus* nauplii (larval feeding stages) exposed to low food supply corresponded to 0.7, compared to a ratio of 1.9 in nauplii under 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.

2.6. Statistical analysis

A nested ANOVA was used to determine if statistically significant differences in settlement/supply ratio (dependent variable) existed between species/regions (*C. bisinuatus* in the SSC vs. *C. montagui* in PS in the English Channel). A balanced design with sites (Site 1, Site 2) nested within region was used. With this approach, we tested the hypothesis that larger settlement/supply ratios would be expected in areas with greater productivity (i.e. PS in the English Channel, see Section 3) with regional differences overwhelming any small-scale variation. Using the same design, differences in larval lipid 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 juvenile size between species were tested using 2-sample independent Kolmogorov-Smirnov tests, to understand whether selection pressures on morphological larval traits differed among subtropical and temperate environments. To investigate the relationship between morphophysiological larval traits, a Pearson correlation matrix was computed for the variables cyprid size, lipid droplet cover, total lipid content and TAG/PL ratio. R software (version 3.6.1; R Core Team 2019) and the packages 'ggplot2' (Wickham 2016) and 'ggpubr' (Kassambara 2019) were used to conduct statistical analysis and as a graphical tool.

3. RESULTS

3.1. Local conditions: trophic regime, supply and settlement

Monthly estimates of pelagic primary production (chl *a*) for 2015 (Fig. 1) revealed that sampling was carried out during a peak of chl *a* for both the SSC (March, 2.85 mg m⁻³) and PS (August, 5.17 mg m⁻³). Throughout the sampling period, daily estimates averaged 1.31 ± 0.4 (SE) mg m⁻³ for the SSC, contrasting with the higher chl *a* concentration of PS, 3.09 ± 1.2 mg m⁻³ (Fig. 3A). Larval supply (Fig. 3B) was around 3 times higher at the temperate shores (158 ± 46 ind. m⁻³) compared to the subtropical ones (46 ± 14 ind. m⁻³). The same pattern held true for

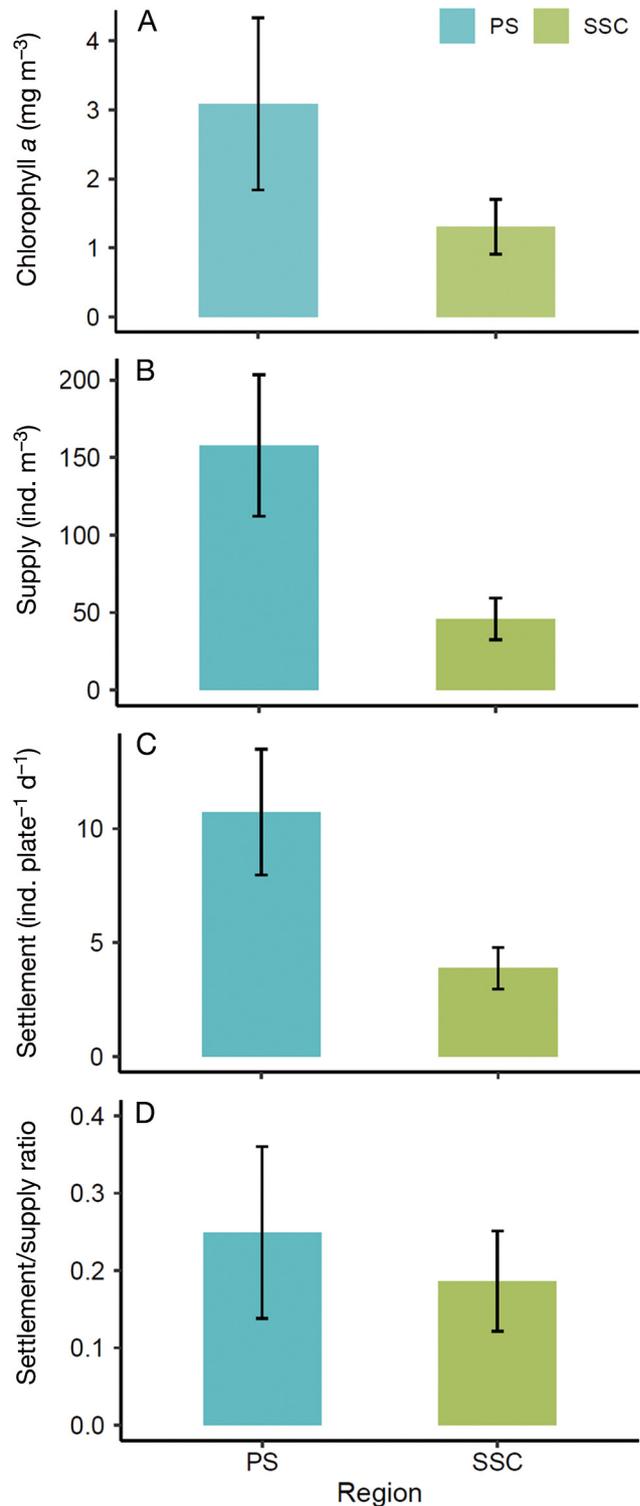


Fig. 3. Daily parameters (means ± SE) found at each sampling location: (A) chlorophyll *a* concentration, i.e. estimate of primary productivity; (B) supply, i.e. cyprid abundance; (C) settlement rate; and (D) settlement/supply ratio, i.e. a measure of settlement success. SSC: São Sebastião Channel (*Chthamalus bisinuatus*); PS: Plymouth Sound in the English Channel (*C. montagui*)

daily settlement rates (Fig. 3C), with *Chthamalus montagui* settling at higher rates (~ 11 settlers plate $^{-1}$ d $^{-1}$) compared to its subtropical congener *C. bisinuatus* (~ 4 settlers plate $^{-1}$ d $^{-1}$). However, settlement/supply ratios (Fig. 3D) were similar, with no differences found between regions, nor between sites within regions ($p > 0.05$; Table 1). This indicates comparable rates of larval settlement per number of larvae in the water column, that is, *C. bisinuatus* cyprids settled in the same proportion as those of *C. montagui* despite developing in less productive waters. However, within-region variability (coefficient of variation; % CV) was high for supply (SSC: 68%; PS: 72%) and settlement (SSC: 73%; PS: 56%) patterns, suggesting that the generality of these results must be interpreted with caution, in light of the variability observed.

3.2. Morpho-physiological larval traits

The size-frequency distribution curves differed between species for the cyprid stage (Kolmogorov-Smirnov $K_d_{170,153} = 7.00$, $p < 0.0001$; Fig. 4A) and juvenile stage ($K_d_{148,442} = 7.50$, $p < 0.0001$; Fig. 4C), with *C. montagui* being consistently larger than its subtropical congener. Lipid droplet cover distributions also differed ($K_d_{170,153} = 5.04$, $p < 0.0001$; Fig. 4B), with *C. bisinuatus* having greater covers than the temperate *C. montagui*. *C. bisinuatus* averaged 224 ± 2.2 μm in cyprid carapace length, $24 \pm 0.6\%$ in lipid droplet cover and 226 ± 1.8 μm in juvenile size (means \pm SE). *C. montagui* averaged 290 ± 5.7 μm in cyprid carapace length, $14 \pm 0.6\%$ in lipid droplet cover and 283 ± 1.9 μm in juvenile size.

A detailed description of the lipid classes found in the larval tissue of both chthamalids is given in Table 2. Total lipid content averaged 50 μg cyprid $^{-1}$ for *C. bisinuatus*, and 48.5 μg cyprid $^{-1}$ for *C. montagui*. TAGs and PLs were the major lipid classes of both species, constituting $>90\%$ of the total larval-

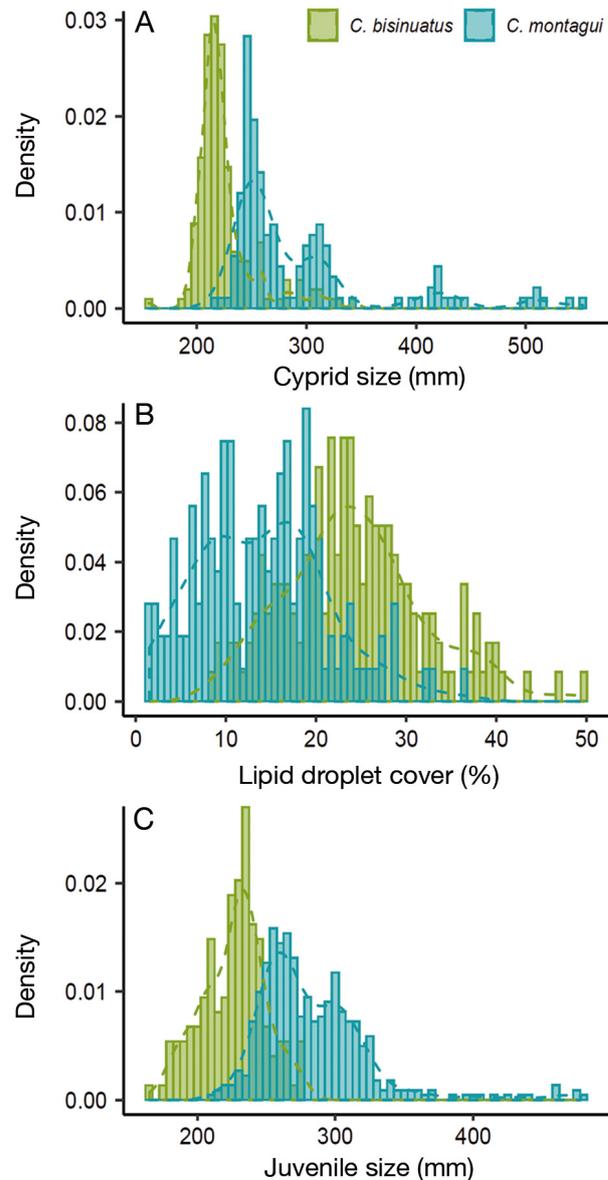


Fig. 4. Density curves of the frequency distributions for *Chthamalus bisinuatus* (subtropical) and *C. montagui* (temperate): cyprid larvae (A) carapace length and (B) % lipid droplet cover; and (C) rostro-carinal diameter of early (1 d old) juveniles

Table 1. Nested ANOVA summary table for regional/species differences (São Sebastião Channel/*Chthamalus bisinuatus* versus Plymouth Sound/*C. montagui*) in supply to settlement patterns and in lipid allocation in cyprid tissues given by the triacylglycerol/phospholipid (TAG/PL) ratio

Source	Settlement/supply ratio					TAG/PL ratio				
	df	SS	MS	F	p	df	SS	MS	F	p
Region	1	0.01	0.01	0.40	0.59	1	0.36	0.36	2.80	0.11
Site (Region)	2	0.05	0.03	0.37	0.71	2	0.91	0.47	3.57	0.05
Residuals	4	0.28	0.07			20	2.55	0.13		

lipid content. The TAG/PL ratio was similar for both species (1.2 for *C. bisinuatus* and 1.3 for *C. montagui*; $p > 0.05$, Table 1), indicating that larvae similarly allocated lipids to energy storage and cellular structure, and that larvae had previously accumulated TAG reserves during their naupliar development. However, there was considerable intra-specific variation (% CV) in this condition index (30% in *C. bisinuatus* and 32% in *C. montagui*), which might

Table 2. Lipid class composition (%) of the cyprids collected from subtropical (*Chthamalus bisinuatus*) and temperate (*C. montagui*) nearshore waters. Lipid classes, divided into triacylglycerol (TAG), phospholipid (PL) and acetone mobile polar lipids (AMPLs), constitute the larval total lipid content (shown in $\mu\text{g larva}^{-1}$). The TAG/PL ratio, i.e. the ratio of storage to membrane lipids, is provided for both species. Values are given as means \pm SE

Lipid class	<i>C. bisinuatus</i>	<i>C. montagui</i>
TAG	49.8 \pm 1.8	52.8 \pm 2.1
PL	41.3 \pm 1.4	41.6 \pm 1.9
AMPLs	8.8 \pm 0.9	5.6 \pm 0.4
Total lipid content	50.0 \pm 1.9	48.5 \pm 2.3
TAG/PL ratio	1.21	1.27

explain the nearly significant effect of site in the ANOVA comparisons ($p = 0.05$; Table 1).

The relationship between the TAG/PL ratio and other larval traits shows that cyprids with similar physiological status may vary in size (Fig. 5A), lipid droplet cover (Fig. 5B), and total lipid content (Fig. 5C); yet, some patterns emerged. The highest ratios (TAG/PL ≥ 1.5) found in *C. montagui* cyprids comprised small-sized cohorts ($<300 \mu\text{m}$) with lipid droplets covering a greater area of the body ($\geq 20\%$ cover). This was confirmed by the positive relationship between TAG/PL ratio and lipid droplet cover ($p = 0.01$, $r = 0.90$; Table 3). The lipidic costs associated with larger sizes can be further evidenced by the (nearly significant) negative relationship between size and total lipid content ($p = 0.05$, $r = -0.74$; Table 3). No significant relationships were detected among *C. bisinuatus* larval traits (Table 3). *C. bisinuatus* cyprids had a similar size range (200–245 μm) regardless of TAG/PL ratio, but similar to their temperate counterparts, the highest ratios were found in cyprids that had a greater lipid droplet cover ($\geq 20\%$ cover).

4. DISCUSSION

In this study, we show that similar lipid allocation in late-stage barnacle larvae can occur in *Chthamalus* species exposed to contrasting trophic regimes, contrary to the expected depletion of TAG reserves in cyprid larvae from less productive waters. This points to an energetic threshold 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 TAG/PL ratio was reflected in our finding of similar supply/settlement ratios in *C. bisinuatus* and *C. montagui*. We highlight the challenges

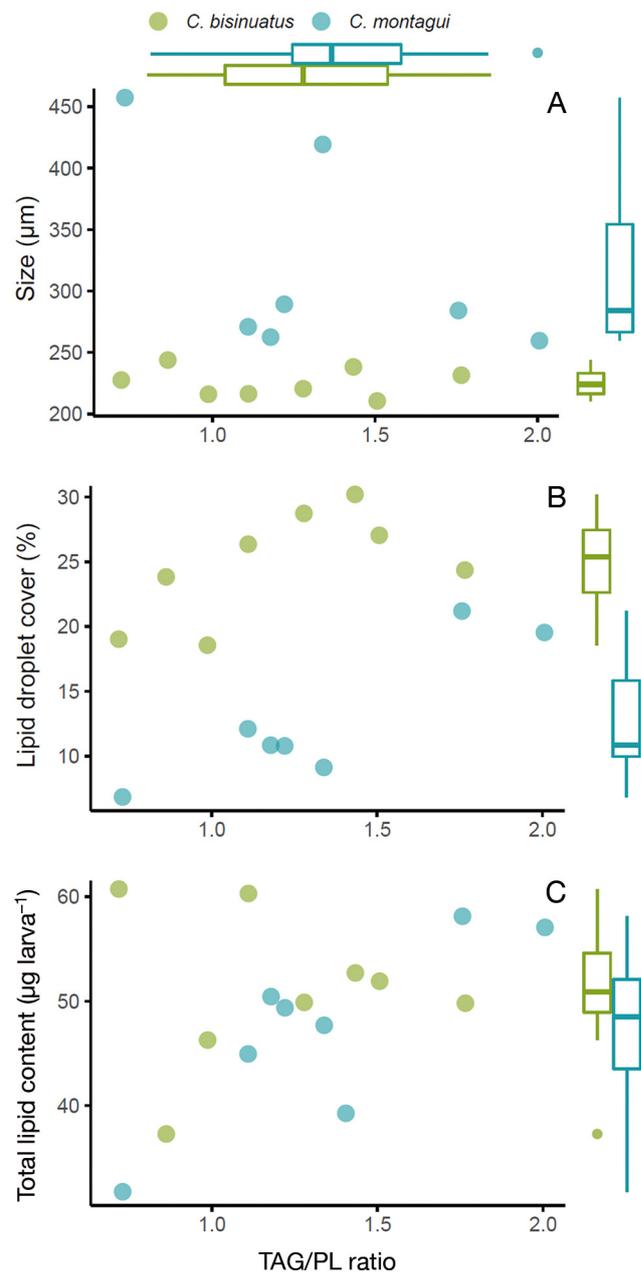


Fig. 5. Triacylglycerol/phospholipid (TAG/PL) ratio of daily *Chthamalus bisinuatus* (subtropical) and *C. montagui* (temperate) cyprid cohorts (daily average of 2 replicate plankton tows per site) plotted against other morpho-physiological traits: (A) size, (B) % lipid droplet cover and (C) total lipid content. Box plots include the 25th percentile, the median (line within the box) and the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles

of directly relating estimates of primary productivity with food supply and larval physiology, as lower chl a concentrations do not necessarily indicate food limitation for barnacle nauplii. We also propose a conceptual model for the integration of morpho-

Table 3. Pearson correlation coefficients for cyprid morpho-physiological traits. Each sample (n) corresponds to the daily average of 2 replicate plankton tows, representing daily cohorts. TAG: triacylglycerol; PL: phospholipid. Significance denoted as ***p = 0.01; **p < 0.05; *p < 0.1

Variable	<i>Chthamalus bisinuatus</i>			<i>Chthamalus montagui</i>			
		Size	Droplet cover	Total lipid	Size	Droplet cover	Total lipid
TAG/PL ratio	r	-0.10	0.60	0.00	-0.55	0.90	0.82
	p	0.81	0.11	0.99	0.20	0.01***	0.01***
	n	8	8	8	7	7	8
Size	r		0.08	-0.43		-0.64	-0.741
	p		0.85	0.9		0.12	0.05*
	n		8	8		7	7
Droplet cover	r			0.04			0.84
	p			0.93			0.02**
	n			8			7

physiological traits when assessing lipid allocation (energetic to structural lipids) in the tissues of cyprid larvae.

Total lipids and TAG are predictors of settlement success, post-metamorphic growth and survival in other species (e.g. *Amphibalanus amphitrite*; Miron et al. 2000, Thiyagarajan et al. 2005, Tremblay et al. 2007), and therefore an excellent reference index for larval quality. TAG adjusts quickly to changes in food supply and temperature (Lochmann et al. 1995, Freitas et al. 2002), whereas PL provides structural-type functions (e.g. membrane stability) that are maintained fairly constant (Fraser 1989) and correlate with the size of larvae, as demonstrated in fish and crustaceans. We argue that the use of a lipid-based condition index (such as the TAG/PL ratio) is useful to estimate the physiological condition of barnacle cyprids by informing how larvae allocate lipids in their tissues to energy storage and/or cellular structure (Fig. 6). We found similar ratios for both chthamalid

4.1. Ratio of storage to membrane lipids: TAG/PL

As reported in studies with other benthic invertebrates (Delaunay et al. 1992, Abad et al. 1995), TAG and PL were the main lipid classes found in cyprids of both *Chthamalus* species tested here. TAG alone accounted for 50% of the total lipid content in *C. bisinuatus*, and 53% in *C. montagui*. These values are similar to those reported by Holland & Walker (1975) for balanid cyprids, where neutral lipids comprised 66% of the total lipid fraction. The need for TAG accumulation in pre-metamorphic stages is easily understood, given the reliance on endogenous energy during metamorphosis (Lucas et al. 1979, Thiyagarajan et al. 2003). Moreover, higher TAG accumulation reduces sinking rates, which has been suggested to reduce energy costs of swimming cyprids to maintain optimal depth (Burrows et al. 1999) and allow for a more prolonged search for a suitable substrate (Lucas et al. 1979).

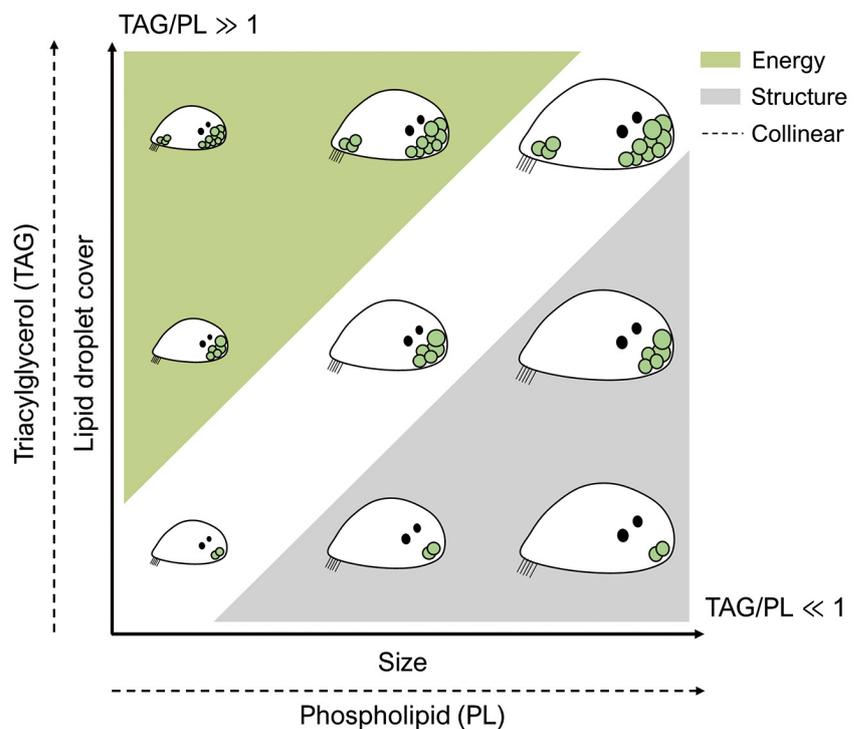


Fig. 6. Conceptual model for the integration of morpho-physiological traits when assessing lipid allocation (energy, structure) in late-stage barnacle larvae. The triacylglycerol (TAG) to phospholipid (PL) ratio can be used as an index of physiological condition. For instance, larvae with a TAG/PL = 1 (non-shaded area of the scheme) are similar in physiological condition, although they have different sizes and variable lipid reserves, illustrating that larger sizes incur higher metabolic costs. Larvae with TAG/PL > 1 accumulate more energetic (TAG) reserves as opposed to larvae showing TAG/PL < 1. This model clarifies how cyprid physiological condition can be estimated with visual traits, when size and lipid droplet cover are integrated together

barnacle species, with a TAG/PL ratio >1 (1.2 for *C. bisinuatus*, 1.3 for *C. montagui*), indicating that larvae had accumulated energetic (TAG) reserves during their previous naupliar feeding stages and/or through maternal allocation. Estimates of the TAG/PL ratio at the point-of-no-return (i.e. of non-viable larvae in the wild) are still lacking for chthamalid cyprids and are necessary to make well-grounded inferences on larval physiological status using lipid-based condition indices. We argue that analysing several morpho-physiological traits together leads to a better understanding of larval condition (Fig. 6). For instance, focussing on size metrics alone would render large *C. montagui* cyprids the ones with the highest quality, which appears not to be the case. Subtropical *C. bisinuatus* cyprids with 200 μm carapace length had higher TAG reserves than *C. montagui* with 400 μm or more. We hypothesize that the larger the cyprid, the bigger the structural and energetical lipidic demands it has. For the balanid barnacle *Balanus glandula*, Hentschel & 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 with a slower feeding rate and a longer naupliar duration, demonstrating the plasticity in size and age at metamorphosis, and suggested it to be an adaptation to environmental variability. As suggested for other barnacle groups (balanids, Hentschel & Emlet 2000, Giménez et al. 2017), the physiological condition of both chthamalids described here likely reflects local selection for larval phenotypes that match the requirements for juvenile survival (see Section 4.3).

4.2. Effects of food supply

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

ever, herbivorous zooplankton are rarely limited by food (Strathmann 1996), and barnacle nauplii are unlikely to be strongly affected by competition (Bashevkin & Morgan 2020). To our knowledge, limited information is available on particle sizes ingested by barnacle nauplii, or on selective feeding behaviour, and for chthamalids the only evidence was published by Stone (1989), under laboratory conditions. This gap in the scientific literature was also recently pointed out by Bernal & Anil (2019). These authors found that picoplankton ($<2.0 \mu\text{m}$), particularly the picocyanobacteria *Synechococcus*, occupy a key position in the naupliar diet of *Amphibalanus amphitrite*. Unlike larger phytoplankton (nano and micro), picocyanobacteria and other picophytoplankton are better adapted to low-nutrient conditions, contributing significantly to the bulk of primary production in oligotrophic waters (Bernal & Anil 2019). Even species adapted to more productive regimes, such as the nauplii of *C. montagui* and *B. perforatus*, have been grown successfully when fed on small cells ($<5 \mu\text{m}$) of *Micromonas pusilla* and *Isochrysis galbana* (Stone 1989). Vargas et al. (2006) also reported that nauplii of 2 barnacle species, *Jehlius cirratus* and *Notobalanus flosculus* from central Chile, primarily graze on picophytoplankton and small nanophytoplankton ($<5 \mu\text{m}$) when incubated in natural seawater. While low amounts of phytoplanktonic food resources are present over the SSC, detrital pathways (e.g. phytodetritus, bacteria) prevail in these subtropical waters (Leal et al. 2018). It is therefore possible that naupliar stages of barnacles over the SSC are adapted to explore other sources of food in their diet to build their energetic (TAG) reserves. Recent studies on the dietary 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 microalgae (I. Leal et al. unpubl.). In *C. bisinuatus* for instance, a large proportion of energetic reserves contain trophic tracers for terrestrial detritus and macrophytes (I. Leal et al. unpubl.). TAGs, the main constituent of larval reserves, are triesters consisting of a glycerol bound to 3 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. ω -3 and ω -6 essential fatty acids). As the fatty acid composition of lipid classes was not characterized in this study, it is not possible to ascertain what type of fatty acids were the 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* nauplii, whereas in subtropical waters detrital matter (saturated) makes up most of the diet of *C. bisinuatus* nauplii. That would mean that, although both species have similar TAG contents, their fatty acid composition is probably different. We therefore urge further research to reconcile the trophic ecology (e.g. feeding behaviour, feeding rates, dietary fatty acid composition) of chthamalid barnacles.

Shifts in food during the ultimate naupliar instar (VI) particularly affect the cyprid's lipid accumulation, stored in TAG droplets (Hentschel & Emlet 2000). Considering the duration of pelagic larval development from nauplius to cyprid of both *Chthamalus* species, i.e. 12 d for *C. bisinuatus* (Barbosa et al. 2016) and 14–17 d for *C. montagui* (Burrows et al. 1999), cyprid cohorts sampled might have been exposed to different food supplies that reflect a lag in their TAG/PL ratios. For example, scattered events of high primary productivity during short periods (1–2 d) have been reported for the SSC (Kasten & Flores 2013). Such 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 for cohorts with high TAG reserves may occur in subtropical shores. The physiological condition of cyprids is also largely determined by maternal allocation (Kasten et al. 2019b). In subtropical *C. bisinuatus*, carry-over effects of maternal allocation seem to have the same effects as pelagic inputs to larvae in the determination of cyprid size (c.a. 13–14% size increase; Kasten et al. 2019b), which may compensate for the low food supply in these subtropical shores.

4.3. Implications

4.3.1. Physiological thresholds in cyprid larvae

We suggest that there may be a threshold above which greater sizes are achieved in detriment of lipid reserves, a cost that is likely too high for species that are exposed to low food supply. Lipid accumulation has been suggested to be an adaptive strategy for species inhabiting habitat-limited shores (e.g. maternal provisioning is essential on crowded shores, Kasten et al. 2019b). This has also been suggested for *Semibalanus balanoides* larvae along the coast of West Scotland by 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 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 reserves (stored as lipid droplets) for settlement and metamorphosis may offset the detrimental 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 faster (Leal et al. 2020). On cold-temperate shores, peaks of *C. montagui* settlement follow major phytoplanktonic blooms. Thus, food is likely not limiting at the onset of juvenile feeding, and larvae may invest in size to counterbalance the slower growth rates imposed by low temperatures. The impact of the observed physiological traits on metamorphic performance will also depend on the post-metamorphic environment. Post-settlement processes (e.g. predation, desiccation, microhabitat availability) can accentuate the patterns set before or during settlement (Bohn et al. 2013a,b), making a comprehensive understanding of larval physiology in supply-side ecology critical.

A caveat of our study lies in comparing 1 species each in 2 locations, albeit with local replication using a nested design, if wanting to draw wider conclusions about latitudinal patterns. Ideally, more than 1 species should be used in each locality, with several localities on both sides of the ocean. Unfortunately, only 1 chthamalid barnacle species occurs in southeast Brazil. In Europe, *C. stellatus* has a more offshore larval distribution as well as longer larval life with greater dispersal (Burrows et al. 1999, Pannacciulli et al. 1997) and is more prevalent on exposed headlands (Crisp et al. 1981, Jenkins 2005). The field-based results from our comparative study, however, plus the final proposed conceptual 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 energetics in shaping settlement of this important group of habitat-forming species.

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 a similar

TAG/PL ratio for barnacle cyprids exposed to considerably different levels of chl *a* ($1.31 \pm 0.4 \text{ mg m}^{-3}$ in the SSC, southeastern Brazil, vs. $3.09 \pm 1.2 \text{ mg m}^{-3}$ in PS, southwestern British Isles), for a period spanning 2 wk prior to 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 Fig. A1 in the Appendix), resulting from differences in nutrient and light availability for phytoplankton growth set by regional and 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 translate directly into primary production nor exportation rates for phytoplankton carbon. Mechanisms related specifically to food quality (e.g. phytoplankton species, organic matter composition) cannot be directly associated to chl *a* patterns. Further, nearshore trophic subsidies to rocky shore communities are often overlooked. For instance, surf-zone hydrodynamics play a role as subsidies of phytoplankton (sensu Morgan et al. 2018) by increasing the nutrient uptake, light exposure and suspension of surf diatoms from the sediment into the water column. The high concentrations of phytoplankton—far higher concentrations of phytoplankton in surf zones than offshore (Shanks et al. 2016, 2017)—particulate organic matter and detritus can sustain 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 nutrient apport to nearshore marine communities fails to be detected by remote estimates of primary productivity. This compromises assumptions made on food supply for suspension feeders, especially in areas with strong detrital-based nearshore food webs, typical of low latitudes (e.g. Leal et al. 2018, 2019).

4.4. Conclusions

Quantifying the lipid class fractions (TAG, PL) that are present in the total lipid content of larvae gives important information regarding the way lipid compounds are allocated (energy storage/cellular structure). The similar TAG/PL ratio observed for 2 closely related barnacle species exposed to different levels of surface chl *a* highlights the challenges of directly relating estimates of primary productivity with larval physiological status, and the need to better describe

trophic resources available for pelagic naupliar stages. We hope to motivate further research, ideally with more replicate species, to unveil latitudinal patterns in lipid allocation in this important group of barnacles.

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LITERATURE CITED

- Abad M, Ruiz C, Martínez D, Mosquera G, Sánchez JL (1995) Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibrán (Galicia Spain). *Comp Biochem Physiol* 110:109–118
- Allen RM, Marshall DJ (2010) The larval legacy: cascading effects of recruit phenotype on post-recruitment interactions. *Oikos* 119:1977–1983
- Arts MT, Robarts RD, Evans MS (1993) Energy reserve lipids of zooplanktonic crustaceans from an oligotrophic saline lake in relation to food resources and temperature. *Can J Fish Aquat Sci* 50:2404–2420
- Barbosa ACC, Gomes CC, Pereira GC, Bueno M, Flores AAV (2016) Local biological drivers, not remote forcing, predict settlement rate to a subtropical barnacle population. *Mar Ecol Prog Ser* 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
- Bashevkin SM, Morgan SG (2020) Predation and competition. In: Anger K, Harzsch S, Thiel M (eds) *The natural history of the Crustacea: developmental biology and larval ecology*, Vol 7. Oxford University Press, New York, NY, p 360–382
- Bemal S, Anil AC (2019) Picophytoplankton *Synechococcus* as food for nauplii of *Amphibalanus amphitrite* and *Artemia salina*. *Hydrobiologia* 835:21–36
- Bertness MD, Gaines SD, Stephens EG, Yund PO (1992) Components of recruitment in populations of the acorn barnacle *Semibalanus balanoides* (Linnaeus). *J Exp Mar Biol Ecol* 156:199–215
- Bohn K, Richardson CA, Jenkins SR (2013a) The importance of larval supply, larval habitat selection and post-settlement mortality in determining intertidal adult abundance of the invasive gastropod *Crepidula fornicata*. *J Exp Mar Biol Ecol* 440:132–140
- Bohn K, Richardson CA, Jenkins SR (2013b) Larval microhabitat associations of the non-native gastropod *Cre-*

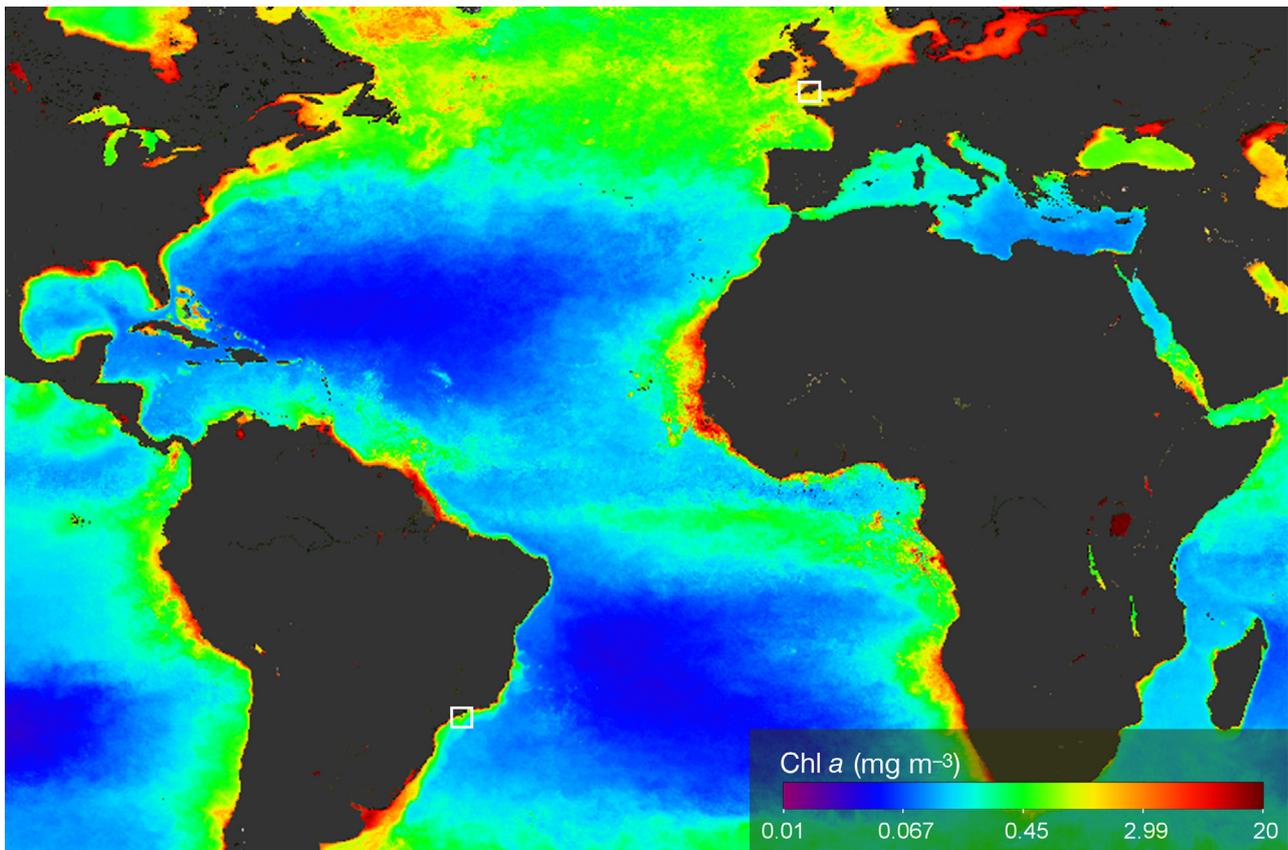
- pidula fornicata* and effects on recruitment success in the intertidal zone. *J Exp Mar Biol Ecol* 448:289–297
- Bueno M, Moser GAO, Tocci BRC, Flores AAV (2010) Retention-favorable timing of propagule release in barnacles and periwinkles. *Mar Ecol Prog Ser* 414:155–165
- Burrows MT, Hawkins SJ, Southward AJ (1992) A comparison of reproduction in co-occurring chthamalid barnacles, *Chthamalus stellatus* (Poli) and *Chthamalus montagui* Southward. *J Exp Mar Biol Ecol* 160:229–249
- Burrows MT, Hawkins SJ, Southward AJ (1999) Larval development of the intertidal barnacles *Chthamalus stellatus* and *Chthamalus montagui*. *J Mar Biol Assoc UK* 79: 93–101
- Burrows MT, Jenkins SR, Robb L, Harvey R (2010) Spatial variation in size and density of adult and post-settlement *Semibalanus balanoides*: effects of oceanographic and local conditions. *Mar Ecol Prog Ser* 398:207–219
- Ciotti AM, Garcia CAE, Jorge DSF (2010) Temporal and meridional variability of satellite-estimates of surface chlorophyll concentration over the Brazilian continental shelf. *Pan-Am J Aquat Sci* 5:236–253
- Colombo SM, Wacker A, Parrish CC, Kainz MJ, Arts MT (2017) A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. *Environ Rev* 25:163–174
- Connell JH (1961) Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecol Monogr* 31:61–104
- Connell JH (1985) The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. *J Exp Mar Biol Ecol* 93:11–45
- Crisp DJ (1955) The behaviour of barnacle cyprids in relation to water movements over a surface. *J Exp Biol* 32: 569–590
- Crisp DJ (1976) Settlement responses in marine organisms. In: Newell RC (ed) *Adaptation to environment. Essays on the physiology of marine animals*. Butterworth-Heinemann, London, p 83–124
- Crisp DJ, Spencer CP (1958) The control of the hatching process in barnacles. *Proc R Soc B* 149:278–299
- Crisp DJ, Southward AJ, Southward EC (1981) On the distribution of the intertidal barnacles *Chthamalus stellatus*, *Chthamalus montagui* and *Euraphia depressa*. *J Mar Biol Assoc UK* 61:359–380
- Delany J, Myers AA, McGrath D, O’Riordan RM, Power AM (2003) Role of post-settlement mortality and ‘supply-side’ ecology in setting patterns of intertidal distribution in the chthamalid barnacles *Chthamalus montagui* and *C. stellatus*. *Mar Ecol Prog Ser* 249:207–214
- Delaunay F, Marty Y, Moal J, Samain JF (1992) Growth and lipid class composition of *Pecten maximus* (L.) larvae grown under hatchery conditions. *J Exp Mar Biol Ecol* 163:209–219
- Ewers-Saucedo C, Pappalardo P (2019) Testing adaptive hypotheses on the evolution of larval life history in acorn and stalked barnacles. *Ecol Evol* 9:11434–11447
- Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
- Fraser JA (1989) Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Can J Fish Aquat Sci* 46:1868–1873
- Freitas L, Fernández-Reiriz MJ, Labarta U (2002) Lipid classes of mussel seeds *Mytilus galloprovincialis* of subtidal and rocky shore origin. *Aquaculture* 207:97–111
- Freuchet F, Tremblay R, Flores AAV (2015) Interacting environmental stressors modulate reproductive output and larval performance in a tropical intertidal barnacle. *Mar Ecol Prog Ser* 532:161–175
- Giménez L (2010) Relationships between habitat conditions, larval traits, and juvenile performance in a marine invertebrate. *Ecology* 91:1401–1413
- Giménez L, Torres G, Pettersen A, Burrows MT, Estévez A, Jenkins SR (2017) Scale-dependent natural variation in larval nutritional reserves in a marine invertebrate: implications for recruitment and cross-ecosystem coupling. *Mar Ecol Prog Ser* 570:141–155
- Gorman D, Pucci M, Soares LS, Turra A, Schlacher TA (2019) Land–ocean connectivity through subsidies of terrestrially derived organic matter to a nearshore marine consumer. *Ecosystems* 22:796–804
- Håkanson JL (1993) Nutritional condition and growth rate of anchovy larvae (*Engraulis mordax*) in the California Current: two contrasting years. *Mar Biol* 115:309–316
- Harding GC, Fraser AJ (1999) Application of the triacylglycerol/sterol condition index to the interpretation of larval lobster *Homarus americanus* distribution in close proximity to Georges Bank, Gulf of Maine. *Mar Ecol Prog Ser* 186:239–254
- Hawkins SJ, Hartnoll RG (1982) Settlement patterns of *Semibalanus balanoides* (L.) in the Isle of Man (1977–1981). *J Exp Mar Biol Ecol* 62:271–283
- Hentschel BT, Emler RB (2000) Metamorphosis of barnacle nauplii: effects of food variability and a comparison with amphibian models. *Ecology* 81:3495–3508
- Holland DL, Walker G (1975) The biochemical composition of the cypris larva of the barnacle *Balanus balanoides* L. *ICES J Mar Sci* 36:162–165
- Jarrett JN (2003) Seasonal variation in larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. *Ecology* 84:384–390
- Jenkins SR (2005) Larval habitat selection, not larval supply, determines settlement patterns and adult distribution in two chthamalid barnacles. *J Anim Ecol* 74:893–904
- Jenkins SR, Norton TA, Hawkins SJ (1999) Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. *J Exp Mar Biol Ecol* 236:49–67
- Jenkins SR, Murua J, Burrows MT (2008) Temporal changes in the strength of density-dependent mortality and growth in intertidal barnacles. *J Anim Ecol* 77:573–584
- Kassambara A (2019) ggpubr: ‘ggplot2’ Based Publication. Ready Plots. R package version 0.2.2. <https://CRAN.R-project.org/package=ggpubr>
- Kasten P, Flores AAV (2013) Disruption of endogenous tidal rhythms of larval release linked to food supply and heat stress in an intertidal barnacle. *Mar Ecol Prog Ser* 472: 185–198
- Kasten P, Tremblay R, Flores AAV (2019a) Mismatched seasonal patterns of larval production and quality in subtropical barnacle populations along a coastal trophic gradient. *Estuar Coast Shelf Sci* 224:43–50
- Kasten P, Jenkins SR, Tremblay R, Flores AAV (2019b) Evidence for enhanced late-stage larval quality, not survival, through maternal carry-over effects in a space monopolizing barnacle. *Hydrobiologia* 830: 277–286
- Kattner G, Graeve M, Calcagno JA, Lovrich GA, Thatje S, Anger K (2003) Lipid, fatty acid and protein utilization during lecithotrophic larval development of *Lithodes*

- santolla* (Molina) and *Paralomis granulosa* (Jacquinot). J Exp Mar Biol Ecol 292:61–74
- Knight-Jones EW (1953) Decreased discrimination during setting after prolonged planktonic life in larvae of *Spirorbis borealis* (Serpulidae). J Mar Biol Assoc UK 32: 337–345
- ✦ Laptikhovsky V (2006) Latitudinal and bathymetric trends in egg size variation: a new look at Thorson's and Rass's rules. Mar Ecol 27:7–14
- ✦ Lawrence JM (1976) Patterns of lipid storage in post-metamorphic marine invertebrates. Am Zool 16:747–762
- ✦ Leal I, Bouchard É, Flores AAV, Tremblay R (2018) Trophic cues as possible triggers of mussel larval settlement in southeastern Brazil. Aquat Living Resour 31:26
- ✦ Leal I, Flores AAV, Collin R, Tremblay R (2019) Drifting in the Caribbean: hints from the intertidal bivalve *Isognomon alatus*. Estuar Coast Shelf Sci 227:106333
- ✦ Leal I, Flores AAV, Archambault P, Collin R, Tremblay R (2020) Response of tropical and subtropical chthamalid barnacles to increasing substrate temperatures. J Exp Mar Biol Ecol 524:151281
- ✦ Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306
- ✦ Leslie HM, Breck EN, Chan F, Lubchenco J, Menge BA (2005) Barnacle reproductive hotspots linked to near-shore ocean conditions. Proc Natl Acad Sci USA 102: 10534–10539
- ✦ 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
- ✦ Lucas MI, Walker G, Holland DL, Crisp DJ (1979) An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar Biol 55:221–229
- ✦ Marshall DJ, Keough MJ (2003) Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Mar Ecol Prog Ser 255:145–153
- ✦ Marshall DJ, Keough MJ (2004) Variable effects of larval size on post-metamorphic performance in the field. Mar Ecol Prog Ser 279:73–80
- ✦ Mazzuco ACA, Christofoletti RA, Pineda J, Starczak VR, Ciotti AM (2015) Temporal variation in intertidal community recruitment and its relationships to physical forcings, chlorophyll-*a* concentration and sea surface temperature. Mar Biol 162:1705–1725
- ✦ Menge BA, Daley BA, Wheeler PA, Dahlhoff E, Sanford E, Strub PT (1997) Benthic–pelagic links and rocky intertidal communities: bottom-up effects on top-down control? Proc Natl Acad Sci USA 94: 14530–14535
- ✦ Minchinton TE, Scheibling RE (1991) The influence of larval supply and settlement on the population structure of barnacles. Ecology 72:1867–1879
- ✦ Miron G, Walters LJ, Tremblay R, Bourget E (2000) Physiological condition and barnacle larval behavior: a preliminary look at the relationship between TAG/DNA ratio and larval substratum exploration in *Balanus amphitrite*. Mar Ecol Prog Ser 198:303–310
- ✦ Moran AL, Emlet RB (2001) Offspring size and performance in variable environments: field studies on a marine snail. Ecology 82:1597–1612
- Morgan SG (1995) Life and death in the plankton: larval mortality and adaptation. In: McEdward LR (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, FL, p 279–322
- ✦ Morgan SG, Shanks AL, MacMahan JH, Reniers AJ, Feddersen F (2018) Planktonic subsidies to surf-zone and intertidal communities. Annu Rev Mar Sci 10:345–369
- ✦ Moysse J (1963) A comparison of the value of various flagellates and diatoms as food for barnacle larvae. ICES J Mar Sci 28:175–187
- O'Connor JD, Gilbert LI (1968) Aspects of lipid metabolism in crustaceans. Am Zool 8:529–539
- ✦ Pannacciulli FG, Bishop JDD, Hawkins SJ (1997) Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. Mar Biol 128:73–82
- Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer, New York, NY, p 4–20
- ✦ Pechenik JA, Rittschof D, Schmidt AR (1993) Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. Mar Biol 115:287–294
- ✦ Pernet F, Tremblay R, Langdon C, Bourget E (2004) Effect of additions of dietary triacylglycerol microspheres on growth, survival, and settlement of mussel (*Mytilus* sp.) larvae. Mar Biol 144:693–703
- ✦ Power AM, Piyapattanakorn S, O'Riordan RM, Iyengar A and others (1999) Verification of cyprid size as a tool in the identification of two European species of *Chthamalus* barnacles using mtDNA-RFLP analysis. Mar Ecol Prog Ser 191:251–256
- R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rainbow PS, Walker G (1977) The functional morphology of the alimentary tract of barnacles (Cirripedia: Thoracica). J Exp Mar Biol Ecol 28:183–206
- Rass TS (1941) Geographic parallelisms in morphology and development of teleost fish of northern seas. MOIP, Moscow
- ✦ Rodríguez F, Fernández E, Head RN, Harbour DS, Bratbak G, Heldal M, Harris RP (2000) Temporal variability of viruses, bacteria, phytoplankton and zooplankton in the western English Channel off Plymouth. J Mar Biol Assoc UK 80:575–586
- ✦ Rodríguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. Mar Ecol Prog Ser 97: 193–207
- ✦ Roughgarden J, Iwasa Y, Baxter C (1985) Demographic theory for an open marine population with space-limited recruitment. Ecology 66:54–67
- ✦ Schindelin J, Arganda-Carreras I, Frise E, Kaynig V and others (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682
- ✦ Shanks AL, Morgan SG, MacMahan J, Reniers AJHM and others (2016) Variation in the abundance of *Pseudonitzschia* and domoic acid with surf zone type. Harmful Algae 55:172–178
- ✦ Shanks AL, Morgan SG, MacMahan J, Reniers AJ (2017) Alongshore variation in barnacle populations is determined by surf zone hydrodynamics. Ecol Monogr 87: 508–532
- ✦ Skákala J, Ford D, Brewin RJW, McEwan R and others (2018) The assimilation of phytoplankton functional types for operational forecasting in the northwest European shelf. J Geophys Res Oceans 123:5230–5247

- Southward AJ (1991) Forty years of changes in species composition and population density of barnacles on a rocky shore near Plymouth. *J Mar Biol Assoc UK* 71:495–513
- Stone CJ (1989) A comparison of algal diets for cirripede nauplii. *J Exp Mar Biol Ecol* 132:17–40
- Strathmann RR (1996) Are planktonic larvae of marine benthic invertebrates too scarce to compete within species? *Oceanol Acta* 19:399–407
- Thiagarajan V, Harder T, Qiu JW, Qian PY (2003) Energy content at metamorphosis and growth rate of the early juvenile barnacle *Balanus amphitrite*. *Mar Biol* 143:543–554
- Thiagarajan V, Hung OS, Chiu JMY, Wu RSS, Qian PY (2005) Growth and survival of juvenile barnacle *Balanus amphitrite*: interactive effects of cyprid energy reserve and habitat. *Mar Ecol Prog Ser* 299:229–237
- Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biol Rev Camb Philos Soc* 25:1–45
- Toupoint N, Gilmore-Solomon L, Bourque F, Myrand B, Pernet F, Olivier F, Tremblay R (2012) Match/mismatch between the *Mytilus edulis* larval supply and seston quality: effect on recruitment. *Ecology* 93:1922–1934
- Tremblay R, Olivier F, Bourget E, Rittschof D (2007) Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. *Mar Ecol Prog Ser* 340:1–8
- Underwood AJ, Fairweather PG (1989) Supply-side ecology and benthic marine assemblages. *Trends Ecol Evol* 4:16–20
- Vargas CA, Manríquez PH, Navarrete SA (2006) Feeding by larvae of intertidal invertebrates: assessing their position in pelagic food webs. *Ecology* 87:444–457
- Wickham H (2016) *Ggplot2: elegant graphics for data analysis*. Springer-Verlag New York, NY

Appendix.

Fig. A1. Surface chlorophyll *a* (chl *a*) concentration (mg m^{-3}) map, produced with data from the 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. Coasts sampled in this study are indicated (São Sebastião Channel, Brazil, and the English Channel, British Isles)



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