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Novel insights into the ecophysiological processes shaping settlement dynamics of rocky shore foundation species

Nouvelles connaissances sur les processus écophysiologiques qui façonnent la dynamique de recrutement des espèces fondatrices du milieu intertidal rocheux

Thèse présentée
dans le cadre du programme de doctorat en océanographie
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À minha família,

Por tudo.

Guincho, Portugal
By Inês Leal

MAR SONORO

“Mar sonoro, mar sem fundo, mar sem fim.
A tua beleza aumenta quando estamos sós
E tão fundo intimamente a tua voz
Segue o mais secreto bailar do meu sonho.
Que momentos há em que eu suponho
Seres um milagre criado só para mim.”

– Sophia de Mello Breyner Andresen

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ABSTRACT

This thesis presents novel insights into supply-driven population dynamics in the intertidal realm by integrating key aspects of ecology with physiology. Through a series of studies, I integrate key ecological drivers with intrinsic physiological factors of early life stages of rocky shore foundation species to explain settlement dynamics. A considerable amount of work was done through a comparative approach, i.e., by comparing the physiology/response of organisms exposed to different environmental conditions. This provided novel information on the ecophysiology of six keystone species: the bivalves *Isonogmon alatus* and *Brachidontes solisianus*, and the barnacles *Chthamalus proteus*, *Chthamalus bisinuatus*, *Chthamalus montagui*, and *Semibalanus balanoides*. The trophic environment strongly shaped the ecophysiology of these species, including effects on larval nutritional composition and physiological response, which has profound implications for comparative studies of intertidal species.

The first series of studies in Chapter 1 describe the plasticity in size at settlement and metamorphosis in populations of the low-latitude bivalves *I. alatus* and *B. solisianus*. The analysis of shell morphometry (Prodissoconch II and Dissoconch shells) revealed that post-larval drifting behavior, i.e., mobility of settlers after the first settlement, was commonplace in the studied areas and likely an integral part of the life cycle of these species. In southeastern Brazil, trophic subsidies deriving from pulsed natural disturbances, i.e., rainfalls, appeared to trigger the settlement response of *B. solisianus*. This was suggested by the strong positive correlation of precipitation (> 10 mm) and fatty acid tracers of terrestrial origin (10-13 % of nearshore suspended matter composition) with primary settlement estimates. In the Caribbean waters of northwestern Panama, juvenile drifting was commonplace in the *I. alatus* population, with post-larval drifters reaching nearly 20 % of all pelagic individuals. This species, highly adapted to oligotrophy, did not appear to respond to trophic triggers. It is possible that in this tropical system, individuals get more competent with age, i.e., drifters settle as they age. These studies highlight that the spatial distribution of competent larval stages and/or early settlers might not reflect their final distribution. This information is crucial for managing and exploiting natural stocks, as indices of spatfall, i.e., settlement and attachment, provide managers the possibility of predicting major fluctuations in recruitment.

The second series of studies in Chapter 2 highlight the advantages of using lipidomics to characterize the physiological condition of settling larvae (e.g., triacylglycerol [TAG] to phospholipid [PL] ratios) and the composition of dietary sources entering coastal food webs (e.g., Fatty Acid Trophic Markers). Cyprid larvae of closely related species from the genus *Chthamalus* (subtropical *C. bisinuatus* vs. temperate *C. montagui*) similarly stored lipid reserves (TAG ≥ 50 % of total lipid content) despite being exposed to considerably different levels of primary productivity as given by chlorophyll-*a* (Chl-*a*) estimates. This finding flagged the challenges of relating common estimates of primary productivity with food supply, particularly close to shore. Mechanisms related specifically to food quality (e.g., phytoplankton species, organic matter composition) cannot be directly associated to Chl-*a* patterns. For instance, saturated fatty acids (SFAs; trophic markers of detritus and bacteria) largely contributed to the composition of nearshore suspended matter sampled over multiple latitudes (ranging 55-60 % in low-latitudes and 40-45 % in high-latitudes), reflecting a tight land-ocean coupling that drives strong detrital-based food webs close to shore. A thorough profiling of the fatty acids of low-latitude *Chthamalus* spp. and high-latitude *Semibalanus* sp. cyprids also revealed that SFAs play an important role as energy fuel during settlement and metamorphosis that should not be overlooked. Further, both *Chthamalus spp.* and *Semibalanus sp.* cyprids contained higher essential fatty acid levels than the relative share in their environment, and the low levels of 18-carbon precursor fatty acids in food resources (≤ 5 % of total lipid content) suggested selective retention rather than biosynthesis. Such retention varied as a function of the trophic environment, and was particularly noticeable for the essential docosahexaenoic acid (DHA; 22:6ω3), signaling food deprivation for tropical Caribbean populations. Future research on "tipping points" is necessary to understand the threshold(s) up to which this acclimatory response, i.e., selective retention, increases performance and survival. Importantly, greater energy reserves might boost the growth of metamorphosed juveniles and ameliorate the effects of increasingly warmer environments, as evidenced for the subtropical chthamalid *C. bisinuatus*. This chapter finishes with a fundamental study on the metamorphic process of *S. balanoides* cyprids, i.e., once triggered, metamorphosis proceeds with or without attachment to a substrate. Pioneer ecologists called attention to this process three quarters of a century ago, and this study now opens the door for future research on the physiological mechanisms driving desperate metamorphosis in this important group of space-monopolizing species.

These studies show the importance of reconciling the trophic ecology with the physiology of marine benthic invertebrates. The interaction of larvae with a range of ecological drivers affects their physiology, and determines where settlement occurs and at what intensity, ultimately setting the scene for the distribution, abundance, and dynamics of the adult populations that follow. The use of lipidomics brings immense opportunities to clarify the physiological processes mediating this pelago-benthic transition. It is my contention that uncovering the processes shaping the early life-history of marine benthic invertebrates not only advances community ecology, but also has serious implications in the management of natural resources.

Keywords: Supply-side ecology, Complex life-cycles, Larval physiology, Lipidomics, Fatty acid trophic markers, Metamorphosis, Rocky intertidal, Marine benthic invertebrates

RÉSUMÉ

Cette thèse présente de nouvelles connaissances sur la dynamique des populations dans le domaine intertidal en intégrant des aspects d'écologie et de physiologie. Grâce à une série d'études, j'intègre les principaux facteurs écologiques aux facteurs physiologiques intrinsèques des premiers stades de vie des espèces fondatrices du milieu intertidal rocheux pour expliquer la dynamique de fixation. Un travail considérable a été effectué grâce à une approche comparative, c.-à-d. en comparant la physiologie et la réponse de plusieurs espèces exposées à différentes conditions environnementales en milieu naturel. Je fournis de nouvelles informations sur l'écophysiologie de six espèces clés : les bivalves *Isonogmon alatus* et *Brachidontes solisianus*, et les balanes *Chthamalus proteus*, *Chthamalus bisinuatus*, *Chthamalus montagui* et *Semibalanus balanoides*. L'environnement trophique a fortement façonné l'écophysiologie de ces espèces, dont je souligne les effets sur la composition nutritionnelle des larves et la réponse physiologique, ce qui a des profondes implications pour les études comparatives des espèces intertidales.

La première série d'études du Chapitre 1 décrit la plasticité de la taille des individus au moment de la fixation et de la métamorphose des populations de bivalves de basse latitude *I. alatus* et *B. solisianus*. L'analyse de la morphométrie des coquilles (Prodissoconch II et Dissoconch) a révélé que le comportement de dérive post-larvaire, c.-à-d. la mobilité des juvéniles après la première fixation, était courant dans les zones étudiées et faisait probablement partie intégrante du cycle de vie de ces espèces. Dans le sud-est du Brésil, les apports trophiques dérivant de perturbations naturelles liées aux pluies semblent déclencher la fixation de *B. solisianus*. Cela a été suggéré par la forte corrélation positive des précipitations (> 10 mm) et des d'acides gras d'origine terrestre (10-13 % de la composition des matières en suspension près du littoral) avec les estimations de fixation primaire. Dans les eaux caribéennes du nord-ouest du Panama, la dérive des juvéniles était courante dans la population d'*I. alatus*, avec des juvéniles en dérive atteignant près de 20 % de tous les individus pélagiques. Cette espèce, adaptée à l'oligotrophie, ne semble pas répondre aux déclencheurs trophiques. Il est possible que dans ce système tropical, les individus deviennent plus compétents avec l'âge. Ces études soulignent que la distribution spatiale des stades larvaires compétents et/ou des premiers juvéniles fixés pourrait ne pas refléter leur distribution finale. Cela représente une information cruciale pour la gestion et l'exploitation des stocks naturels, car les indices de *spatfall*, c.-à-d. la fixation et l'attachement, est souvent utilisé pour prédire les fluctuations majeures du recrutement.

La deuxième série d'études du Chapitre 2 met en évidence les avantages de l'utilisation de la lipidomique pour caractériser l'état physiologique des larves (par ex., le triacylglycérol [TAG] : phospholipide [PL] ratio) et la composition des sources alimentaires côtières (par ex., marqueurs trophiques d'acides gras). Les larves cypridés de *C. bisinuatus* et *C. montagui* ont stocké des réserves lipidiques de manière similaire (TAG ≥ 50 % de la teneur totale en lipides) malgré l'exposition à différents régimes trophiques estimés par la chlorophylle-*a* (Chl-*a*). Cela a mis en évidence les défis de relier directement des estimations de la productivité primaire avec l'approvisionnement alimentaire, en particulier près des côtes. Les mécanismes liés spécifiquement à la qualité de la nourriture (par ex., les espèces de phytoplancton, la composition de la matière organique) ne sont pas directement associés aux estimations de Chl-*a*. Par exemple, les acides gras saturés (AGS; marqueurs trophiques de détritus et de bactéries) ont largement contribué à la composition des matières en suspension échantillonnées près du littoral (allant de 55 à 60 % dans les basses latitudes et de 40 à 45 % dans les hautes latitudes), reflétant un couplage terre-océan générant des réseaux trophiques à base de détritus près des côtes. Des profils complets en acides gras des cypridés de *Chthamalus* spp. et *Semibalanus* sp. ont également révélé que la contribution des AGS en tant que carburant énergétique lors de la fixation et de la métamorphose ne doit pas être négligée. En outre, les cypridés contenaient des niveaux d'acides gras essentiels plus élevés que la part relative dans leur environnement. Combiné aux faibles niveaux d'acides gras précurseurs de 18 carbones dans les ressources trophiques (≤ 5 % de la teneur totale en lipides), ces résultats suggéraient une rétention sélective plutôt qu'une biosynthèse. Cette rétention variait en fonction de l'environnement trophique, et était notable pour l'acide docosahexaénoïque (DHA; 22:6ω3), signalant un déficit nutritionnel pour les populations tropicales des Caraïbes. Des recherches futures sur les «points de bascule» sont nécessaires pour comprendre le(s) seuil(s) où cette réponse d'acclimatation, c.-à-d. la rétention sélective, augmente les performances et la survie. Des réserves d'énergie plus importantes pourraient stimuler la croissance des juvéniles et améliorer les effets d'environnements de plus en plus chauds, comme indiqué pour *C. bisinuatus*. Ce chapitre se termine par une étude fondamentale sur le processus métamorphique des cypridés de *S. balanoides*, c.-à-d. qu'une fois déclenchée, la métamorphose se déroule avec ou sans attachement à un substrat, un processus sur lequel les écologistes pionniers ont attiré l'attention il y a trois quarts de siècle et qui ouvre la porte pour de futures recherches sur les mécanismes physiologiques conduisant à une métamorphose désespérée.

Ces études montrent l'importance de concilier l'écologie trophique avec la physiologie des invertébrés benthiques marins. L'interaction des larves avec une gamme de facteurs écologiques affecte leur physiologie et détermine le lieu de la fixation et son intensité, définissant la distribution, l'abondance et la dynamique des populations adultes. L'utilisation de la lipidomique offre d'immenses opportunités pour clarifier les processus physiologiques sous-jacents à cette transition pélago-benthique. Je soutiens que la découverte des processus qui façonnent les premiers stades de vie des invertébrés benthiques marins fait non seulement progresser l'écologie des communautés, mais a également de sérieuses implications dans la gestion des ressources naturelles.

Mots clés : Théorie «Supply-side ecology», Cycles de vie complexes, Physiologie larvaire, Lipidomique, Marqueurs trophiques, Métamorphose, Intertidale rocheuse, Invertébrés benthiques marins

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARA	Arachidonic acid (20:4ω6)
Chl *a*	Chlorophyll *a*
DHA	Docosahexaenoic acid (22:6ω3)
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic acid (20:5ω3)
FA	Fatty Acid
FATM	Fatty Acids Trophic Marker
GC-MS	Gas Chromatography–Mass Spectrometry
GLS	Generalized Least Squares
IR	Infra-red
LNA	Linolenic acid (18:3ω3)
LOA	Linoleic acid (18:2ω6)
MTFA	Mass of Total Fatty Acids
MUFA	Monounsaturated Fatty Acid
PCA	Principal Component Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance

PERMDISP	Permutational Dispersion Analysis
PII	Prodissoconch II
PL	Phospholipid
POM	Particulate Organic Matter
PS	Plymouth Sound
PUFA	Polyunsaturated Fatty Acid
RDA	Redundancy Analysis
SD	Standard Deviation
SE	Standard Error
SFA	Saturated Fatty Acid
SIMPER	Similarity Percentage Analysis
Sp.	Species (singular)
Spp.	Species (plural)
SSC	São Sebastião Channel
TAG	Triacylglycerol
TPM	Total Particulate Matter
TSM	Total Suspended Matter

GENERAL INTRODUCTION

SETTLEMENT OF MARINE BENTHIC INVERTEBRATES

The population structure of marine benthic species with a biphasic life-cycle, that is, that spend a certain period of their life-history as planktonic free-living larvae before reaching the benthos, is initially governed by recruitment. In the broadest sense, recruitment comprises the addition of new individuals to the adult, breeding populations (see reviews by Caley et al., 1996; Hunt and Scheibling, 1997). As defined by Hunt and Scheibling (1997) "recruitment is an operational term rather than a biological event", being preceded by larval settlement. Understanding how settlement determines recruitment, and hence adult distribution and abundance, is a central theme in ecology and requires the understanding of the dependencies of settlement rates, i.e., the rate at which pelagic larvae take up permanent contact with the substrate (*sensu* Connell, 1985).

The settlement dynamics of marine benthic invertebrates have been extensively studied for the past decades (e.g., Connell 1961; Crisp 1976; Hawkins, 1981; Roughgarden et al., 1985; Olson and Olson, 1989; Pawlik, 1992; Rodriguez et al., 1993; Jenkins et al., 1999; Barbosa et al., 2016). A vast array of factors has been documented to affect settlement (e.g., shoreline configuration, hydrodynamics, predation, competition, habitat selection, larval condition, among others; Pineda, 1994; Gosselin and Qian, 1997; Miron et al., 1999; Archambault and Bourget, 1999; Tremblay et al., 2007a,b) and linked to variability in population dynamics of many benthic organisms (e.g., Connell, 1985; Roughgarden et al., 1985).

Since the 1980's, there has been growing interest on the role of larval input on rocky shore community dynamics, baptized as "supply-side ecology" by Lewin (1986), which catalyzed a shift in research with a focus on biological interactions and disturbance, to an emphasis on larval processes in determining community structure. Supply-side ecology suggests that variable larval input determines the abundance of local adult populations and may also determine the relative importance of post-settlement factors, such as predation and competition, in shaping communities. Larval behaviour (e.g., gregariousness; first reported in field populations of the barnacle *Elminius modestus*; Knight-Jones and Stevenson, 1950) has been suggested as one of the main sources of decoupling between larval supply and settlement, with strong consequences for population connectivity (Pineda, 1994; Pineda et al., 2010). Pineda (1994) suggested that at scales of 100 m, *temporal* variability in settlement rate may be related to larval pool and physical transport processes, while *spatial* variability may be associated with behavioral response and substrate availability. Further, evidence from laboratory (Jarrett and Pechenik, 1997), and field studies (Miron et al., 1999; Jarret, 2003), advanced that settling larvae may exhibit dramatic differences in recruitment success due to underlying differences in the physiological quality of individuals making up daily cohorts, making the bridge between ecological and physiological processes shaping settlement.

THE PLANKTONIC LIFE: DISPERSING

> *"Marine invertebrate larvae [...] may spend as little as a few minutes or as long as several months in the plankton before metamorphosing to adult form and habitat."*
>
> – Pechenik, 1990

Marine benthic invertebrates encompass an incredibly diverse suite of taxa, and most – sponges, flatworms, mollusks, crustaceans, echinoderms, to name a few – exhibit a complex life cycle that involves a planktonic larval phase followed by a benthic juvenile and adult phase (Thorson, 1950). Larvae of marine benthic invertebrates may hatch from embryos that result from gamete fertilization in the pelagic environment, where embryonic and larval development may also occur, or from embryos that are brooded by females, i.e., cross-fertilization, a feature exhibited by taxa with some degree of parental care. Thorson (1964) estimated that c.a. 90,000 species of marine invertebrates produce planktonic larvae. The replenishment of these marine populations is thus largely dependent on the larval supply from the pelagos (Caley et al., 1996). The planktonic stages are very important in linking adult populations genetically and contributing to local population sizes (St-Onge et al., 2015), but it comes at a cost. The mortality for species with a planktonic larval development has been estimated to be 5–8 times greater than for species with direct development (Rumrill, 1990).

As described by Pechenik (1990) the duration of the planktonic life of marine benthic invertebrates may vary considerably, from only minutes to months. Throughout this time, larvae drift freely, dispersing from their place of origin to meters or hundreds of kilometers away (Jablonski and Lutz, 1983; Pawlik, 1992). The small sizes of these larvae allow them to be easily transported and mixed by water currents. However, the view of invertebrate larvae as “inert” particles has long been abandoned. In 1955, Crisp demonstrated that balanid cyprids can swim at a speed of 4 to 5 cm/sec, and such sustained speed may not even represent their maximum swimming capacity (Crisp, 1955).

Planktonic larvae may be divided in two main groups with contrasting developmental strategies concerning nutrition mode: planktotrophy and lecithotrophy (Thorson, 1950). Planktotrophic larvae feed while developing in the plankton, depending on particulate organic matter, including bacteria, eukaryotic phytoplankton, and protists, for nutrition (Thorson, 1950; Jablonski and Lutz, 1983). These larvae generally require a prolonged time for development in the plankton, which in turn results in a high dispersal capacity. This way, species with planktotrophic larvae are plausibly geographically widespread, with low speciation and extinction rates (Jablonski and Lutz, 1983). As for lecithotrophic larvae, the yolk from the eggs provides the nourishment needed for development, so that the larvae do not feed (but see Jaeckle and Manahan, 1989: non-feeding larvae can metabolize dissolved free amino acids from seawater) and remain in the plankton for a much shorter period than planktotrophic larvae, from a few hours to a few days (Thorson, 1950; Jablonski and Lutz, 1983; Havenhand, 1993). Accordingly, lecithotrophic larvae have a lower dispersal capacity than planktotrophic ones, being geographically more restricted, with higher local speciation and extinction rates (Jablonski and Lutz, 1983). During their pelagic development, larvae may also rely on endotrophy, i.e., the use of vitelline reserves exclusively (e.g., while the velum for feeding is being developed). The larval biologist Gunnar Thorson suggested that species from polar and deep-sea regions rarely have planktonic development and that planktotrophy was more common in the tropics than in temperate regions, commonly known as "Thorson's rule" (Thorson 1950).

THE BENTHIC LIFE: ATTACHING AND METAMORPHOSING

The onset of benthic life is a time of extreme vulnerability for marine invertebrates, with a common pattern of exponential decrease in survivorship during the first days or weeks of juvenile life (see reviews by Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Growing evidence suggests that the condition of larvae and young juveniles, determined by the amount of energy reserves, may greatly influence the number of successful recruits (e.g., Gosselin and Qian, 1997; Phillips, 2002; Tremblay et al., 2007a,b; Rayssac et al., 2010).

Before the benthic life of marine invertebrate larvae can begin, they need to become fully-developed, i.e., competent to initiate the metamorphic process (see reviews by Pawlik, 1992; Rodriguez et al., 1993). The attainment of competency may vary according to the larval developmental strategy: minutes to days in the case of most lecithotrophic larvae, and weeks to months for most planktotrophic larvae (Pawlik, 1992). The competent stage ends when larvae receive environmental cues that indicate the presence of a suitable habitat (e.g., Bishop et al., 2006a,b). After finding a suitable substrate on which to attach, the competent larva undergoes a radical transformation, upon which larval structures are lost and the adult body begins to form (Pawlik, 1992; Pechenik, 2006).

"Metamorphosis is a time of great revolution – commonly it is a dramatic transition to a new life in a new place in a new body."

– Pechenik, 2006

It is precisely the transition from a pelagic larval stage to a benthic juvenile stage (i.e., pelago-benthic transition) that the settlement of marine benthic invertebrates entails. Settlement involves a series of events that begins with the behavioural search for a suitable habitat and ends with metamorphosis, a radical change in morphology and physiology that is tightly controlled by environmental signals and hormones (Pawlik, 1992; Rodriguez et al., 1993; Bishop et al., 2006a). Although a dramatic transition to a new body, “metamorphosis is not a new beginning” (Pechenik et al., 1998). Larval experience influences juvenile performance through “latent” or “carry-over effects”, i.e., effects that have their origins in early development but that are first exhibited in juveniles or adults (Pechenik, 2006). This ecological latent manifestation is also known as “larval legacy” (Allen and Marshall, 2010).

IS THERE A SPECIFIC CUE THAT TRIGGERS SETTLEMENT?

It has long been recognized that marine invertebrate larvae require specific habitat cues for settlement (Wilson, 1953). Presumably, larvae respond to a hierarchy of abiotic and biotic cues in the course of habitat selection, such as substratum texture or thermal capacity, the presence of conspecifics or biofilms (see reviews by Pawlik, 1992; Rodriguez et al., 1993; Thiyagarajan, 2010). Such cues are key in the small-spatial scale exploration and in the decision to accept or reject a settlement site (Miron et al., 1996). For example, the composition of the bacterial community of biofilms may inhibit or stimulate the attachment of the larvae of the barnacle *Balanus amphitrite,* thus modulating substrate selection behaviour in this species (Olivier et al., 2000; Thiyagarajan, 2010). Recent evidence suggests that underwater sound may also play a role in the settlement of crustaceans and mollusks, by guiding larvae to settlement habitats (Radford et al., 2008; Jolivet et al., 2016).

Using the acorn barnacle *Balanus amphitrite* as a reference (a model organism for investigating the transition from a pelagic life to a benthic life in marine invertebrates; e.g., De Gregoris et al., 2012), several studies have shown that habitat selection is strongly influenced by the physiological condition of larvae (e.g., Miron et al., 2000; Tremblay et al., 2007b; Thiyagarajan, 2010). Indeed, under laboratory conditions, Miron et al. (2000) showed that habitat selection in *B. amphitrite* cyprids (the last larval stage) involves substratum type, hydrodynamics, and physiological condition (which was shown to decrease with larval age). Moreover, Tremblay et al. (2007b) clearly demonstrated that habitat selection is controlled by larval physiological quality in the field. The authors found that as energetic reserves of *B. amphitrite* cyprids decreased, larvae risked settling in poorer quality sites rather than postponing settlement. Although considerations of larval condition trade-offs continue to be refined, our understanding of its underlying causes remains limited due, in part, to a strong need for experimental studies carried out directly in the field (e.g., capture the conditions offspring experience before reaching the local adult habitat; Emlet and Sadro, 2006).

Pelagic food quantity and quality have been shown to determine larval physiological quality, and influence juvenile growth and survival (Pechenik et al., 1998; Emlet and Sadro, 2006; Thiyagarajan, 2010). Importantly, food might be abundant and still be nutritionally deficient. For example, Lasker (1981) found that although phytoplankton was abundant for anchovy larvae, recruitment was low during years when poor trophic resources, *Gymnodidium spp.*, predominated. The probability of recruitment failure due to limiting food resources has been shown for several taxa, including the barnacle *Balanus balanoides* and the mussel *Mytilus edulis* (reviewed by Olson and Olson, 1989).

THE TROPHIC SETTLEMENT TRIGGER HYPOTHESIS

Marine invertebrates have specific nutritional demands in terms of survival, growth, and reproduction. Lipids, the primary source of energetic reserves, have been linked to the physiological quality of organisms (e.g., Pernet and Tremblay, 2004; Tremblay et al., 2007a,b). Toupoint et al. (2012a) found that, over consecutive years, major settlement peaks of *Mytilus edulis* were synchronized with phytoplanktonic pulses rich in essential fatty acids (EFAs). Following this evidence, the authors applied Cushing's match-mismatch hypothesis (Cushing, 1990) to *M. edulis* settlement and suggested the "trophic settlement trigger" hypothesis: phytoplanktonic pulses rich in polyunsaturated fatty acids may act as a settlement trigger for mussel larvae. This suggests a bottom-up influence of primary production and its dietary quality on recruitment success. A following study on the effect of biofilm on the settlement of *M. edulis* concluded that settlement depends both on planktonic and biofilm-derived cues in a hierarchical order, with planktonic cues being more influential if present (Toupoint et al., 2012b).

The trophic settlement trigger hypothesis and nutritional role of plankton stimulating settlement of *M. edulis* were further confirmed in a controlled experiment using labelled ^{13}C picoeukaryote microalgae species to demonstrate their assimilation and incorporation in membrane cells of settled mussels (Jolivet et al., 2016). This hypothesis has also been validated for the larval settlement of the Pacific oyster *Crassostrea gigas* in Mediterranean lagoon (Sète), but induced by diatom blooms (Lagarde et al., 2017). Cushing's match-mismatch hypothesis has also been applied for barnacle populations, in the context of "recruitment windows", whereby recruitment failure occurs when barnacle nauplii fail to develop at the same time as the spring algal bloom (Barnes, 1956; Pineda et al., 2006).

METAMORPHOSIS DELAY

The first evidence of flexibility in the timing of metamorphosis of benthic invertebrates was published almost a century ago by Mortensen (1921). Since then, evidence has accumulated on the ability of pelagic larvae to prolong their planktonic life for a variable period of time until finding a suitable substratum (see reviews by Pechenik, 1990; Pawlik, 1992). The ability to postpone metamorphosis varies considerably among larvae, both inter- and intra-specifically, being an example of developmental plasticity (Pechenik, 1990). The term "delay of metamorphosis" precisely describes this plasticity during development (Pechenik, 1990; Bishop et al., 2006a).

Compelling evidence both from laboratory and field studies demonstrates that the ability to delay metamorphosis is present in several groups of marine invertebrates, e.g., Annelida, Mollusca, Arthropoda, Echinodermata, among others (see review by Pechenik, 1990). Recently, Martel et al. (2014) reported that each summer in the Gulf of St. Lawrence a significant number of *Mytilus edulis* larvae delayed metamorphosis, c.a. 15 days, and suggested that the recurring metamorphosis delay observed might be caused by the absence of a suitable substrate or a specific trophic cue to trigger settlement. The authors found that a small size at metamorphosis, associated with shorter larval duration, resulted in greater settlement success rates. Indeed, delaying metamorphosis can result in a depletion of energetic reserves and therefore in a decrease in larval condition, affecting post-metamorphic performance (e.g., Pechenik, 1990; Pechenik et al., 1993; Pechenik et al., 1998; Elkin and Marshall, 2007). Pechenik et al. (1993) stated that "delaying metamorphosis may be a double-edged sword" by finding that the delay of metamorphosis of *Balanus amphitrite* larvae, although increasing larval chances of locating a suitable settlement site, reduced post-metamorphic growth rates and recruitment success. Such results highlight the fitness costs associated with metamorphosis delay, especially for non-feeding, lecithotrophic larvae (Pechenik, 1990; Pechenik et al., 1998; Elkin and Marshall, 2007). Indeed, feeding, planktotrophic larvae are more likely to delay metamorphosis without showing reduced performance later on, since continued feeding may allow them to build up energetic reserves (Phillips, 2002; Elkin and Marshall, 2007).

There is considerable diversity among taxa in the degree to which larvae can delay metamorphosis. Several hypotheses have been drawn in an attempt to explain such variation: the "desperate larva" hypothesis, the "variable retention" hypothesis, and the "death before dishonor" hypothesis (Marshall and Keough, 2003; Bishop et al., 2006a). According to the "desperate larva" hypothesis, lecithotrophic (non-feeding) larvae become increasingly desperate to settle as energetic maternal reserves decline, and metamorphosis occurs in response to a nonspecific cue, or even spontaneously (Knight-Jones, 1953; Bishop et al., 2006a). Planktotrophic (feeding) larvae may also settle and metamorphose in response to nonspecific cues or spontaneously. The "variable retention" hypothesis has been drawn to explain such behaviour, and contrary to the "desperate larva" hypothesis, food limitation is unlikely a factor. This hypothesis states that feeding larvae may be energy depleted due to the increasing metabolic demands caused by juvenile tissue formation, or patchy food resources, and thus metamorphose in the absence of cues (Bishop et al., 2006a). However, some larvae do not decrease their habitat selectivity, or undergo spontaneous metamorphosis, as a function of time without a cue (e.g., Miller and Hadfield, 1990: the nudibranch *Phestilla sibogae,* a specialist grazer of Porites corals, requires its coral cue to settle and metamorphose). This behaviour applies to habitat specialists, and may be explained by the “death before dishonor” hypothesis. According to this hypothesis, larvae retain the larval stage, even if starved, until coming across specific cues or dying (Bishop et al., 2006a). This way, habitat specialists are expected to have higher abilities to delay metamorphosis (Pechenik, 1990). Even though these hypotheses give insights on the diversity of the metamorphic processes among benthic invertebrates, the mechanisms underlying such plasticity during development are still far from known (Bishop et al., 2006a).

POST-METAMORPHIC DRIFTING

Some species of benthic invertebrates can undergo post-metamorphic drifting, also known as secondary settlement, dispersal, or migrations, by re-entering the water column through drifting or crawling even weeks after the first settlement (Armonies, 1992; Olivier et al., 1996; Le Corre et al., 2013; Navarrete et al., 2015; Forêt et al., 2018a,b). Through secondary dispersal, a species may enlarge its geographical range, and presumably select a more suitable habitat to settle (Armonies, 1992). Le Corre et al. (2013) observed that secondary dispersal of blue mussels *Mytilus* spp. significantly contributed to local recruitment dynamics, i.e., several peaks throughout the recruitment season, and highlighted the need to distinguish between primary and secondary settlers so as not to bias estimates of recruitment. Other researchers have suggested that settlement-relocation is continuous in mytilids, whereby postmetamorphs are transported onshore through the surf-zone by wave-driven near-bed transport, i.e., “tumbling under the surf”, which may take from a few hours to several days, with larvae arriving at the shoreline in a wide range of sizes at any given time (Navarrete et al., 2015). Recently, Forêt et al. (2018a) reported secondary migrations in 25 bivalve taxa (spanning Mytilidae, Nuculidae, Semelidae, Mactridae, and Lucinidae). The authors demonstrate that the intense post-settlement dispersal observed at the end of the recruitment season was not synchronized with periods of high hydrodynamic stress but rather to a change in the structure of phytoplanktonic assemblages, particularly nanoeukaryotes. The authors suggested that these acted as pelagic cues triggering secondary migrations, resulting from an increased demand for energy required for active migratory behaviour. Therefore, food quality acts not only in primary settlement (as a trophic *settlement* trigger; Toupoint et al., 2012a), but also in secondary settlement (as trophic *migration* trigger; Forêt et al., 2018a).

LATITUDINAL VARIATION IN ENVIRONMENTAL CONDITIONS

"The few facts known… tend to show that the development proceeds at a conspicuously quicker rate at the higher temperature of the tropics than in the cooler climate of extratropical regions… here is a problem worth studying."

– Mortensen, 1921

Marine benthic invertebrates develop in waters that extend from the poles to the equator, experiencing a full range of environmental conditions that vary markedly over space and time (Hoegh-Guldberg and Pearse, 1995; Phillips, 2002). Temperature is a well-documented factor known to exert a strong selective pressure upon marine populations distributed over various scales, from small, intertidal scales to broad, latitudinal ones (e.g., Bertness and Gaines, 1993; Rayssac et al., 2010; Sanford and Kelly, 2011). Rayssac et al. (2010) found that the thermal tolerance exhibited by larvae of coexisting mussel species, *Mytilus edulis* and *M. trossulus*, reflected the biogeography of the species. The authors concluded that the selective effects of temperature on mussel populations act primarily during early ontogeny, so that temperature influences not only the distribution of benthic communities, but also controls their dynamics before these organisms reach the benthos (Rayssac et al., 2010).

Temperature effects need to be considered, however, in the context of nutritional availability (Thorson, 1950; Hoegh-Guldberg and Pearse, 1995). Developmental rates can be boosted by higher environmental temperatures only if the larva gets enough food to sustain increased metabolic rates. Food supply has been shown to be a major factor determining population dynamics of marine invertebrates in polar (e.g., Cavallo and Peck, 2020), temperate (e.g., Toupoint et al., 2012a) and subtropical environments (e.g., Kasten and Flores, 2013). In the southern coast of Brazil, Kasten and Flores (2013) found that food supply and temperature were positively correlated with larval release of the subtropical intertidal barnacle *Chthamalus bisinuatus*. In a subarctic system, the Gulf of St. Lawrence, Le Corre et al. (2013) described a bimodal spawning/settlement pattern for the marine mussels *Mytilus* spp. that likely reflected seasonal variability in water temperature and food availability. Temperature and food are therefore two of the most important variables influencing larval development and settlement of benthic invertebrates (Hoegh-Guldberg and Pearse, 1995; Kasten and Flores, 2013; Toupoint et al., 2012a,b; Cavallo and Peck, 2020).

PHENOTYPIC PLASTICITY

Benthic ecologists have long abandoned the assumption that all larvae produced by different parents within the same species display the same ability to survive and recruit. There is ample evidence of inter-individual variability among marine invertebrate larvae in traits related to dispersal (e.g., Miron et al., 1995; Olivier and Retiere, 1998; Nanninga and Berumen, 2014) as well as physiological condition (e.g., Miron et al, 1999, 2000; Tremblay et al., 2007b; Kasten et al., 2019a). For instance, using genetic markers, St-Onge et al. (2015) identified 5 larval cohorts of the soft-shell clam *Mya Arenaria* within the same reproductive season. Of those cohorts, the first one was the most successful to survive and recruit, and represented more than 80 % of the recruits in the population.

Phenotypic heterogeneity/plasticity can be described as the potential for an organism to produce a range of different (fit) phenotypes in multiple environments (DeWitt et al., 1998). During early ontogeny, intraspecific variation in larval phenotypes can derive from a variety of plastic larval attributes (physiological, nutritional, behavioral) that have been identified as determinants of population connectivity and successful onshore recruitment (Nanninga and Berumen, 2014). Differential behaviour (e.g., habitat selection; Jenkins, 2005) and quality (e.g., energy reserves; Tremblay et al., 2007b) of settling larvae may even play a more relevant role on the regulation of marine populations than variations in larval supply. Importantly, phenotypic plasticity allows to produce adaptive phenotypes in heterogeneous environments (Burgess and Marshall, 2011; Sanford and Kelly, 2011).

A CHANGING CLIMATE

Benthic organisms inhabiting the intertidal zone, especially sessile ones, are exposed to air on a daily or twice-daily basis, experiencing abiotic constraints of both terrestrial and marine environments (Helmuth, 1998). In these habitats, conditions fluctuate to extremes in just a few hours. During low tide, an intertidal invertebrate may increase its body temperature 15ºC above water temperature, or conversely decrease to freezing temperatures after aerial exposure (Gosselin and Qian, 1997; Helmuth et al., 2006). As early as 1954, Southward and Crisp (1954) identified *Semibalanus balanoides* and *Chthamalus* spp. as indicator species for investigating changes in the environment, by recognizing that effects of thermal changes were visible in barnacle populations sooner than in other sessile invertebrates. The body temperature of barnacles closely mirrors that of the rock surface (Bertness and Gaines, 1993), and recent studies estimate that the body temperature of subtropical chthamalids could reach temperatures higher than 40ºC during hot events, exceeding their thermal tolerance threshold (Kasten and Flores, 2013). During such extreme events, the authors found that adult barnacles released their stored larvae as a means to ensure reproductive output. Further, Freuchet et al. (2015) observed that thermally-stressed *C. bisinuatus* ultimately restricted resource allocation to embryos, producing low quality larvae (Freuchet et al., 2015).

Marine benthic invertebrate communities may face increasingly stressful conditions as the environment continues to warm. Global surface temperature is expected to continue rising over the 21st century, and extreme events like heat waves and storm-induced precipitation are expected to become more frequent and intense in many regions (IPCC, 2014). Careful consideration must, however, be given to the small-spatial scale interplay of environmental conditions, i.e., topographic complexity, type of substratum, local weather, local tides, which influence organismal physiological responses more than global averages (Helmuth, 1998; Helmuth, 2002; Helmuth et al., 2006; Miller and Dowd, 2019). Under stressful conditions, only the highest quality larvae may persist (Phillips, 2002; Thiyagarajan, 2010), necessitating research on the interplay between temperature and physiological larval traits (i.e., nutritional) of marine benthic invertebrates.

ECOPHYSIOLOGY AS A COMPREHENSIVE TOOL FOR STUDYING SETTLEMENT

A clear understanding of the physiological mechanisms underlying organismal responses to environmental conditions over space and time is necessary to make well-grounded inferences on species-specific habitat requirements, preferences, and tolerances (see review by Horodysky et al., 2015). As physiology is the link between environmental conditions and animal behaviour and fitness, great scientific gains can be made through the integration of physiology and ecology. Such interdisciplinary integration may lead to a better understanding of environment-organism-ecosystem relationships, by linking processes from cells to populations in an appropriate ecosystem context (Horodysky et al., 2015).

INTERPLAY LARVAE-TROPHIC ENVIRONMENT

The population dynamics of marine invertebrates is strongly influenced by the interactions with their environment. The minuscule pelagic larvae inhabit a three-dimensional habitat, the planktonic environment, being interactively affected by both extrinsic (e.g., water temperature, food availability) and intrinsic factors (e.g., lipid reserves) (Fig. 1). For instance, a rise in water temperature causes an increase in metabolic activity as well as feeding efficiency, resulting in high growth rates as long as stored energy reserves (e.g., lipid droplets) and/or food availability meet energetic demands. A deeper knowledge of the trophic environment surrounding marine benthic communities is necessary if appropriate conclusions regarding the role of larval physiological conditions in structuring the population dynamics are to be drawn.

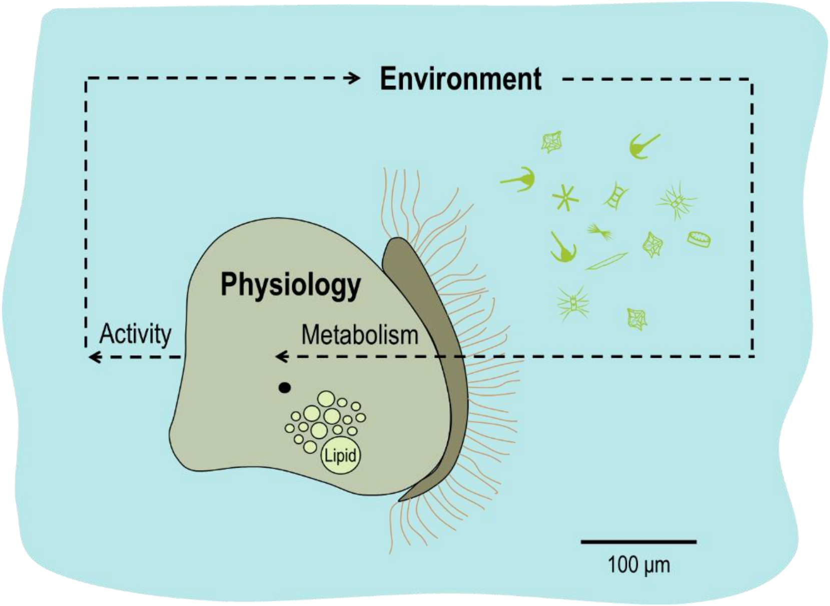


Figure 1. A simple illustration of the integrated study of marine invertebrate larval development. The environment operates on the larva’s activity through its metabolism. Drawings based on the pediveliger larvae of the tropical flat-tree oyster *Isognomon alatus*.

LIPIDOMICS

The study of lipids in marine food webs precisely brings ecology and physiology together. Organisms store lipids when they take in more energy than can be immediately consumed. This energy surplus is packaged and stored for later use, when the need for energy surpass available nutrient supply (see review by Ducharme and Bickel, 2008). Lipids are mainly stored in cellular organelles named lipid droplets (Fig. 2), which are found distributed in the body cavity of many invertebrate larvae (Holland and Walker, 1975; Lee et al., 2006; Tremblay et al., 2007b; Ben Kheder et al., 2010). Their unique structure provides a separation of the aqueous and organic phases, so that, histochemically, stored lipids can be recognized as droplets in the body fluid. Long considered to be inert, lipid droplets have been increasingly recognized as dynamic and diverse structures at the center of lipid and energy metabolism, which interact with other organelles, e.g., peroxisomes, endoplasmic reticulum, and mitochondria (e.g., Bartz et al., 2007; Guo et al., 2009). Adding to this complexity, different lipid droplets can contain different proteins with different rates of acquiring triacylglycerol (Ducharme and Bickel, 2008), suggesting that distinct types of lipid droplets may have specialized functions (Guo et al., 2009).

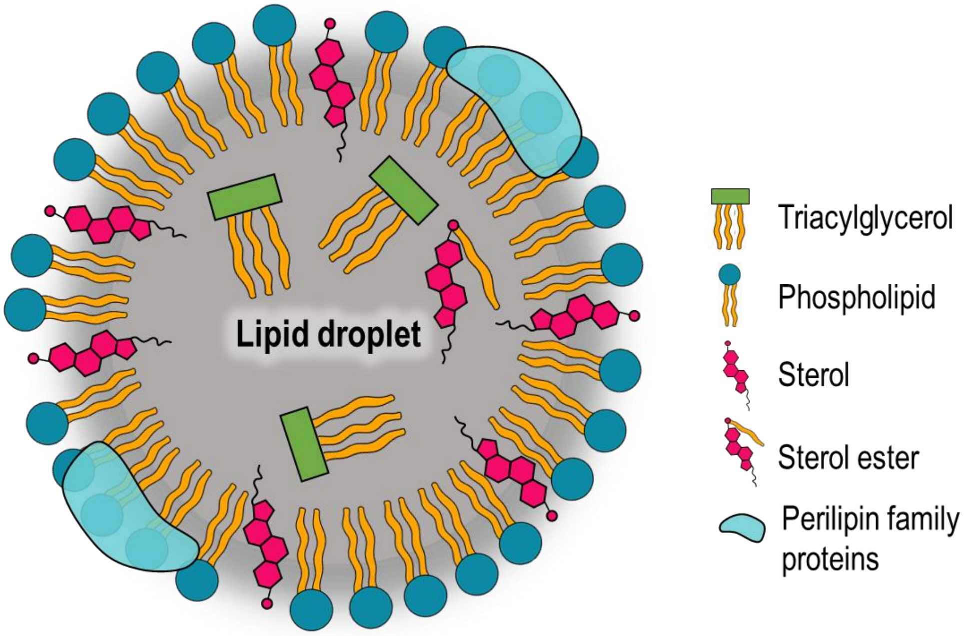


Figure 2. Schematic diagram of the architecture structure of lipid droplets. Lipid droplets are storage organelles that have a unique architecture consisting of a hydrophobic core of neutral lipids (mainly triacylglycerols and sterol esters), which is enclosed by a phospholipid monolayer that is decorated by a specific set of proteins, including structural proteins of the perilipin family, which bind to lipid droplet surfaces as peripheral membrane proteins.

Lipids stored in lipid droplets can be rapidly metabolized for energy, membranes, and hormones (Lee and Walker, 1995), according to physiological needs. Triacylglycerols, composed of a glycerol backbone esterified with three fatty acids, are the most common storage lipid (Fig. 3). For instance, the non-feeding (lecithotrophic) larvae of barnacles contain numerous triacylglycerol droplets, which are gradually consumed until the larva finds a suitable settlement site and metamorphoses (e.g., *Balanus balanoides*, Lucas et al., 1979; *Balanus amphitrite*, Tremblay et al., 2007b). Building blocks of the main types of lipids (e.g., triacylglycerols, phospholipids, wax esters), fatty acids are carboxylic acids with a long aliphatic chain, i.e., a hydrocarbon chain with a carboxyl group (–COOH) at one end, and a methyl group (–CH3) at the other end. Fatty acids are usually named using the following notation: A:BωX (e.g., 20:5ω3, eicosapentaenoic acid), where A indicates the number of carbon atoms in the molecule, B is the number of double bonds, and X is the position of the first double bond relative to the terminal methyl group of the molecule. In living organisms, common fatty acids vary in chain-length and in number of double bonds (Dalsgaard et al., 2003). The number of double bonds determines the degree of desaturation of the molecule (Fig. 3). Increasing desaturation decreases the solidifying point (i.e., freezing temperature) of the molecule. Saturated fatty acids (SFAs) do not contain any double bond between carbon atoms. Monounsaturated fatty acids (MUFAs) have one double bond, which may be present in different positions. Polyunsaturated Fatty Acids (PUFAs) are compounds with two or more double bonds, that are mostly produced by primary producers.

Fatty acids have been used to investigate predator–prey relationships in the marine environment as they elucidate patterns of resource allocation (i.e., Fatty acid trophic markers [FATM], Dalsgaard et al., 2003; Kelly and Scheibling, 2012). The FATM concept stems from the logic that lipid components are in many circumstances incorporated into the tissues of consumers in a conservative manner, and transferred up the food web, comprising a well-established tool in studies of food web structure. *De novo* synthesis of EFAs by primary producers – linoleic acid (LIN; 18:2ω6) and α-linolenic acid (LNA; 18:3ω3), as well as the longer chain components derived from them, arachidonic acid (ARA; 20:4ω6), eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3) – ensure food quality for marine consumers, and are vital for maintaining somatic growth, survival, and reproductive success (e.g., Pernet and Tremblay, 2004). Either biosynthesis (e.g., Kelly et al., 2008) or selective fatty acid retention (e.g., Pernet et al., 2005; Thériault and Pernet, 2007; Gendron et al., 2013) may be postulated when consumer tissues contain higher concentrations of EFAs than does their food. Both strategies tend to occur when the diet is nutritionally deficient. Importantly, integrating the study of lipids with ecology allows for a scale up from their very specific biochemical and physiological roles to their broader ecological effects, e.g., energy flow in food webs.

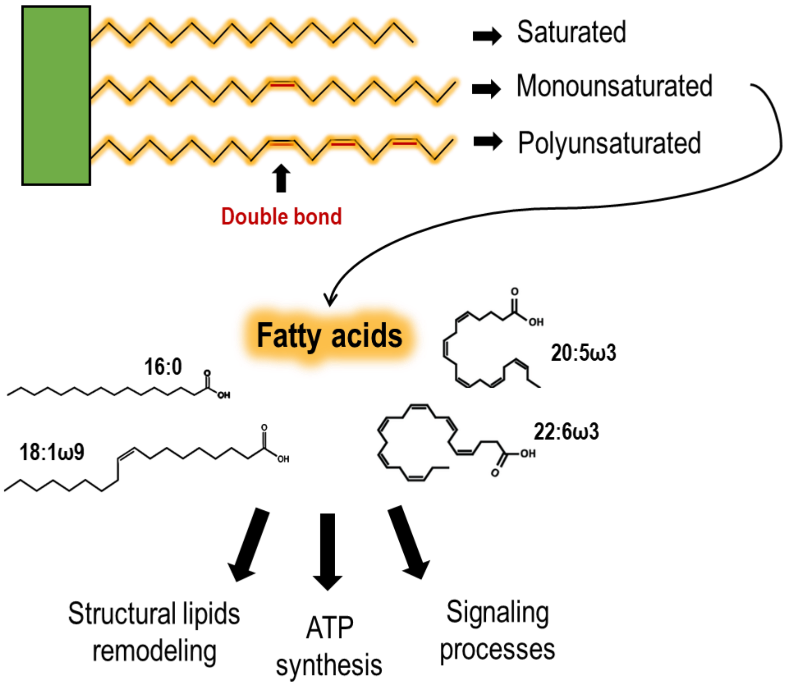


Figure 3. Fatty acids are building blocks of lipids and the main fuel for larval metabolism. Triacylglycerols (TAG) contain three fatty acids attached to a glycerol molecule. During times of nutrient scarcity, TAG stored within lipid droplets is catabolized into free fatty acids and glycerol in a process known as lipolysis (see review by Ducharme and Bickel, 2008). The glycerol and free fatty acids liberated from lipid droplets are used for energy provision via mitochondrial β-oxidation and the generation of ATP. Storage lipids can also be used as substrate for synthesis of other important cellular molecules, such as membrane phospholipids and eicosanoids.

The following citation from Horodysky et al. (2015) elegantly summarizes this last topic:

“Physiological processes reflect an organism’s internal ecology; an interacting milieu of cells, tissues, and organ systems, each with their own defined roles that act within a broader ecosystem – the individual – that can scale to populations and ecosystems through effects on individual fitness”.

– Horodysky et al., 2015

RESEARCH OBJECTIVES

SCOPE

Most larval stages of marine benthic invertebrates share no resemblance to their adult forms, and the two life-history phases have long been studied in isolation. At present, researchers are shifting to an integrated approach to pinpoint the linkages between these contrasting life-history phases and to gain new insights on the population dynamics of marine benthic invertebrates. The timing of food supply pulses (e.g., food availability, or a shift from high to low food quality) during larval development can affect larval size, energy, and behaviour, and significantly impact settlement and post-settlement performance of young metamorphosed juveniles. A better understanding of the trophic ecology of settling larval stages of marine benthic invertebrates allows to better understand the role played by carry-over effects on the life cycles of these taxa.

STUDY MODELS: ROCKY SHORE FOUNDATION SPECIES

As model organisms I used bivalve mollusks ([Mollusca: Bivalvia] with a competent planktotrophic larval stage) and sessile crustaceans ([Arthropoda: Cirripedia] with a competent lecithotrophic larval stage) (example in Fig. 4). The species studied include the bivalves *Brachidontes solisianus* (d'Orbigny, 1842) and *Isognomon alatus* (Gmelin, 1791), and the acorn barnacles *Chthamalus proteus* Dando and Southward, 1980, *Chthamalus bisinuatus* Pilsbry, 1916, *Chthamalus montagui* Southward, 1976, and *Semibalanus balanoides* (Linnaeus, 1767).

As sessile forms throughout their adulthood, these organisms are ecosystem engineers (*sensu* Jones et al., 1994) that build three-dimensional structures, providing substrate, food, and habitat complexity. They can change the environment via their own physical structure (autogenic engineers: e.g., barnacles), or by transforming biotic or abiotic habitat features (allogenic engineers: e.g., mussel beds or oyster reefs). These species play a similar role in the intertidal community, being vital for generating and maintaining local species diversity in the intertidal. The upper and lower midlittoral zones and surrounding nearshore waters were therefore the targets of the sampling protocols carried out in my doctoral research.

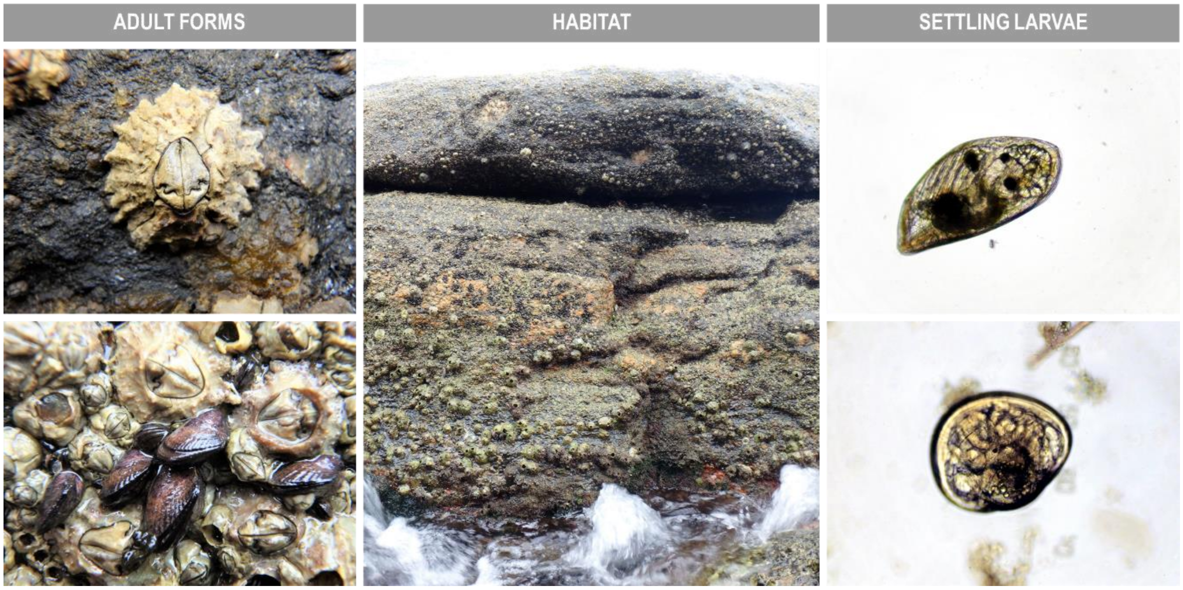


Figure 4. An illustration of the biological models used in this study. The multi-panel picture displays a typical intertidal zonation across a subtropical rocky shore, from the upper midlittoral (dominated by the barnacle *Chthamalus bisinuatus*) to the lower midlittoral zone (where patches of the mussel *Brachidontes solisianus* prevail). The competent larval stages comprise the settling individuals.

STUDY AREAS: FROM THE ARCTIC TO THE TROPICS

I have sampled coastal shores spanning multiple latitudes (Fig. 5): (1) Arctic, in the western coast of Greenland, (2) Subarctic, in the southeastern coast of Canada, (3) Tropical, in the northwestern coast of Panama, and (4) Subtropical, in the southeastern coast of Brazil.

Figure 5. Map of sampling locations, (1) Nuuk, Greenland, (2) Antigonish, Canada, (3) Bocas del Toro, Panama, and (4) São Sebastião, Brazil. The multi-panel picture displays the rocky shores sampled at each location.

Field work was carried out at the Greenland Institute of Natural Resources (GINR, Nuuk), the Department of Biology of St. Francis Xavier University (StFX, Nova Scotia), the Smithsonian Tropical Research Institute’s Bocas del Toro Research Station (STRI, Panama), and the Center for Marine Biology of the University of São Paulo (CEBIMar, Brazil). Samples were analyzed at the Institute of Ocean Sciences of Rimouski (ISMER, Québec).

SPECIFIC OBJECTIVES & HYPOTHESES

The main objective of my doctoral research was to better understand the ecophysiological mechanisms underlying settlement dynamics of rocky shore foundation species (see conceptual model in Fig. 6). I was particularly interested in the pelago-benthic transition, i.e., larval settlement (Figs. 7,8). The transitional mechanisms (i.e., nutritional, physiological) preceding the colonization of the new benthic habitat affect the number of settlers and the performance of recently-metamorphosed juveniles, and therefore the input of new individuals to the adult populations. I argue that these mechanisms, still understudied, are a determinant of population dynamics of marine benthic organisms.

Pelagic larvae need to have enough energy to fuel metabolism and prepare for settlement and metamorphosis, which is a ubiquitous feature across types of nutritional development, i.e., planktotrophy and lecithotrophy. The dynamics of larval development under contrasting trophic environments may have consequences to observed settlement patterns in nature. My research helps to answer the general question:

How do plastic larval traits driven by contrasting trophic conditions shape natural populations?

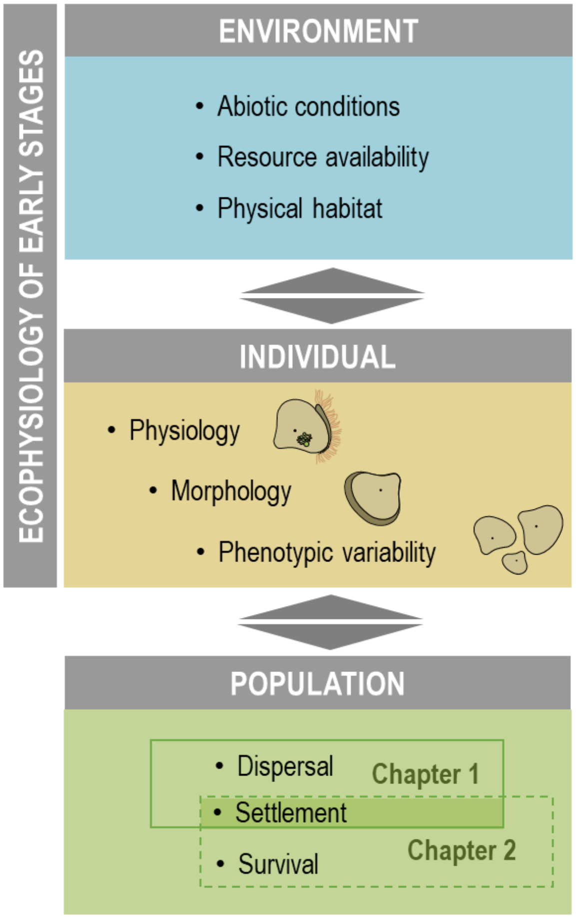


Figure 6. Conceptual model setting the scene for the thesis objectives. The physiological state of an individual is directly determined by the environment, and determines the population structure by affecting dispersal, settlement, and survival. Feedbacks among these will affect ecological dynamics at the population and community-level.

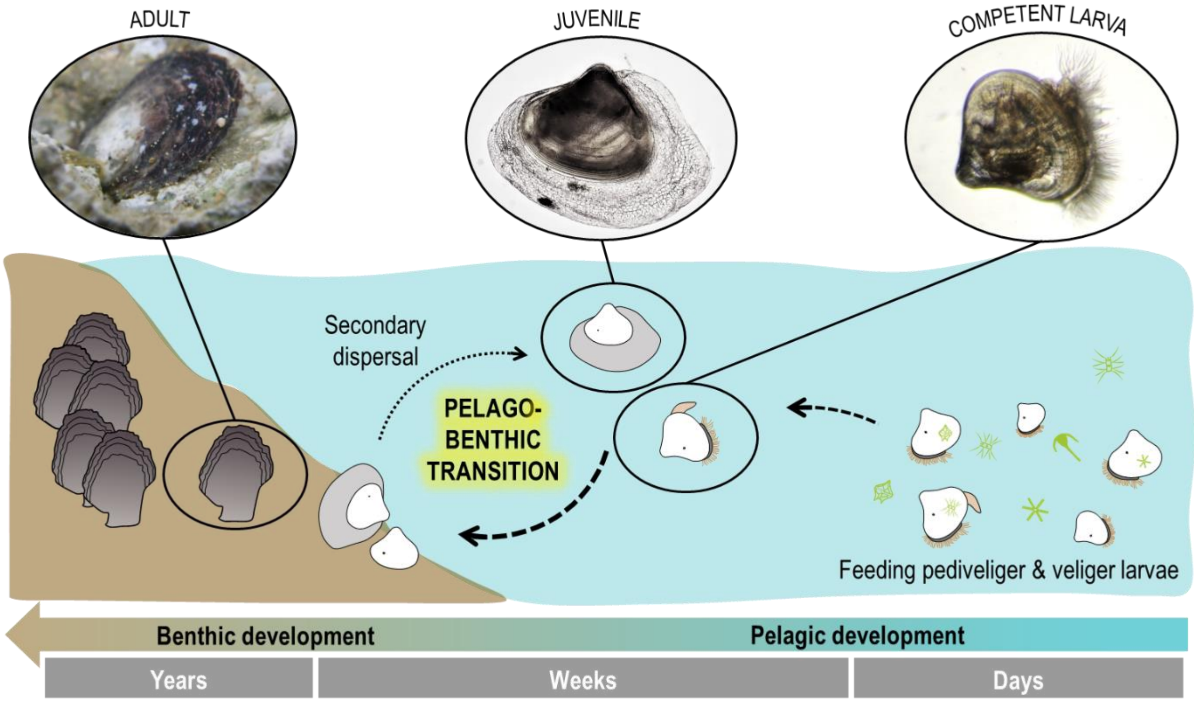


Figure 7. Conceptual scheme illustrating the pelago-benthic transition in planktotrophic bivalves, with underlying role of larval trophic ecology. The pediveliger (ped = foot) is the final stage prior to settlement and metamorphosis. The transition to the benthos is not quite definite in these taxa, with secondary migrations typically occurring as a small-scale dispersal mechanism after first settlement. The juvenile (or post-larva) and pediveliger (or competent larva) stages were the targets of the studies here presented. Photographs correspond to the tropical flat-tree oyster *Isognomon alatus*.

The studies in this chapter are founded on the trophic settlement trigger hypothesis elaborated by Toupoint et al. (2012a), which states that trophic resources rich in essential fatty acids trigger settlement in *Mytilus edulis* larvae. The body of research done in settlement dynamics mediated by trophic triggers of benthic bivalves has focused on temperate species (e.g., Toupoint et al., 2012a; Forêt et al., 2018a). In this chapter, I focus on low-latitude regions, typically nutrient-depleted year-round. In these areas, the lack of high-quality food sources may impose a challenge for competent planktotrophic larvae and early postlarvae.

These early-life stages may drift in the plankton for several months, and larger sizes at metamorphosis as well as pronounced secondary dispersal may be a red flag signaling exposure to suboptimal trophic conditions. I hypothesized the following:

(1) Under the effects of trophic triggers, settlers should be characterized by lower post-competency growth, i.e., restricted drifting, and lower variation of shell morphometrics, i.e., response of similarly-sized individuals. In the absence of such cues, a considerable fraction of larvae and postlarvae would remain adrift building up their shell size.

(2) Competent larvae that remain adrift in the plankton, i.e., delaying metamorphosis, are expected to display poorer physiological condition compared to those that colonize the benthos at smaller sizes, as they become energy-depleted due to increasing metabolic demands caused by juvenile tissue formation and/or patchy food resources.

These hypotheses were tested through different studies in Chapter 1. Settlement, nearshore larval supply, and environmental parameters were assessed at a fine temporal-scale (daily). This ecological data was coupled with morphometrics (i.e., Prodissoconch II [PII] and Dissoconch shell lengths) of settlers as well as physiological data derived from lipidomics (i.e., fatty acid profiles) of nearshore pelagic food sources and competent larvae, to understand the role of trophic triggers and larval nutritional status in mediating settlement. I used multivariate data analysis tools spanning Permutational Multivariate Analysis of Variance (PERMANOVA) to Principal Component Analysis (PCA) and Redundancy Analysis (RDA) to explain variability in the datasets. Altogether, the studies included in this chapter provide new insights into dispersal among pelagic stages (i.e., PII size variability), settlement (i.e., distinction between primary and secondary settlement), and food sources (i.e., fatty acid trophic markers) entering benthic food webs in tropical and subtropical shores.

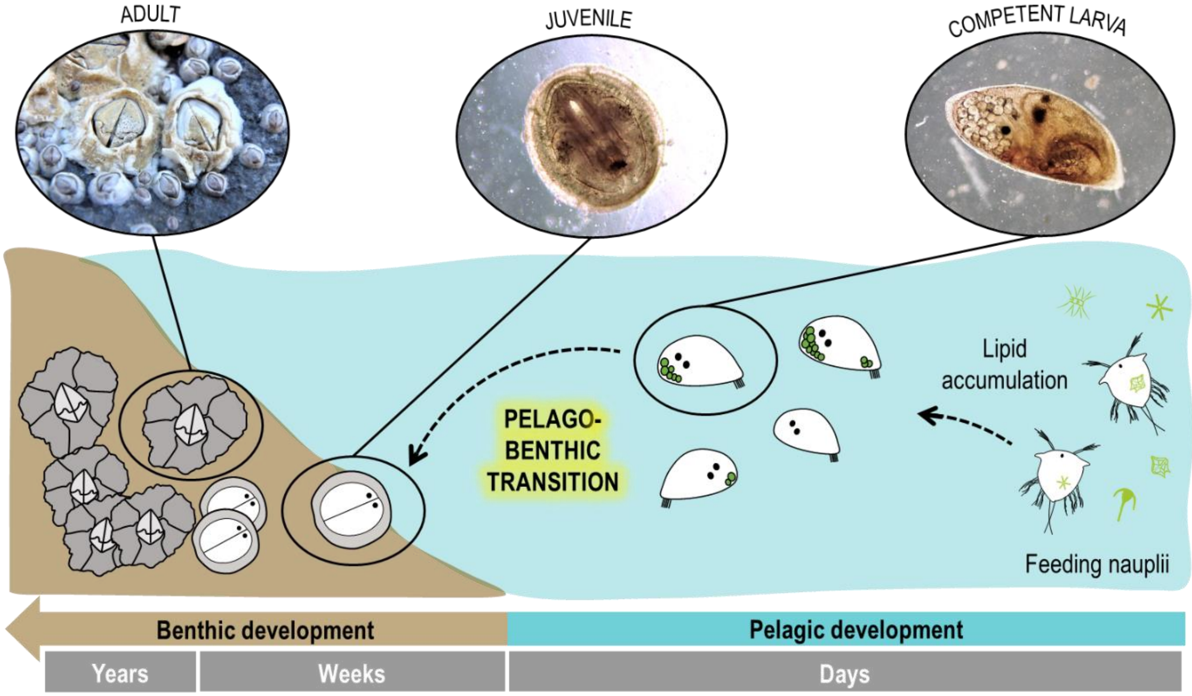


Figure 8. Conceptual scheme illustrating the pelago-benthic transition in lecithotrophic barnacles, with underlying role of larval energetic reserves. The non-feeding cyprid is the final larval stage, specialized for settlement and metamorphosis. The cyprid relies on the lipid accumulation taking place over the previous VI naupliar stages to fuel metamorphosis and permanent attachment to the benthos. The juvenile and cyprid (or competent larva) stages were the targets of the studies here presented. Photographs correspond to the acorn barnacle *Semibalanus balanoides*.

Non-feeding larval stages rely on stored energy reserves to power settlement and metamorphosis. Poor food supply may condition the resources available for maternal investment during embryonic development, and to planktonic feeding nauplii, so that the competent larvae that follow may be unable to overcome such deficiencies and fail to reach the energetic thresholds that allow them to undergo metamorphosis. Although advances have been made regarding late-stage barnacle larval energetic effects on settlement and post-settlement performance (e.g., Thiyagarajan et al., 2003; Tremblay et al., 2007b), little is known regarding how the accumulation of larval lipid reserves occurs under different trophic conditions. I hypothesized the following:

(1) The physiological condition of late-stage barnacle larvae should reflect the trophic environment in which the previous (naupliar) stages developed. Within the same population, variability in pelagic conditions should be visible in cohort-specific physiological status, so that poorer food supply would lead to cohorts with poorer larval physiological condition. Further, the physiological status of larvae (i.e., stored energetic reserves) should be reflected in their settlement and post-metamorphic performance.

(2) Late-stage barnacle larvae should remodel their lipids (energetic/structural) in the face of environmental constraints, i.e., selectively allocate essential fatty acids (EFAs) in their energy storage/cellular structure. The greatest the environmental stress, the greatest should larval effort in selectively retaining EFAs be. I would expect that lipid remodeling should be more pronounced in species inhabiting the tropics, that need to sustain high metabolic rates driven by higher temperatures while oligotrophic conditions during development simultaneously pose a challenge for lipid accumulation.

These hypotheses were tested through a series of studies in Chapter 2. This chapter includes multi-latitudinal/multi-species comparisons, spanning tropical, subtropical, temperate, subarctic, and arctic barnacle species. Similar to Chapter 1, settlement, larval supply, and environmental parameters were assessed at a fine temporal-scale (daily). This data was coupled with larval morphometrics (i.e., size, lipid droplets), and lipidomics (i.e., lipid classes and fatty acid profiles) of larval cohorts and nearshore dietary sources. As indices of physiological condition, I used the TAG/PL ratio, EFA ratios (larvae/diet), and lipid droplet cover (lipid droplet area/body area). Besides using multivariate analysis tools as PERMANOVA and PCA, I also used Generalized Linear Models to predict post-settlement responses. I conclude the chapter by presenting new findings on barnacle metamorphosis that might reflect “larval desperation”. Altogether, the findings of the studies presented in this chapter advance on how lipid allocation occurs late-stage barnacle larvae tissues, the strategies used by different species to tackle local environmental constraints, and the fatty acid signatures of food sources entering benthic food webs over different latitudes.

THESIS OUTLINE

This thesis summarizes my doctoral research, which has resulted in 7 scientific articles. The thesis follows a logical order, and whilst each chapter is comprised by separate scientific articles that can be reflected on independently, the body of work builds naturally to form a comprehensive analysis of the ecophysiological processes shaping settlement dynamics of rocky shore foundation species, as follows:

CHAPTER 1 PLANKTOTROPHIC BIVALVES

Article 1

Leal, I., Bouchard, É., Flores, A.A.V. and Tremblay, R., 2018. Trophic cues as possible triggers of mussel larval settlement in southeastern Brazil. *Aquatic Living Resources*, *31*, p.26.

Article 2

Leal, I., Tremblay, R. and Flores, A.A.V., *To be submitted to Oikos*. Allochthonous subsidies drive settlement of subtropical foundation species.

Article 3

Leal, I., Flores, A.A., Collin, R. and Tremblay, R., 2019. Drifting in the Caribbean: Hints from the intertidal bivalve *Isognomon alatus*. *Estuarine, Coastal and Shelf Science*, 227, p.106333.

CHAPTER 2 LECITHOTROPHIC BARNACLES

Article 4

Leal, I., Bohn, K., Hawkins, S.J., Jenkins, S.R., Flores, A.A.V. and Tremblay, R. *In Review in MEPS*. Lipid allocation in late-stage barnacle larvae from subtropical and temperate waters.

Article 5

Leal, I., Flores, A.A.V., Archambault, P., Sejr, M.K., Thyrring, J., Collin, R., Scrosati, A.R. and Tremblay, R. *To be submitted to Ecology*. Physiological lipid remodeling as a function of latitude in late-stage barnacle larvae.

Article 6

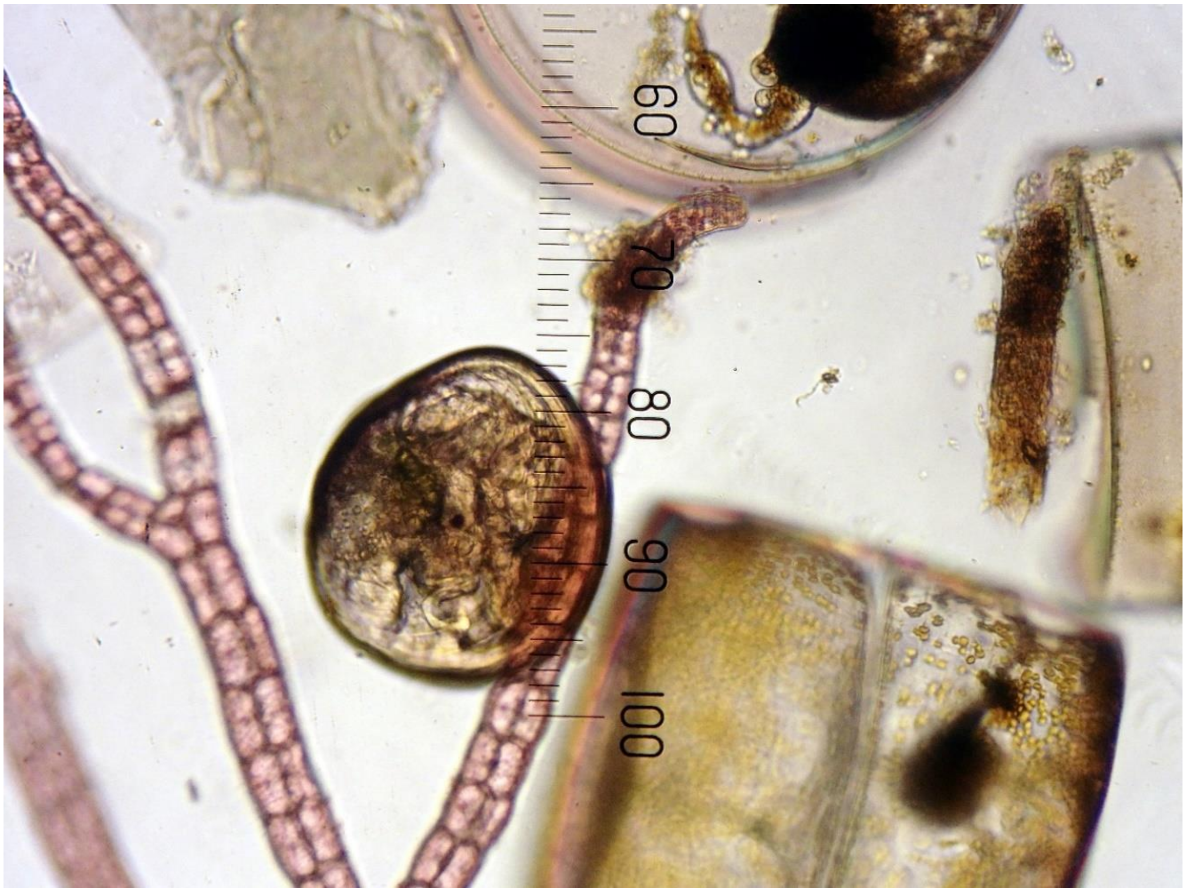
Leal, I., Flores, A.A., Archambault, P., Collin, R. and Tremblay, R., 2020. Response of tropical and subtropical chthamalid barnacles to increasing substrate temperatures. *Journal of Experimental Marine Biology and Ecology*, 524, p.151281.

Article 7

Leal, I., Flores, A.A.V., Scrosati, R.A. and Tremblay, R., 2020. Cyprid larvae of the acorn barnacle *Semibalanus balanoides* (Linnaeus, 1767) (Cirripedia: Sessilia: Archaeobalanidae) can metamorphose to juveniles without being permanently attached to a substrate. *The Journal of Crustacean Biology*, 40(2), pp.209-212.

CHAPTER 1

PLANKTOTROPHIC BIVALVES



Brachidontes solisianus pediveliger larva, sampled at the Center for Marine Biology of the University of São Paulo.

ARTICLE 1

TROPHIC CUES AS POSSIBLE TRIGGERS OF MUSSEL LARVAL SETTLEMENT IN SOUTHEASTERN BRAZIL

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1.1 ABSTRACT

Oligotrophic conditions may impose a nutritional challenge for the larval and early post-larval development of bivalves during the search for a suitable benthic habitat. Here we investigated what settlement cues might be important for mytilid populations in southeastern Brazil. Our results point to a trophic trigger mediating larval settlement that may include an effect of saturated fatty acids, probably linked to organic detritus and bacterial production deriving from terrestrial inputs. The prevalence of drifting in this population suggests it may be a strategy for individuals to delay final settlement until encountering favorable trophic environmental conditions.

Keywords: *Brachidontes solisianus*, Shell morphometry, Prodissoconch II, Dissoconch, Juvenile drifting

1.2 RÉSUMÉ

Les conditions oligotrophes peuvent imposer un défi nutritionnel pour le développement larvaire et post-larvaire précoce des bivalves lors de la recherche d’un habitat benthique approprié. Dans cette étude, nous avons examiné quels signaux environnementaux pourraient être importants pour la fixation des populations de mytilidés dans le sud-est du Brésil. Nos résultats indiquent un déclencheur trophique favorisant la fixation larvaire basé sur les acides gras saturés, probablement lié à des détritus organiques et à la production bactérienne provenant de sources terrestres. La prévalence des migrations secondaires, par dérive poste-larvaire, dans cette population suggère une stratégie de retard de l’établissement final des individus jusqu’à ce qu’ils rencontrent des conditions environnementales trophiques favorables.

Mots clés : *Brachidontes solisianus*, Morphométrie de la coquille, Prodissoconch II, Dissoconch, Dérive juvénile

1.3 INTRODUCTION

Most marine benthic invertebrates exhibit a complex life cycle that involves a planktonic larval phase followed by a benthic juvenile and adult phase (Thorson 1950). The duration of the planktonic phase may vary considerably, from only minutes to months (Pechenik 1990). Throughout this time, larvae may drift away from their place of origin, covering distances from meters to hundreds of kilometers (Jablonski and Lutz 1983; Pawlik 1992). Larvae need to become competent, i.e., fully-developed and able to metamorphose, before reaching the benthos (Pawlik 1992; Rodriguez et al. 1993). Frequently, the competent stage ends when larvae receive a highly specific settlement cue that indicates the presence of a suitable habitat (e.g., Satuito et al. 1997; Bishop et al. 2006a; Thiyagarajan 2010).

Larvae may respond to a series of stimuli (environmental cues) over the course of habitat selection, such as substratum texture and/or thermal capacity, presence of conspecifics, biofilms, among others (Pawlik, 1992; Rodriguez et al., 1993; Dobretsov 1999; Thiyagarajan, 2010). For example, the composition of the bacterial community of biofilms may inhibit or stimulate the attachment of benthic invertebrates (Olivier et al., 2000). For mytilid populations, biofilm-derived cues have been shown to act in a hierarchical order together with planktonic ones, the last being more influential if present (Toupoint et al., 2012b). Importantly, in the absence of such cues, larvae remain adrift in the plankton before metamorphosing to adult form, i.e., delaying metamorphosis (Pechenik 1990; Pawlik 1992). Recently, Martel et al. (2014) reported that *M. edulis* larvae typically delay metamorphosis in natural conditions c.a. 15 days. The authors found that a small size at metamorphosis, associated with shorter larval duration, resulted in greater settlement success rates. Indeed, delaying metamorphosis may be a “double-edged sword” due to a depletion of energetic reserves and consequent decrease in larval condition (Pechenik et al. 1993; Elkin and Marshall 2007). Yet, for planktotrophic larvae, it may be beneficial for a short-period of time; larvae may increase their energy reserves in the presence of a patch of high food resources and therefore increase their chance of finding a suitable adult habitat (Philips 2002).

The trophic conditions that larvae experience during their planktonic development may greatly influence juvenile performance (Pechenik et al. 1998; Philips 2002; Emlet and Sadro 2006; Thiyagarajan 2010). The lipid content of pre-metamorphic larvae has been shown to be one of the factors explaining settlement success of bivalves (pectinids, Pernet et al. 2004; mytilids, Rayssac et al. 2010). Toutpoint et al. (2012a) found that, over consecutive years, major settlement peaks of *Mytilus edulis* were synchronized with phytoplanktonic pulses rich in essential fatty acids. Based on this evidence, the authors proposed the "trophic settlement trigger" hypothesis, suggesting a bottom-up influence of primary production and dietary lipid quality on recruitment success (Toupoint et al. 2012a). Moreover, Martel et al. (2014) suggested that the recurring metamorphosis delays observed for the same species might be caused precisely by the absence of a suitable substrate or a specific trophic cue to trigger settlement.

Even weeks after the first settlement, early mussel settlers can re-suspend in the water column through drifting (e.g., byssus, threads) or crawling, a process called secondary dispersal (Lane et al. 1985; Armonies 1992; Shanks and Shearman 2011; Le Corre et al. 2013). An analogous behaviour found in terrestrial systems would be the "ballooning" activity for the dispersal of many juvenile spiders (e.g., Humphrey 1987) and in marine systems the mucous threads used for drifting by some gastropods (e.g., Martel and Chia 1991). Le Corre and co-authors (2013) reported that secondary dispersal of *Mytilus* spp. greatly contributed to local recruitment dynamics, with several peaks throughout the summer in a boreal estuary. Through secondary dispersal, post-larvae may select a more suitable adult habitat, possibly far from the initial settlement site, thus increasing their distributional range (Shanks and Shearman 2011). Despite its contribution to the population dynamics of an array of benthic invertebrates, the relative importance of post-larval drifting is not well documented in the literature and may be more common, at least for bivalves, than previously assumed (e.g., Martel and Chia 1991; Baker and Mann 1997; Le Corre et al. 2013).

The aim of this study was primarily to gain insights on bivalve settlement dynamics in a subtropical region, where populations of benthic invertebrates that dominate the upper shores are often subjected to low food supply conditions (Kasten and Flores 2013; Kasten et al. 2019a,b). Such conditions may impose a challenge for competent planktotrophic larvae and early post-larvae of intertidal bivalves during their search for a suitable benthic habitat, raising the question: what settlement cues might be acting in subtropical oligotrophic regions? Here, we addressed this question for mytilid populations in southeastern Brazil by 1) characterizing nearshore trophic conditions, and 2) examining shell variables of mussel post-larvae indicative of drifting. We expect that under the effects of settlement triggers, recruits will be characterized by lower post-competency growth, i.e., restricted drifting, and lower variation of shell morphometrics.

1.4 MATERIAL AND METHODS

Populations of the mytilid *Brachidontes solisianus* (Orbigny 1846) were sampled in the southeastern coast of Brazil. *Brachidontes solisuanus* dominates the mid intertidal zone of the rocky shores of São Paulo State, together with chthamalid barnacles (Petersen et al. 1986; Eston et al. 1986). During February and March 2015, samplings were carried out at 7 random dates at two different sites on the São Sebastião Channel (SSC): Baleeiro Head (23°49'45"S, 45°25'25"W), located in the enclosed CEBIMar area, and Feiticeira Beach (23°50'45.7"S, 45°24'34.79"W), located in Ilhabela. Samples were collected at the Center for Marine Biology of the University of São Paulo (CEBIMar/USP) and processed at the Institute of Ocean Sciences of Rimouski (ISMER/UQAR).

To characterize the trophic resources available for the seston-feeding post-larvae, three replicate samples (2 L per replicate) were obtained at each sampling day and site. Samples were filtered on a 20 µm mesh sieve, stored in opaque bottles and subsequently filtered on GF/F 25mm Whatman filters for estimation of particulate organic matter (POM; $mg \cdot L^{-1}$), mass of total fatty acids of seston (MTFA; $\mu g \cdot mg^{-1}$ POM) and its composition (% fatty acids), following the protocol described in Toupoint et al. (2012a). MTFA were further discriminated into saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. To test the variability in trophic conditions in time (days) and space (sites separated by a few km), two-way random effects ANOVAs were run on POM and MTFA data. Because SFAs made up most of the lipid contents (see results), this fraction was further decomposed into specific fatty acids. The analogous PERMANOVA routine was run to test for temporal and spatial variations. Euclidian distances and 9,999 permutations were used to calculate pseudo-*F* ratios. These analyses provided a measure of the consistency of seston SFA classes in the area.

To collect *Brachidontes solisianus* post-larvae, ten multifilament nylon scouring pads (tuffy pads) were randomly installed over a 100-m stretch along the lower midlittoral zone, where adults predominate. Tuffy pads were replaced daily at each site, and settlers extracted with a high-pressure jet of seawater into a 100 µm mesh sieve. After being identified under a stereomicroscope (Monteiro-Ribas et al. 2006), individual post-larvae were preserved for morphometric analyses, which included the estimation of total shell length, and its two components: prodissoconch II (PII) length and dissoconch length (µm) (following the method described in Martel et al. 1995, 2014). PII shell is secreted once the veliger can feed in the plankton and until the competent pediveliger metamorphoses into a post-larva (size at first settlement). Dissoconch shell is secreted after this first settlement, when the post-larva is crawling over the benthic habitat or re-suspended in the water column (here considered “drifting”). The ratio between the total shell length and PII length was used as a proxy of drift and was compared between sites and among daily cohorts. A total of 246 individual post-larvae were collected and measured. Multiple linear regression analyses were carried out to identify the environmental parameter(s) (POM, SFAs, PUFAs, MUFAs) with the highest explanatory power for the observed variation of post-metamorphic drifting. Mass of total fatty acids was collinear to the mass of SFAs and was not included in the analysis. No collinearity was observed between any other pairs of the variables tested ($r < |0.70|$; Dormann et al. 2011).

1.5 RESULTS AND DISCUSSION

Our results on the trophic status of the nearshore water column agree with previous studies indicating that the SCC is principally a meso-oligotrophic environment (Ciotti et al. 2010; Kasten and Flores 2013; Barbosa et al. 2016), considering the relatively low POM (1.02 ± 0.03 $mg \cdot L^{-1}$) and MTFA measured over this study (MTFA; 17.68 ± 1.06 $\mu g \cdot mg^{-1}$ POM). Overall, organic inputs (POM) showed some spatial coherence ($F_{site} = 1.11$; $p = 0.332$), with similar variation at both sites across time (Fig. 9A), as suggested by the lack of interactive site *vs*. date effects ($F_{dayXsite} = 1.00$, $p = 0.445$) in spite of significant overall temporal variation ($F_{day} = 4.50$, $p = 0.045$). However, the trophic quality of the organic inputs (as MTFAs) was markedly inconsistent across the spatial and temporal scales examined (Fig. 9B), with spatial contrasts found at some sampling dates but not in others ($F_{dayXsite} = 4.29$; $p = 0.003$). Such inconsistencies are typical of the effects of scattered and short-term oceanographic processes (1 to 2 days), leading to localized vertical mixing and relative surplus production above low baseline levels (Ciotti et al. 2010; Kasten and Flores 2013). In other words, while the overall quantity of potential food sources varied consistently through time at sites separated by a few km, the lipid budget, which has been shown to be critical for a number of invertebrate larvae (e.g., Phillips 2002; Pernet et al. 2004; Rayssac et al. 2010) is far more erratic.

A more detailed assessment of nearshore seston lipids shows that, compared to most other coastal systems studied worldwide (e.g., Toupoint et al. 2012a; Cabrol et al. 2015), the fraction of saturated fatty acids (SFAs) in the seston is stable and remarkably high (87 %; 15.47 ± 6 $\mu g \cdot mg^{-1}$ POM) and much higher than monounsaturated fatty acids (MUFAs; 1.07 ± 0.62 $\mu g \cdot mg^{-1}$ POM) and polyunsaturated fatty acids (PUFAs; 1.14 ± 0.63 $\mu g \cdot mg^{-1}$ POM) (Fig. 9B). The high concentrations of SFAs were attributed to the high contribution of 16:0 and 18:0 fatty acids (Fig. 9C), whose joint share remained around 91 % (ranging from 82 to 96 %), with differences between sites on some dates (PERMANOVA; pseudo-$F_{dayXsite} = 2.93$, $p = 0.002$). The amount of SFAs reported here differ considerably from those reported for temperate (40–70 %; Toupoint et al. 2012a) and subarctic systems (≈ 30 %; Cabrol et al.

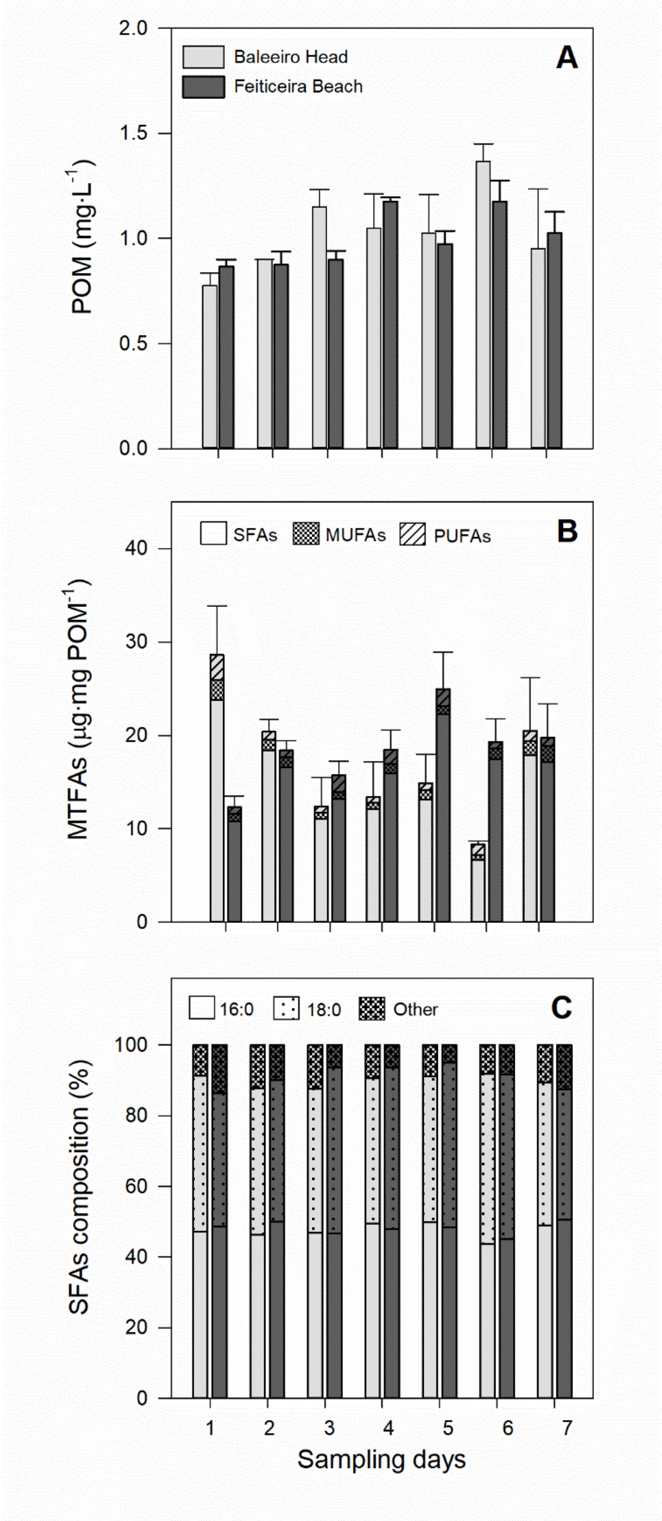

Figure 9. Trophic conditions in the São Sebastião Channel throughout the sampling period. A) Particulate organic matter (POM); B) Mass of total fatty acids (MTFA); C) Composition of seston’s saturated fatty acids (SFA). Values shown are averages and error bars are SE.

2015). The SFA-dominated lipid composition within our study area suggests that the main food sources for benthic suspension feeders in the SCC seemed to be bacteria and detritus, unlike several temperate productive systems, in which phytoplankton blooms make the bulk of primary production (Kelly and Scheibling 2012). We observed that fatty acid trophic markers (Parrish 2013) of diatoms (20:5n3) and dinoflagellates (22:6n3) represented each less than 2 % comparatively to the 5.9 % for fatty acids specific of bacteria (15:0, 17:0, 17:1 and 16:1n7) and 6 % fatty acids markers of mangroves, vascular and terrestrial plants (18:1n9, 18:2n6, 18:3n3, 24:0). Because sampling took place close to the end of the rainy season, it is probable that this heterotrophic production had its origin on land. Indeed, recent studies show that seasonal changes in fluvial forcing drive terrestrial inputs of nutrients and particulate organic matter in this system (Gorman et al. 2019).

Table 1. Summary statistics of the multiple linear regression analyses explaining the variation of juvenile drifting according to the trophic parameters of nearshore waters (particulate organic matter: POM; saturated: SFA; monounsaturated: MUFA; and polyunsaturated fatty acids: PUFA).

Variable	**Coefficient**	**Std. Error**	***t***	***p***
POM	-1.034	0.391	-2.64	**0.027**
SFAs	-0.058	0.016	-3.60	**0.006**
MUFAs	0.100	0.144	0.69	0.507
PUFAs	0.173	0.088	1.95	0.082
R-squared (R^2)	0.633	Sum of Squares (SS)	0.388	
Adj. R-squared	0.470	F-statistic	3.878	
SE of regression	0.158	Prob(F-statistic)	0.042	

Multiple regression analyses indicate that both POM and SFAs (not PUFAs or MUFAs), may possibly trigger settlement of *B. solisianus*, as assumed by the correlation of these variables with estimates of post-metamorphic drift ($R^2 = 0.63$; $p < 0.05$; Table 1; Fig. 10A, B). These trophic signals appear to be correlated with an early response of settlers within a narrow size range and limited post-metamorphic drift (% CV; $R^2 = 0.65$; $p < 0.001$; Fig. 10C). Based on previous studies reporting shell morphometry on different bivalve species (Martel et al. 2014), our results strongly suggest that post-larval drifting behavior is commonplace in the area. Decreasing coefficient of variation with increasing average estimates of drift (Fig. 10C) suggests that, in the presence of any positive settlement triggers, the whole larval pool in nearshore waters will respond and settle, while in the absence of such triggers only larvae that cannot further delay metamorphosis will settle. Assuming that nearshore populations of competent larvae would be comprised mostly by no- or early-drifters, settlers responding to triggers would be of very similar size. Differently, in the absence of such triggers, only long-term drifters, with remarkably different size, would respond and settle. Although there was a similar variation in shell morphometrics at both sites across time (except at Feiticeira at days 1 and 3; Fig. 11), dissoconch lengths ranged from 13 up to 2,000 µm. The importance of trophic processes in determining settlement in the meso-oligotrophic SSC has been already suggested for barnacles (Barbosa et al. 2016), but, so far, the origin and importance of seston nutrients remains unknown. The share of SFAs in lipid profiles has been shown to be higher in marine subtropical organisms compared to temperate or polar ones (Colombo et al. 2017). Specifically, organic detritus have been suggested to be an important energy source for mussels at times when primary production is scant (Rodhouse et al. 1984). High levels of 16:0 and 18:0 SFAs have been observed in rocky shore mytilids inhabiting environments abundant in detritic matter and bacterial load, presumably having a structural-type function (Galap et al. 1999; Freites et al. 2002). SFAs accumulation has also been shown to be important for zooplankton species (Cabrol et al. 2015). The authors suggested that accumulating SFAs may be advantageous under high energetic demands (e.g., demanding osmoregulatory processes), given their efficient oxidation and high energy yield.

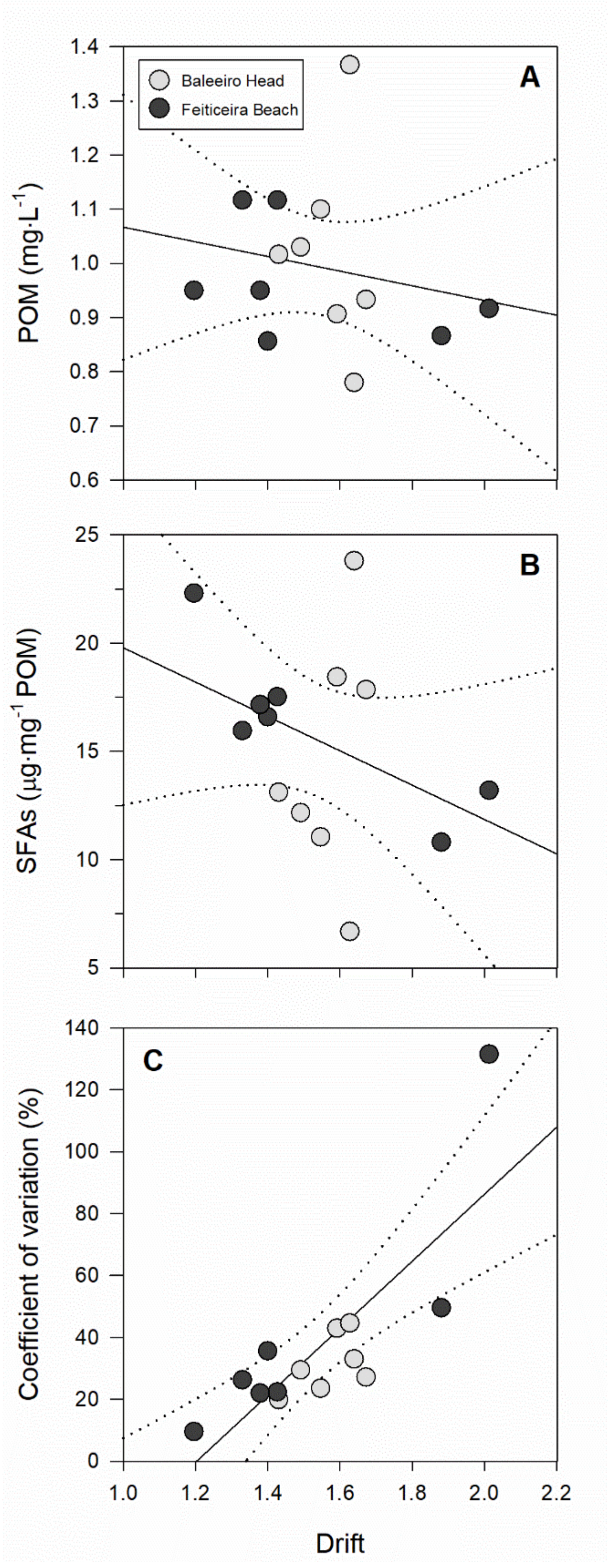

Figure 10. Variables explaining juvenile drifting behaviour: A) Particulate organic matter (POM), B) Saturated fatty acids (SFA). Panel C depicts increasing variance with drift estimates. Dotted lines represent confidence intervals (95 %).

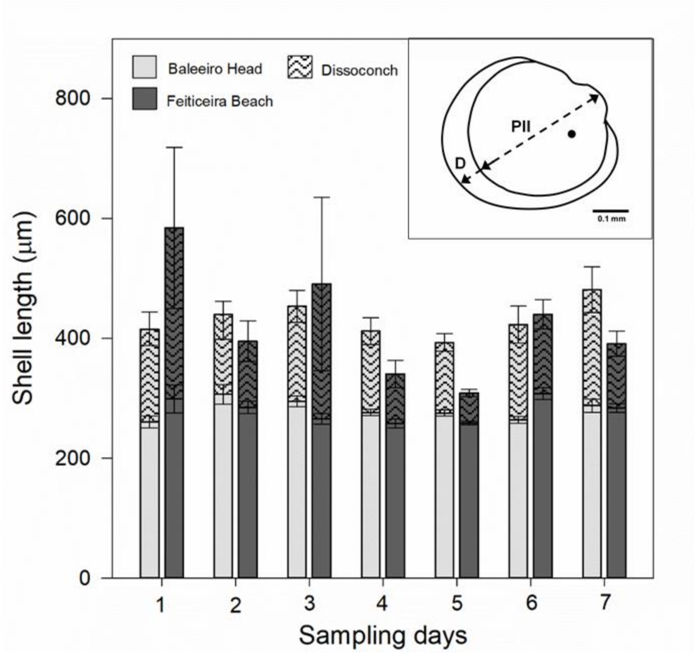

Figure 11. Shell morphometry of *Brachidontes solisianus* post-larvae at Baleeiro Head and Feitieira Beach throughout the sampling period. Averages and respective SEs are shown for prodissoconch II (PII) and dissoconch (D) shells (marked in lined pattern). The indent shows the delimitation of both shells drawn from an image of a primary *B. solisianus* settler.

In oligotrophic conditions, patches of high quantity and quality of food may thus be critical for the initial growth of post-larvae. Other trophic cues have been shown to mediate settlement in other mytilids. For instance, picoplankton species involved in the diet of competent mussel larvae (pediveligers) act as a trophic settlement trigger for *Mytilus edulis* (Jolivet et al. 2016). Here, we propose that SFAs may play a similar role, inducing larval settlement in the subtropical mussel *B. solisianus* by signaling a favorable benthic environment on a typically nutrient-depleted region. Such cues appear to derive from terrestrial inputs, which have been suggested to be an important energetic subsidy to the nearshore food webs in this area (Gorman et al. 2019). Yet, planktonic-derived cues (i.e., phytoplanktonic exoproducts) have previously been suggested as chemical cues potentially acting in the settlement of other mytilids (Toupoint et al. 2012a). Thus, one should not overlook the multitude of cues that may influence (inducing or inhibiting) the settlement of benthic invertebrates (i.e., conspecifics, biofilm, substrate characteristics), that were not investigated here. Other cues, together with planktonic-derived ones (i.e., nutritional cues), may well stimulate the settlement of *B. solisianus* in this subtropical region. This study presents preliminary evidence suggesting the importance of SFAs as a trophic trigger, but other stimuli not addressed here may be important. Future studies should consider the interplay of trophic and non-trophic signaling in the mediation of larval settlement of marine benthic invertebrates.

1.6 CONCLUSION

We present results that preliminary support the hypothesis of a trophic (i.e., nutritional) trigger mediating larval settlement of the subtropical mytilid *B. solisianus*. It is important to note, however, that the evidence we present here is correlational, not necessarily involving a cause-effect relationship. Such a trigger may include an effect of SFAs, commonly linked to organic detritus and bacterial production, possibly acting together with other local cues (i.e., presence of conspecifics and/or biofilm). In the absence of these cues, a considerable fraction of settlers were late post-larvae that probably remained adrift for some time (building up a shell size of up to 2 mm, as reported). The prevalence of drifting suggests this may be a strategy for individuals to delay final settlement until encountering favorable trophic conditions, which may be particularly adaptive in meso to oligotrophic environments. From an ecological perspective, drifting may also be an alternative means of transportation, allowing for benthic species to better exploit available habitat, escape adverse conditions and potentially colonize new areas (Martel and Chia 1991). Further work should aim longer term sampling to better assess seasonal trends of nearshore trophic conditions that may mediate juvenile drifting and settlement in this region.

1.7 ACKNOWLEDGMENTS

We sincerely thank Nathalie Gauthier for all assistance during field work and morphometric analyses and Jean-Bruno Nadalini and Mathieu Babin for their help and expertise with lipid analyses (UQAR/ISMER). This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery grant awarded to Réjean Tremblay).

ARTICLE 2

ALLOCHTHONOUS SUBSIDIES DRIVE SETTLEMENT OF SUBTROPICAL FOUNDATION SPECIES

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TO BE SUBMITTED TO OIKOS

[RESEARCH ARTICLE]

2.1 ABSTRACT

Terrestrial nutrients flushed to sea via coastal runoff may provide a trophic subsidy to filter-feeders, which might be particularly relevant in oligotrophic waters. In this study, we investigated how allochthonous subsidies might modulate the larval physiology, dispersal, and settlement of the mytilid *Brachidontes solisianus* by comparing wet and dry periods in Southeastern Brazil. Analysis of long-term precipitation data revealed that rainfall events of comparable and large intensity (> 10 mm) occurred more frequently from February to April (annual average of 1,268 mm). These rainfall events were strongly correlated with nearshore fatty acid trophic markers of terrestrial origin, as detected by redundancy analyzes, which also identified these as drivers of *B. solisianus* primary settlement. The portion of terrestrial matter entering benthic food webs was considerable (10-13 % of suspended matter [TSM]), and together with detrital matter (> 50 % TSM) these sources contributed to most of the dissimilarity between periods, and appear to play a predominant role in the trophic ecology of *B. solisianus* larvae and postlarvae via coastal plumes. During dry conditions (1) *B. solisianus* larvae selectively incorporated the essential 22:6ω3 docosahexaenoic acid (DHA) into membrane phospholipids at the expense of reserve lipids, indicative of a potential dietary deficit, and (2) secondary settlement (drifting and reattachment of postlarvae) prevailed. We propose that precipitation-driven allochthonous subsidies modulate settlement and dispersal of this subtropical mytilid. These pulsed natural disturbances majorly control energy sources entering Brazilian benthic food webs at the land-ocean interface.

Keywords: Habitat coupling, Fatty acid trophic markers, Detrital food webs, Larval physiology, Shell morphology, Secondary dispersal, Southeast Brazil

2.2 RÉSUMÉ

Les apports d’origine terrestre déversés dans les eaux côtières par les pluies peuvent fournir une source d’énergie aux consommateurs marins, ce qui pourrait être particulièrement pertinent dans les zones côtières peu productives. Dans cette étude, nous avons étudié comment les apports allochtones impactent la physiologie, la dispersion et la fixation des larves de l’espèce de mytilidé *Brachidontes solisianus* en comparant des périodes de pluies et de sècheresses dans le sud-est du Brésil. Les données sur les précipitations à long terme ont révélé que des événements pluviométriques sporadiques d’une intensité comparable et élevée (> 10 mm) se produisent plus fréquemment de février à avril (moyenne annuelle de 1,268 mm). Ces événements pluviométriques étaient fortement corrélés avec les marqueurs trophiques d’acides gras d’origine terrestre, tels que détectés par les analyses de redondance, qui les ont également identifiés comme des déclencheurs de fixation primaire de *B. solisianus*. La part de matière terrestre entrant dans les réseaux trophiques benthiques était considérable (10 à 13 % de la matière organique en suspension [TSM] près du littoral), et avec la matière détritique (> 50 % de TSM), ces sources ont contribué à la majeure partie de la dissemblance entre les périodes, et semblent jouer un rôle prédominant dans l’écologie trophique des larves de *B. solisianus* et des postlarves via des plumes organiques côtiers. En conditions sèches (1) les larves de *B. solisianus* incorporaient sélectivement l’acide docosahexaénoïque 22:6ω3 (DHA) dans les phospholipides membranaires au détriment des lipides de réserve, indiquant un déficit alimentaire potentiel, et (2) la migration secondaire (c.à.d. dérive et réattachement des postlarves) prévalait. Nous proposons que les apports nutritionnels allochtones induites par les pluies modulent la fixation et la dispersion de ce mytilidé subtropical. Ces perturbations naturelles contrôlent principalement les sources d’énergie qui pénètrent dans les réseaux trophiques benthiques brésiliens à l’interface terre-océan.

Mots clés : Couplage terre-océan, Marqueurs trophiques, Réseaux trophiques détritiques, Physiologie larvaire, Dérive juvénile, Sud-est du Brésil

2.3 INTRODUCTION

Marine and terrestrial ecosystems are connected via transfers of nutrients and organic matter. In coastal areas, freshwater outflows create prominent turbidity plumes, which are areas of high biological activity in the pelagos (Schlacher et al., 2009). Zooplankton production in plume areas can be supported via a trophic pathway linked directly to the incorporation of terrestrial organic matter and/or via the assimilation of fresh carbon fixed by marine producers following nutrient stimulation (Schlacher et al., 2009; Gorman et al., 2019). Shifts in the relative influence of terrestrial and oceanic sources have been described in several tropical and subtropical regions receiving high rainfall (Meziane and Tsuchiya, 2000; Wai et al., 2008; Gorman et al., 2017). These sources can be highly variable in low-latitude ecosystems, both seasonally (e.g., monsoonal tropics; Wai et al., 2008) and daily (e.g., subtropical shores; Leal et al., 2018) and affect the trophic base of assemblages relying on pelagic food chains.

Fatty acid signatures have long been used as tracers to show how organic matter from a variety of origins can serve as an energy source for marine communities (e.g., mangrove forests – Bachok et al., 2003; seagrass beds – Kharlamenko et al., 2001; saltmarshes – Meziane et al., 1997; macroalgae – De Cesare et al., 2017; terrestrial plants – Budge and Parrish, 1998). Terrestrially-derived subsidies (in the form of leaf litter and dissolved and/or particulate organic matter) are transported by streams to coastal waters, providing an additional source of energy to marine consumers (Wai et al., 2008). Trophic subsidies to subtidal systems have been linked to rainfall driven pulses (Gorman et al., 2009, 2017), which are particularly relevant within coastal areas of naturally low nutrient status (Burkholder et al., 2004; Herbeck et al., 2011). Storm-induced perturbations can cause a rapid export of freshwater, excess nutrients and total suspended matter (TSM) into nearshore regions, resulting in hyposalinity, reduced water transparency, siltation, as well as temporary eutrophication, with terrestrial matter as a major organic source of TSM (Valiela et al., 1998; Herbeck et al., 2011). Even though terrestrially-derived leaf litter can be utilized directly by macrozoobenthos, primary production can also be stimulated by nutrients available after fragmentation and heterotrophic biodegradation processes, i.e., recycled through detrital-based food webs (Wafar et al., 1997; Meziane and Tsuchiya, 2000). Cross-habitat flows of detrital subsidies often produce bottom-up effects in marine, freshwater, and terrestrial systems, with many consumers directly and indirectly heavily relying on terrestrially produced detritus as a major energy source (Polis et al., 1997). For instance, in the oligotrophic south-western Australian waters, growth of the mussel *Mytilus edulis* has been shown to be higher where detritus were present, which occurred predominantly close to shore (shallow areas < 15 m; Bearham et al., 2020).

Filter-feeding benthic invertebrates are an important link between the planktonic ecosystem and the rock-bound ecosystems close to shore, so their thriving response to natural subsidies is expected (Palumbi, 2003). These organisms are often ecosystem engineers and foundation species in many coastal communities (Jones et al., 1994; Gutierrez et al., 2003), and the stability of their populations strongly relies on supply of settlers from the plankton (e.g., Sigurdsson et al., 1976; Olivier and Retière, 1998; Forêt et al., 2018). The transition from the pelagos to the benthos represents a critical bottleneck in foundation species with a bi-phasic life cycle (Pineda et al., 2009; Nanninga and Berumen, 2014). If larvae fail to encounter a suitable substratum, they may postpone metamorphosis and examine additional substrata, influencing both dispersal and future juvenile survival (e.g., Crisp, 1974; Scheltema, 1971). The settlement process of most bivalves can be divided in two steps that operate over contrasting spatial scales: (1) initial attachment to a substratum (primary settlement), which takes place at the end of the larval phase and is shortly followed by metamorphosis to the postlarval stage, and (2) post-metamorphic resuspension and reattachment (secondary settlement). The latter can involve post-settlement movements (drifting or crawling) presumably to select a more suitable substratum or microhabitat (Sigurdsson et al., 1976; Olivier and Retière, 1998; Forêt et al., 2018). Accounting for the overall abundance of settlers, regardless of their size, may thus result in an overestimation of primary settlement (Le Corre et al., 2013) and compromise estimates of local recruitment. Further, the exchange or dispersal of individuals among patchy habitats plays a central role in spatial ecology and metapopulation dynamics (Nanninga and Berumen, 2014), and is particularly poorly understood in the tropics.

The mussel *Brachidontes solisianus* (Bivalvia, Mytilidae) is an intertidal foundation species across southeastern Brazilian coasts, attaching to the rock surface by stout byssal threads and occasionally colonizing shallow subtidal habitats (< 3 m; Monteiro-Ribas et al., 2006). Trophic, i.e., nutritional, triggers seem to mediate larval settlement in subtropical populations of *B. solisianus* (Leal et al., 2018). Such triggers comprise organic matter rich in saturated fatty acids (SFA), commonly linked to organic detritus and bacterial production (e.g., Dalsgaard et al., 2003; Parrish, 2013), that presumably signal a favorable benthic environment on a typically nutrient-depleted region (Leal et al., 2018). The SFA-dominated lipid composition of southeastern Brazilian nearshore waters suggests that the main food sources for benthic suspension feeders derives from a detrital pathway, unlike several temperate productive systems in which phytoplankton blooms provide the bulk of dietary sources for filter-feeders (e.g., Toupoint et al., 2012; Perez et al., 2013; Cabrol et al., 2015). However, the origin of such direct trophic subsidies (i.e., fluxes of energy, detritus, or organisms from productive to less productive habitats; *sensu* Polis et al., 1997) and role in shaping nearshore benthic communities is largely unknown.

In this study, we tested the hypothesis that allochthonous subsidies resulting from pulses of rainfall mediate settlement dynamics of the mytilid *B. solisianus*. In the subtropical coast of Brazil, rainfall events wash considerable amounts of phytodetritus and organic matter to nearshore waters (Gorman et al., 2019). We carried out a temporal comparison spanning wet and dry periods, to ascertain how such subsidies might modulate larval physiology, dispersal, and settlement in this population. By integrating lipidomic and morphometric tools, we aimed to characterize (1) the fatty acid trophic markers in nearshore Brazilian waters (i.e., nutrients available to TSM-feeding larvae and postlarvae), (2) the nutritional status of *B. solisianus* pediveliger larvae (i.e., level of incorporation of essential fatty acids into membrane phospholipids at the expense of reserve lipids), and (3) the settlement and drifting dynamics in the population (i.e., counts and morphometry of primary and secondary postlarval settlers). We hypothesized that under the effects of allochthonous subsidies, arguably more abundant after rainfall events, postlarvae will be characterized by lower size at settlement and metamorphosis and restricted post-metamorphic drift. Terrestrial inputs should trigger the response of similarly-sized *B. solisianus* postlarvae (low intraspecific variation). In the absence of such cues, expected to occur during drier periods, a considerable fraction of settlers would be late postlarvae that remained adrift building up shell size (reported to reach up to 2 mm; Leal et al., 2018), which should be corroborated by lower physiological state of pre-metamorphic individuals. Our study provides novel insights into the trophic resources entering benthic food webs and the settlement drivers shaping *B. solisianus* populations inhabiting a tightly coupled land–ocean interface.

2.4 MATERIAL AND METHODS

2.4.1 Study design, area, and species

The mytilid *Brachidontes solisianus* (Orbigny, 1846) was sampled from the southeastern coast of Brazil. Over 12 consecutive days in late March 2018 and late October 2016, two rocky shores were sampled daily: Baleeiro Head (Site 1; 23°49'46.44"S, 45°25'24.96"W) and Jarobá (Site 2; 23°49'42.38"S, 45°25'18.55"W). Facing the São Sebastião Channel (SSC; northern coast of São Paulo State), these shores are characterized by a steep rocky habitat. *Brachidontes solisianus* dominates the lower mid-intertidal zone, forming patches interspersed by the barnacles *Chthamalus bisinuatus* and *Tetraclita stalactifera* (Petersen et al., 1986; Eston et al., 1986). The SSC is principally a meso-oligotrophic system year-round, with high-quality nutrients varying markedly in space and time (Gianesella and Saldanha-Correa, 2008; Leal et al. 2018). Such inconsistencies are typical of the effects of scattered and short-term oceanographic processes (1–2 days), leading to localized vertical mixing and relative surplus production above low baseline levels (Ciotti et al., 2010; Kasten and Flores, 2013). In other words, while the overall quantity of potential food sources varies consistently through time at sites separated by a few km (Leal et al., 2018), the quality of food, i.e., lipid content of organic matter, is far more erratic. Saturated fatty acids make up most of the dietary lipid content of organic matter at these subtropical shores (Leal et al., 2018), with fatty acid trophic markers indicating the predominance of organic detritus and bacterial production. This detrital mater likely has its origin on land, given the land–ocean connectivity found in this shoreline (Fig. 12).

Figure 12. Shoreline of Baleeiro Head in the São Sebastião Channel showcasing the land–ocean connectivity found throughout Brazilian subtropical shores.

Samples were collected at the Center for Marine Biology of the University of São Paulo (CEBIMar/USP), preserved at -80 °C, and processed at the Institute of Ocean Sciences of Rimouski (ISMER/UQAR). Daily rainfall measurements (mm) were provided by the CEBIMar/USP meteorological station located in Baleeiro Head, that collects data each 10 minutes at 18-meter altitude since 2013. This allowed to gauge the long-term seasonal patterns in these subtropical shores (Fig. 13), as well as identify heavy rainfall events throughout the sampling periods causing the most extensive nearshore organic-rich plumes, i.e., trophic subsidies.

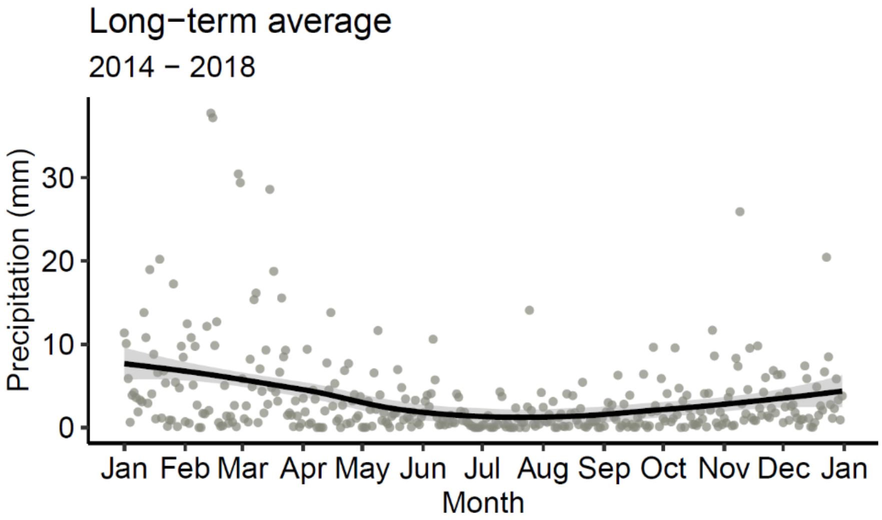


Figure 13. Long-term average of daily rainfall found at the sampled subtropical shores plotted with data provided by the CEBIMar/USP meteorological station spanning 5 years (2014 to 2018), and fitted smoothing spline (black line) showing the yearly rainfall trend.

2.4.2 Shell morphometrics

We collected early-stage individuals of *B. solisianus*, both pelagic and benthic, to understand the settlement and drifting dynamics of this population. The end of the planktonic larval stage in bivalves is marked by a change in shell morphology after metamorphosis, creating a distinct visual demarcation between the prodissoconch II (PII, or larval shell) and the dissoconch (D, or benthic shell) (Martel et al., 1995, 2014), providing valuable information about the larval phase history, especially the size at metamorphosis (i.e., at the time of primary settlement). Pediveligers were identified by the presence of the ciliated velum, well-defined foot, and visible eyespot. Postlarvae were identified by the presence of a retracted velum, fully-developed differentiated gills, and dissoconch shell, which grows as concentric wrinkles building up along its edge. Individuals were identified to species according to Monteiro-Ribas et al. (2006).

A total of 1,045 individual pediveligers and postlarvae (*n* = 635 in March, *n* = 410 in October) were photographed alive after collection under a Nikon microscope (100 ×) and shell measurements made using the software Fiji, an open-source platform for scientific image analysis (Schindelin et al., 2012). Morphometric analyses included the estimation of total shell length, and its two components: prodissoconch II (PII) length and dissoconch length (µm). The PII shell is secreted when the veliger larva begins to feed in the plankton and until the pediveliger metamorphoses into a postlarva, thus indicating the size at settlement and metamorphosis as measured by the longest distance across the anteroposterior axis ending at the PII–dissoconch boundary (Martel et al., 1995, 2014). The dissoconch shell is secreted after settlement and metamorphosis, when the postlarva is crawling over the benthic habitat or re-suspended in the water column (i.e., “drifting”). The ratio between the total shell length and PII length was used as a proxy of drift (herein Drift ratio).

2.4.2.1 Collection of pelagic stages

Pelagic pediveliger larvae and postlarval drifters were sampled from the nearshore sub-surface waters at each diurnal low tide. A 150 µm plankton net (30 cm of net mouth diameter) was towed just below the surface, for a 100-m long-shore transect. A subset of collected pediveligers (*n* = 20 per day) were photographed alive to estimate larval shell length (µm), and preserved in dichloromethane-methanol (2:1, v/v) at -80 °C to later estimate their nutritional status by lipidomic analysis (see below “Lipidomics”). Postlarval specimens drifting the plankton were photographed alive for posterior shell measurements.

2.4.2.2 Collection of settlers

To collect *B. solisianus* settlers, artificial collectors (multifilament nylon pads, SOS ® Products; Menge et al., 1994) were randomly installed (*n* = 10) over a 100-m stretch along the midlittoral zone, where adults predominate. Artificial collectors were placed under running seawater for 1-week prior sampling to allow for biofilm colonization. At the peak of each diurnal low tide, collectors were removed and replaced. Settlers were extracted with a soft jet of seawater into a 100 µm mesh sieve, counted, and photographed alive for later measurements of shell morphology. To avoid overestimating settlement rates, settlers were distinguished between (1) primary settlers, non-fully metamorphosed pediveligers, and (2) secondary settlers, postlarvae with a clear dissoconch shell and newly differentiated gill filaments, which were previously adrift.

2.4.3 Lipidomics

2.4.3.1 Nearshore food sources

To characterize the trophic resources available for the seston-feeding larvae and postlarvae, lipidomic analysis were carried out on total suspended matter (TSM; mg/L) collected from nearshore waters. Replicate ($n = 6$) 2 L seawater samples were collected daily during low tide, filtered through a 100 µm mesh sieve (i.e., the minimal approximate size of the velum for *B. solisianus* pediveliger; I. Leal, pers. obs.), and then through Whatman® glass microfiber filters (GF/F 25 mm, pore size 0.7 µm) preburnt (450 ^{o}C for 2 h) and weighted. Three glass microfiber filters were rinsed with 0.3-M Ammonium formate to dissolve residual salts (Aminot and Chaussepied, 1983), and subsequently heated at 70 °C for 24 h and weighted for estimation of TSM. Three other glass microfiber filters were stored at -80 °C, in dichloromethane-methanol vials (2:1, v/v) for lipid analysis.

Fatty acids (FA) were extracted through direct transesterification (Lepage and Roy, 1984) and analyzed by Gas Chromatography–Mass Spectrometry (GC-MS), to determine the mass of total fatty acids (MTFA; µg/mg TSM) and its composition (% FA), following the protocol described in Toupoint et al., 2012. We used a full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled to a multichannel gas chromatograph “Trace GC ultra” (Thermo Scientific) equipped with an autosampler (model Triplus), a PTV injector and a mass detector model ITQ900 (Thermo Scientific). An Omegawax 250 (Supelco) capillary column was used for separation with high purity helium as a carrier gas. Data were treated using Xcalibur v.2.1 software (Thermo Scientific) and FA identified and quantified with standards Supelco 37. FA were further discriminated into saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids. Fatty acid trophic markers (FATM) considered in this study were attributed to seven trophic sources (bacteria, detritus, terrestrial plants, macroalgae, terrestrial matter, diatoms, and dinoflagellates; Table 2) according to published research on FATM (Budge and Parrish, 1998; Dalsgaard et al., 2003; Kelly and Scheibling, 2012; Parrish, 2013).

Table 2. List of fatty acid trophic markers used in this study, and respective abundance (%) in suspended matter collected from nearshore waters. Precursors and Essential Fatty Acids: Linoleic acid (LOA), α-Linolenic acid (LNA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). *n* stands for the number of samples analysed.

Fatty Acid	**Marker of**	**Mean ± SE**	
		March (*n* = 65)	October (*n* = 44)
∑15:0, 15:1, 17:0, 17:1	Bacteria	1.4 % ± 0.1	1.7 % ± 0.0
∑14:0, 16:0, 18:0	Detritus	46.5 % ± 0.5	48.8 % ± 0.9
	Detrital	**47.9 % ± 0.6**	**51.5 % ± 0.9**
∑22:0, 24:0	Terrestrial plants	3.6 % ± 0.2	3.4 % ± 0.1
∑18:2ω6 (LOA), 18:3ω3 (LNA)	Terrestrial matter	9.4 % ± 0.2	6.9 % ± 0.3
	Terrestrial	**12.9 % ± 0.2**	**9.5 % ± 0.3**
∑18:4ω3, 16:4ω3	Green Macroalgae	0.0 % ± 0.0	0.0 % ± 0.0
∑18:1ω9, 18:3ω6	Brown Macroalgae	13.5 % ± 0.3	11.9 % ± 0.4
	Macroalgal	**13.5 % ± 0.3**	**11.9 % ± 0.4**
∑16:1, 20:5ω3 (EPA)	**Diatoms**	**16.2 % ± 0.2**	**17.6 % ± 0.5**
22:6ω3 (DHA)	**Dinoflagellates**	**3.2 % ± 0.1**	**3.1 % ± 0.1**

2.4.3.2 Larval lipidic composition

To characterize the lipidic composition of daily larval cohorts, pediveligers collected from nearshore waters (*n* = 20 pediveligers, per day) were pooled for lipid analysis (*n* = 3 pooled weekly samples, per site). Given the ability of larvae to transfer fatty acids, and thus utilize lipids without affecting its levels (Giese, 1966), we examined the fractions (i.e., type of lipid) present in the total lipid content of larvae. FA were extracted by a modified Folch procedure (Folch et al., 1957) and the neutral fraction (i.e., energetic fraction, including triglycerides, free FA, but also sterols) was separated from the polar fraction (i.e., structural fraction, composed mainly of phospholipids) (Marty et al., 1992). To eliminate free sterols in the neutral fraction, samples were purified on an activated silica gel with 1 mL of hexane:ethyl acetate (v/v) (Cabrol et al., 2015). FA were analyzed with the GC-MS, as above, to determine the total lipid content in individual larvae (sum of all fatty acids identified; μg/larva) and its composition (% FA, further discriminated into SFA, MUFA, PUFA, EFA).

2.4.3.3 Larval nutritional status: selective EFA retention

Neutral lipids closely reflect short-term variability in available dietary fatty acids, whereas polar lipids are more conservative, reflecting the long-term physiological uptake of dietary fatty acids (Delaunay et al., 1993; Freites et al., 2002). When dietary sources are deficient in the EFA needed to sustain larval physiological needs, particularly for membrane phospholipid building, invertebrate larvae are known to selectively incorporate EFA from the readily-available triacylglycerols in their lipid droplets (Tremblay et al., 2007), through a process known as selective fatty acid retention (Pernet et al., 2005; Thériault and Pernet, 2007; Gendron et al., 2013; Martinez-Silva et al., 2018). If the relative proportion of a given dietary fatty acid in larvae/diet is equal to or less than 1, then the larval requirement for that fatty acid is presumably satisfied. If the relative proportion of fatty acid in larvae/diet is higher than 1, then that fatty acid is selectively incorporated by larvae. Selective incorporation of EFA into membrane phospholipids at the expense of reserve, neutral lipids (i.e., the proportion of EFA in the polar lipids higher than in the neutral lipids and diet; Thériault and Pernet, 2007; Gendron et al., 2013) was tested through a ratio between larval fatty acids and dietary fatty acids, for the essential arachidonic acid (ARA; 20:4ω6), eicosapentaenoic acid (EPA; 20:5ω3), and docosahexaenoic acid (DHA; 22:6ω3), as well as precursors α-linolenic acid (LNA; 18:3ω3), and linoleic acid (LOA; 18:2ω6). The absence of selective retention of EFAs in the cell membrane (ratio < 1) suggests a potential better larval condition (Gendron et al., 2013).

2.4.4 Statistical analyses

Permutational multivariate analysis of variance (PERMANOVA) using PRIMER (v. 7.0.13) was used to make temporal (fixed factor, 2 levels: March, October) and spatial comparisons (fixed factor, 2 levels: Site 1, Site 2) of the FATM present in nearshore suspended matter (detrital, comprising bacteria [∑15:0, 15:1, 17:0, 17:1] and detritus [∑14:0, 16:0, 18:0]; macroalgal [∑18:1ω9, 18:3ω6]; terrestrial, comprising terrestrial plants [∑22:0, 24:0] and terrestrial matter [∑18:2ω6, 18:3ω3]; dinoflagellates [22:6ω3]; diatoms [∑16:1, 20:5ω3]). The analysis was conducted with fatty acids expressed as percentages (% arcsin square-root transformed) using a non-parametric distance-based PERMANOVA based on Bray-Curtis similarities, and 9,999 permutations to calculate pseudo-*F* ratios. Homogeneity was evaluated using the permutation analysis of multivariate dispersion (PERMDISP) (Anderson 2001). Similarity percentage analysis (SIMPER) (Clarke, 1993) was performed on a Bray–Curtis matrix of untransformed data to identify the markers responsible for dissimilarities between months.

To investigate how the surrounding abiotic environment explained (1) available nearshore food sources, given by FATM (%), and (2) settlement dynamics, differentiating between primary and secondary *B. solisianus* settlers, we used redundancy analysis. Redundancy analysis (RDA) is a method to extract and summarize the variation in a set of response variables that can be explained by a set of explanatory variables. It is a direct extension of regression analysis to model multivariate response data (Borcard et al., 2011). The linear correlation between the PCA ordination and the independent environmental parameters was investigated using the *envfit* function of the R package vegan (Oksanen et al., 2019). The angles between variables reflect their correlations, and the arrows length is proportional to the correlation coefficient. The significance of each correlation was assessed by permutation tests and only the variables with *p*-value < 0.05 were displayed.

To test the variability in settlement dynamics in time (days) and space (sites), balanced two-way ANOVAs were run on the response variables size at settlement (postlarval PII shell length) and Drift ratio (postlarval PII/Total length). To visualize the shell morphometrics of *B. solisianus*, size-frequency distributions of postlarvae were plotted.

The R software (version 3.6.1; Core Team, 2019) and packages vegan (Oksanen et al., 2019), ggplot2 (Wickham, 2016), and ggpubr (Kassambara, 2019) were used to conduct statistical analyses and as graphical tools.

2.5 RESULTS

2.5.1 Nearshore conditions

Long-term averages of precipitation found at the sampled subtropical shores (Fig. 13) show that rainfall events are common throughout the year (average annual rainfall of 1,268 mm). However, days with exceedingly higher precipitation rates (> 10 mm) occur more frequently ($p = 0.002$; $U = 3510.0$) from February to April (14 days out of 90), spanning the end of summer and the onset of autumn, compared to September through November (2 days out of 90), comprising the end of winter and onset of spring. These events comprise the ones of most interest since they presumably cause the most extensive nearshore plumes.

Abiotic and biotic conditions found throughout the sampling periods can be found in Figure 14.

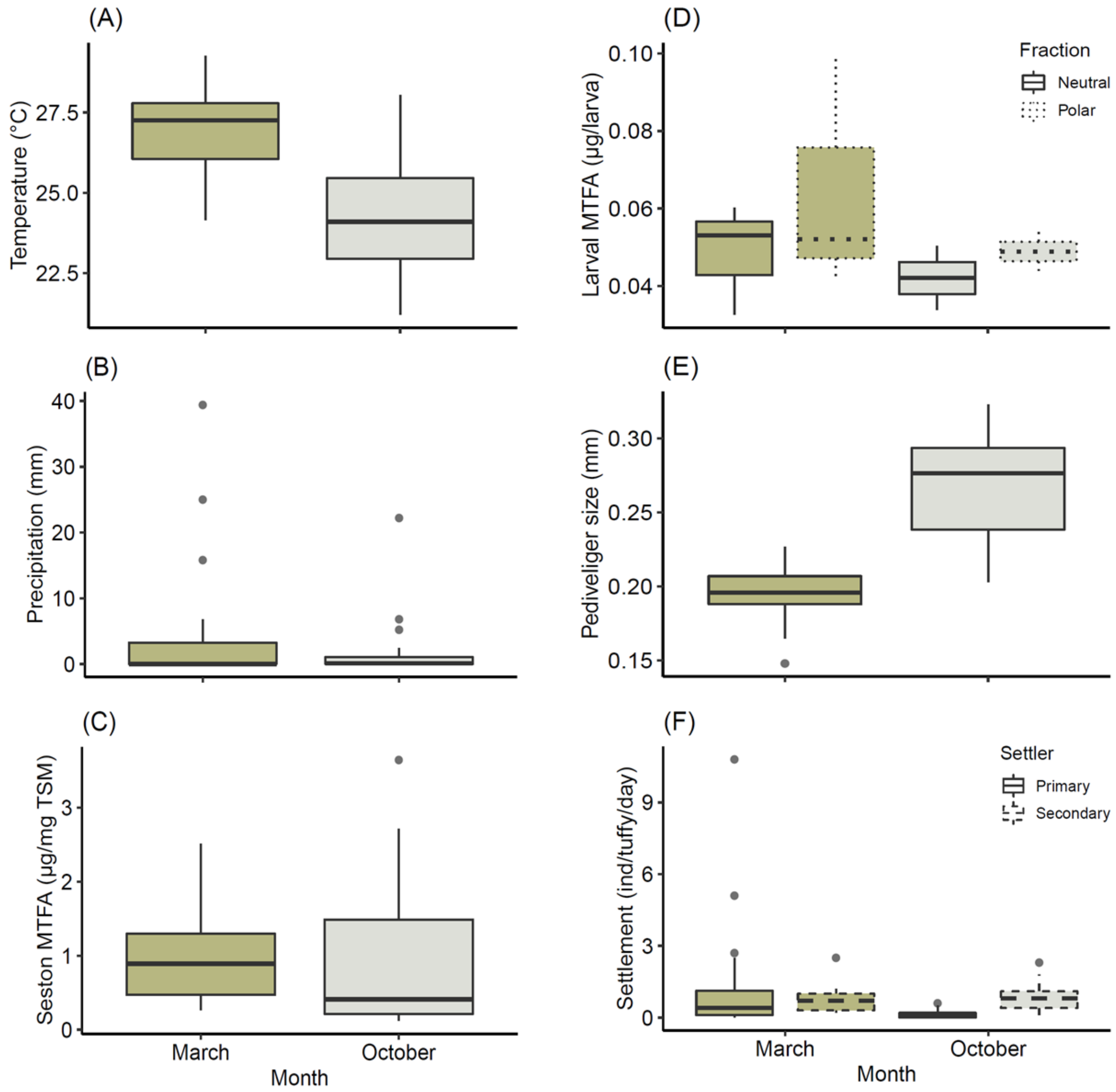


Figure 14. Series of boxplots describing the local abiotic (A–C) and biotic conditions (D–F) at the time of sampling. MTFA: Mass of Total Fatty Acids. TSM: Total Suspended Matter.

Temperatures were higher ($p < 0.001$, $t = -4.74$; Fig. 14-A) in March (27.1 ± 0.4 ^{o}C) compared to October (24.2 ± 0.5 ^{o}C). Rainfall events reached 39.4 mm on a single day in March, and 25 mm on a single day in October (Fig. 14-B). The Mass of Total Fatty Acids (MTFA) of suspended matter (Fig. 14-C) was greater during March (0.98 µg/mg TSM *vs.* 0.92 µg/mg TSM; $p = 0.029$, $U = 1346.0$). In March, *B. solisianus* pediveligers averaged 197 µm in total length (ranging from 148-227 µm), being significantly smaller ($p < 0.001$, $U =$

82.5; Fig. 14-E) than pediveligers sampled in October, which averaged 269 µm in size (ranging from 203-323 µm). This indicates high variability in theoretical competence size for *B. solisianus*, even within sampling periods, oscillating by up to 120 µm between cohorts. As for settlement dynamics, peaks of primary settlers (as per outliers in Fig. 14-F) followed heavy rainfalls (> 20 mm) in March. The heavy rainfall of 39.4 mm in March coincided with a bulk settlement of 150 primary settlers on a single day. In March, a total of 472 settlers were sampled, of which 298 were primary settlers (i.e., 63 %), and 174 were secondary (i.e., 37 %). In October, a total of 250 settlers were sampled, of which 37 were primary settlers (i.e., 15 %), and 213 were secondary (i.e., 85 %).

2.5.2 Lipidomics

2.5.2.1 Nearshore fatty acid trophic markers

Detritus and bacteria alone accounted for more than 50 % of the nearshore suspended matter, being the most abundant FATM in nearshore waters (Table 2). Diatoms and dinoflagellates comprised nearly 20 % of the nearshore suspended matter, indicating that microalgae rich in essential fatty acids (EPA and DHA) were available for larvae and postlarvae in both months. PERMANOVA revealed significant temporal differences in suspended matter sources ($p_{\text{Month}} = 0.007$; Table 3). Markers of terrestrial and detrital matter contributed to most of the dissimilarity between months, together explaining 57 % of the dissimilarity (SIMPER), and being higher in the warmer and rainier month of March.

The redundancy analysis (RDA) carried out on the FATM from the nearshore suspended matter (Fig. 15-A) shows that the environmental variables temperature and precipitation ($p < 0.05$) explained most of the variation in the dataset. Axis 1 concentrated 47.8 % of the data variability, and axis 2 concentrated 57.7 %. Precipitation was positively correlated with tracers for terrestrial inputs, which were most common in March. The RDA carried out on *B. solisianus* settlement estimates (Fig. 15-B) evidenced a strong correlation between primary settlers and precipitation, together with the terrestrial markers in nearshore waters. In this case, axis 1 concentrated most of the variation in the dataset (69.5 %). Importantly, Fig. 15 also evidences that abiotic variables and trophic markers almost completely segregated the samples obtained in the two periods. A detailed description of the fatty acid composition (FA % of MTFA) of nearshore suspended matter can be found in Supplemental Material, Table 5.

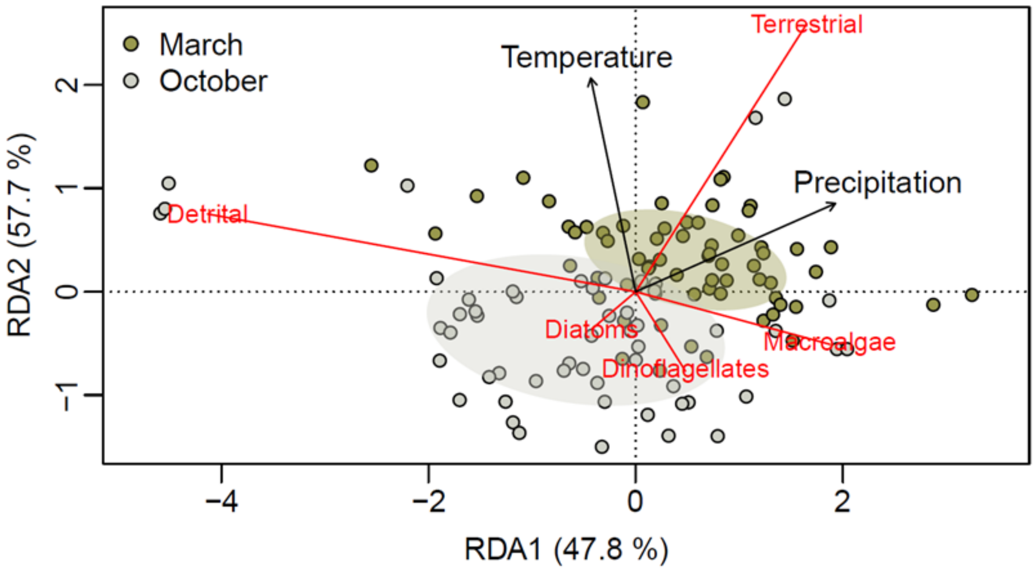
Table 3. PERMANOVA table of results for the comparison of trophic markers (FA %) present in suspended matter. Significance denoted as ‘*’ < 0.05, ‘**’ < 0.01, ‘***’ < 0.001.

	Trophic markers				
Source	*df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*
Month	1	328.6	328.6	22.36	0.000 ***
Site	1	8.4	8.4	0.57	0.569
Month × Site	1	6.2	6.2	0.42	0.681
Residual	115	1689.8	14.7		
Total	118	2036.6			

2.5.2.2 Larval nutritional status

Brachidontes solisianus pediveliger larvae have conspicuous lipid droplets in the anterior part of the body, that function as reservoirs of energetic (neutral) lipids (Fig. 16). Selective retention of essential fatty acids occurred during October, for the essential DHA (Fig. 16). In other words, larvae incorporated DHA into membrane phospholipids at the expense of reserve lipids. This suggests that during this period the neashore suspended matter was below the probable DHA need of larvae (DHA in nearshore suspended matter was ≤ 3 % of MTFA; Supplemental Table 5). This might be also related to the larger larval sizes during this period (Fig. 14-E), which incur greater somatic growth requirements. A detailed description of the fatty acid composition of *B. solisianus* pediveliger larvae (FA % of MTFA) can be found in Supplemental Material, Table 6.

(A) Suspended matter sources



(B) Settlement drivers

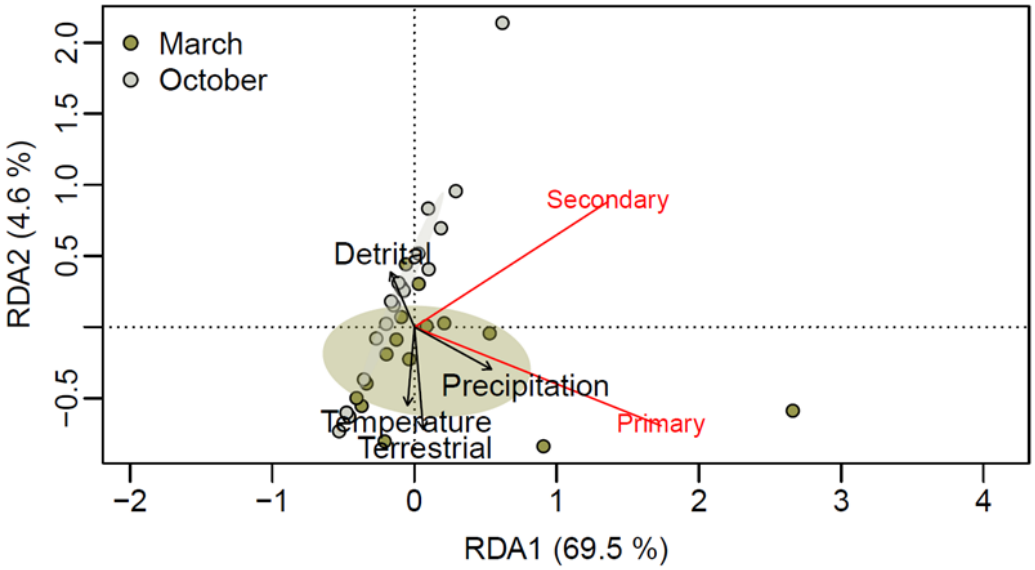


Figure 15. Redundancy analysis (RDA) for the association of abiotic variables and (A) fatty acid trophic markers present in suspended matter in the subtropical nearshore waters, (B) settlement dynamics (primary and secondary *Brachidontes solisianus* settlers). Ellipses represent month-based clustering. Significant correlations between ordination and environmental variables are represented (*envfit* function, p-value < 0.05). Response variables plotted in red, explanatory variables in black.

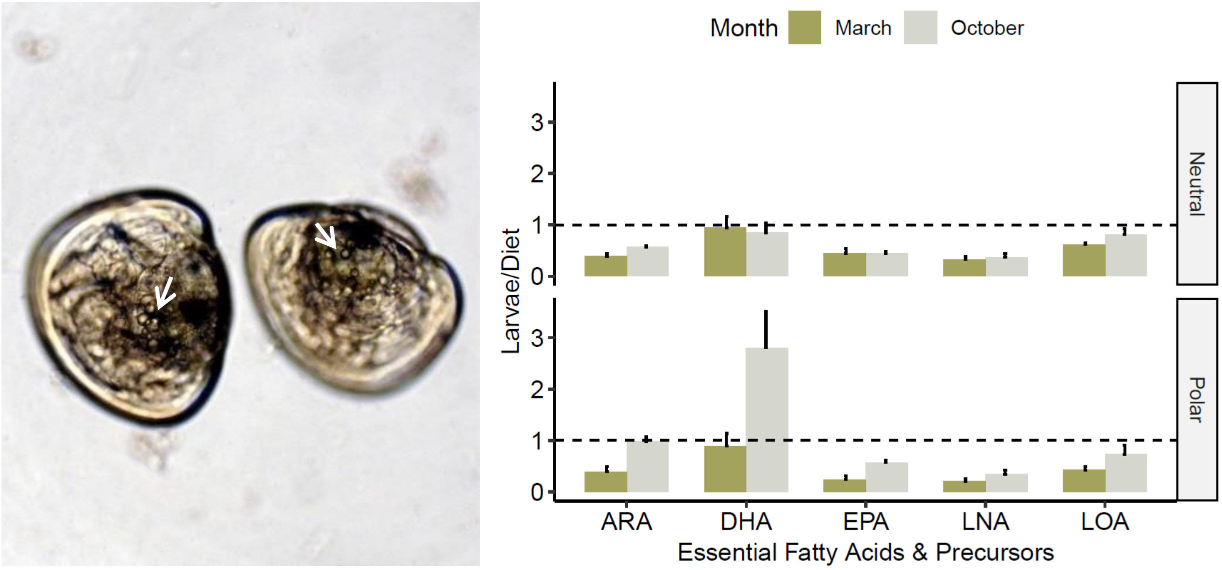


Figure 16. Selective fatty acid retention in *Brachidontes solisianus* pediveliger larvae. The left panel showcases pediveliger larvae with lipid droplets (arrows), their neutral lipids reserves. The right panel shows the ratios Larvae/Diet for essential fatty acids and precursors. Dashed line represents the threshold above which larvae selectively retain a fatty acid. ARA: 20:4ω6 Arachidonic acid; DHA: 22:6ω3 Docosahexaenoic acid; EPA: 20:5ω3 Eicosapentaenoic acid; LNA: 18:3ω3 α-Linolenic acid; LOA: 18:2ω6 Linoleic acid.

2.5.3 Settlement dynamics

2.5.3.1 Size at settlement and post-metamorphic drift

We found that *B. solisuanus* juveniles (postlarvae) can drift up to considerable sizes (up to 2.5 mm). Crawling/pedal-walking may also be a commonly used strategy by this species, as observed in the laboratory (I. Leal, pers. obs.). Prodissoconch (PII) shell was measured in postlarvae smaller than 400 µm, as the growth axis changes after this size, and would not allow for a standardization of measurements. This means that information regarding size at settlement of larger individuals – which accounted for 31 % of the population (203 secondary settlers out of 653) – could not be estimated, and that drifting is likely underestimated.

Brachidontes solisianus size at settlement and metamorphosis (postlarval PII shell length; Fig. 17-A) shifted from an average of 205 µm in March to 299 µm in October. ANOVA results showed significant temporal effects in PII size (Table 4), with no detectable spatial effects ($p_{\text{site}} > 0.05$). As shown in Fig. 17-A, there is high plasticity in size at settlement and metamorphosis of *B. solisianus*, which may range from 165 to > 400 µm. As for drift ratios, indicative of post-metamorphic dispersal in the population (Fig. 17-B), there was an overlap for both sampling periods, averaging 1.16. Contrary to PII size, ANOVA results revealed a significant time-space interaction ($p_{\text{Month} \times \text{Site}} = 0.008$; Table 4) in drifting behaviour, suggesting that temporal effects were not consistent across sites. Drifting appears to vary rather erratically in space and time, and to be a commonplace response of *B. solisianus* postlarvae to stochastic events.

Table 4. Two-way analysis of variance (ANOVA) summary table for temporal and spatial variability in size at settlement and metamorphosis (PII shell length) and post-metamorphic drifting (Drift ratio) of *B. solisianus*. Significance denoted as '*' < 0.05, '**' < 0.01, '***' < 0.001.

	Size at Settlement					**Drift Ratio**				
Source	*df*	*SS*	*MS*	*F*	*p*	*df*	*SS*	*MS*	*F*	*p*
Month	1	0.4	0.4	619.48	0.000 ***	1	0.0	0.0	0.02	0.899
Site	1	0.0	0.0	1.42	0.236	1	0.0	0.0	5.38	0.022 *
Month × Site	1	0.0	0.0	0.48	0.490	1	0.1	0.1	7.26	0.008 **
Residual	164	0.1	0.0			164	1.2	0.0		
Total	168	10.9				168	227.7			

2.5.3.2 A settlement trigger

The intraspecific variation observed in the shell morphometry of *B. solisianus* settlers (coefficient of variation; CV %) shows that a pulse of similarly-sized postlarval drifters settled during March (4 CV %), as opposed to a 13 % variability in drift in the drier period of October. This settlement pulse comprised individuals with a below-average (< 1.1) drifting ratio. The range of variation in post-metamorphic dispersal, i.e., plasticity, signals the scope for different drifting behaviours to occur in this population.

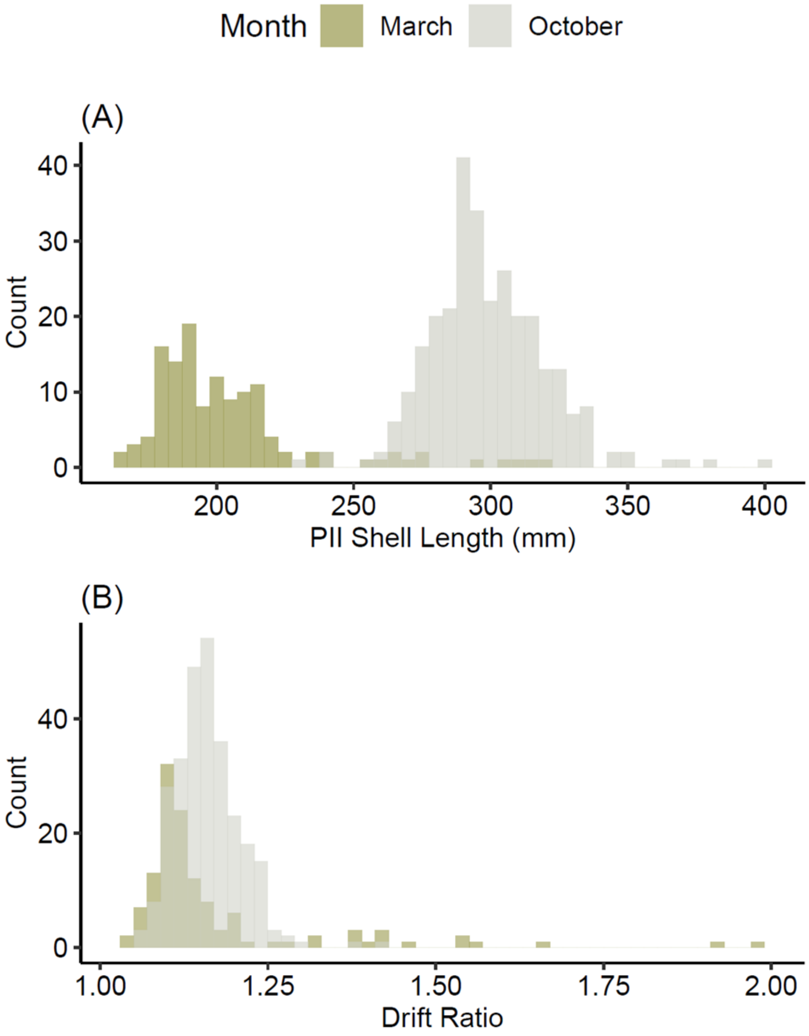


Figure 17. Histograms of postlarval (A) size at settlement and metamorphosis (PII shell length) and (B) post-metamorphic dispersal (Drift ratio), measured on over 400 *Brachidontes solisianus* individuals.

2.6 DISCUSSION

This study suggests that allochthonous subsidies resulting from rainfall pulses mediate settlement dynamics of the subtropical mytilid *Brachidontes solisianus*. We found that coastal runoff during the wet period led to organic-rich plumes, i.e., nearshore surplus of mass of dietary fatty acids, that contrasted with the trophic conditions that prevailed during the dry period. Our analysis of long-term precipitation data revealed that sporadic rainfall events of comparable and large intensity (> 10 mm) frequently occur in the SSC, being more common from February to April. Redundancy analyzes detected a strong positive correlation between precipitation and fatty acid trophic markers of terrestrial origin, and identified these as drivers of *B. solisianus* primary settlement. In paralell, lipidomics revealed that pediveliger larvae strongly retained the essential 22:6ω3 Docosahexaenoic acid (DHA) in membrane phospholipids at the expense of reserve lipids during the dry period, suggesting that dietary sources were likely below the nutritional needs of larvae. We hypothesize that the higher proportion of postlarval drifters/crawlers in the less favorable dry period (85 % of settlers) may have also been a result of poor trophic subsidies, as bivalve juveniles postpone final settlement until encountering habitat conditions suitable for growth and survival in the benthos. We propose that these pulsed natural disturbances, i.e., organic-rich plumes, are an inherent part of the overall system dynamics and an important control of nutrient and material fluxes in the SSC that impact benthic invertebrate communities.

2.6.1 Food sources entering benthic food webs

Our results indicate a high dependence on heterotrophic food chains based on detrital matter, which adds support to previous findings in the study area (Leal et al., 2018). Detritus alone accounted for nearly 50 % of suspended matter composition, and nearly 60 % of larval energetic reserves (Supplemental Tables 5, 6). This is similar to larval diets of other tropical intertidal bivalves (*Isognomon alatus*; Leal et al., 2019). Algae (as phytoplankton, and macroalgae) formed a third of the base of the nearshore food chains. The portion of terrestrial matter entering the benthic food webs was also considerable (10-13 % of nearshore TSM; Table 2). Importantly, terrestrially-derived leaf litter can become nutritionally available after fragmentation and heterotrophic biodegradation processes (Wafar et al., 1997; Meziane and Tsuchiya, 2000), and may contribute to the strong detrital food web observed.

The fatty acids 18:2ω6 Linoleic acid (LOA) and 18:3ω3 α-Linolenic acid (LNA), precursors of long-chain PUFA (Glencross et al., 2009) found in elevated amounts in most littoral vegetation (Budge and Parrish, 1998; Parrish et al., 2000; Copeman and Parrish, 2003; Colombo et al., 2017), have also been considered markers for mangroves (Bachok et al., 2003), seagrass (Kelly and Scheibling, 2012), and macroalgae (De Cesare et al., 2017; Gaillard et al., 2017) in other systems. Yet, while seagrass meadows of *Halophila decipiens* are reportedly expanding in the southwest Atlantic (Gorman et al., 2020), their low abundance in the SSC suggests that their contribution to the coastal food webs is likely small. The macroalgal cover at the mid-shore levels of southeastern Brazil, and especially along the SSC, is mostly restricted to ephemeral species (*Ulva lactuca*, *Porphyra* sp.), with no succession to perennial forms (Flores et al., 2015). The degradation of these ephemeral algae might contribute to the observed pool of detritus. Further, the absence of specific green macroalgae fatty acids markers (18:4ω3 and 16:4ω3, Gaillard et al. 2017) strengthens the use of LOA and LNA as indicators of terrestrial subsidies. The MUFA 18:1ω9 (more abundant in brown algae; Kelly and Scheibling, 2012) was the most abundant macroalgal tracer, accounting for more than 10 % of the suspended matter nearshore, and a considerable part of the diet of *B. solisianus* pediveligers (Supplemental Tables 5, 6). This result is in accordance with reports of the brown weed *Sargassum* comprising the main subtidal vegetated habitat in the study region (Duarte et al., 2016; Figueiredo et al., 2020). Following coastal runoff, the proportion of macroalgal compared to terrestrial inputs is also likely negligible, given the strong coupling of terrestrial habitats and intertidal ones over the SSC coastline (land–ocean interface; Fig. 12).

We suggest that variation in energy sources is likely to be important to the coastal ecosystem functioning in this subtropical system, and may be mediated by precipitation-driven pulses of organic matter (Fig. 15). Conceptually, organic-rich plumes support *B. solisianus* larvae and postlarvae energetically by two trophic pathways: (1) higher abundance of marine phytoplankton stimulated by nutrient surplus, and/or (2) direct trophic subsidies through the uptake of terrestrial organic matter flushed to sea. This agrees with previous studies suggesting that filter-feeders under and near plumes may derive a greater proportion of their diet from terrestrial sources than their pelagic counterparts (Schlacher et al., 2009). In most animals, the ω-3 and ω-6 fatty acid series cannot be synthesized *de novo*, but must come from the diet or as precursors that are present in the food (Glencross et al., 2009). The dietary addition of LOA and LNA has been proven to substantially improve the growth of marine invertebrates (Glencross et al., 2009). In natural settings, zooplankton can ingest large quantities of lithogenic particles carried in turbid plume waters, suggesting opportunistic and omnivorous feeding behaviours (Schlacher et al., 2009). Small plumes have been considered largely ephemeral features that last from several days to weeks at the most and have a limited spatial ambit (Gaston et al. 2006). For instance, plumes from mangrove have been shown to extend to the subtidal zone for approximately 1 km (Meziane and Tsuchiya, 2000). The delivery of terrestrial organic matter to nearshore regions is therefore erratic and short-lived (Gaston et al. 2006; Schlacher et al., 2009). While this may be the case for the SSC, further studies are needed to ascertain the temporal and spatial extent of these organic-rich plumes, which play a predominant role in the trophic ecology of *B. solisianus*.

2.6.2 Larval energetics

Essential fatty acids, particularly, EPA and DHA, are essential for the condition, growth, and survival of suspension-feeding bivalve larvae (e.g., scallop *Placopecten magellanicus*, Pernet and Tremblay, 2004; scallop *Pecten maximus*, Tremblay et al., 2007; oyster *Crassostrea gigas*, Da Costa et al., 2016). DHA is involved in maintaining a suitable membrane structure, whereas EPA fulfils a role as both an energy source and as a precursor of eicosanoids (Marty et al. 1992). It is generally assumed that the synthesis of EFA in bivalves is absent or insufficient to meet metabolic demands, so that these fatty acids have to be supplied exogenously to sustain optimal survival and growth. However, recent evident suggests that some species of bivalve larvae have the ability to desaturase EFA precursors in response to nutritional shortages to produce ARA and EPA, but not DHA (Da Costa et al., 2015). DHA plays a structural role, as it is retained selectively during starvation. Selective retention appears to be a widespread strategy among marine invertebrate larvae (scallops, Pernet et al., 2005; lobsters, Thériault and Pernet, 2007; bivalves, Da Costa et al., 2015; barnacles, Leal et al., *unpublished*). We now provide evidence for selective DHA retention in subtropical *B. solisianus* larvae (Fig. 16). Selective retention of DHA coincided with periods of reduced allochthonous subsidies, as well as with longer planktonic larval duration (longer PII shell lengths; Fig. 17-A). The variability in theoretical competence size suggests that there are important variations in the dispersal patterns of *B. solisianus* in the SSC, and thus in the connectivity among adult populations.

2.6.3 Settlement dynamics

Size at settlement and metamorphosis (PII shell) of *B. solisianus* postlarvae ranged from 165 to > 400 μm showcasing the plasticity in larval duration in this mytilid population. Changes in the dimensions of the PII shell may reflect changes in ambient temperature (i.e., inverse relationship between temperature and maximum PII size; Lutz and Jablonski, 1978). Therefore, we cannot exclude the possibility that the differences in larval size could be related to temperature-driven differential growth patterns. However, given the high variability in larval size of within-season, i.e., same temperature grown cohorts (up to 120 μm), we suggest that this plasticity in size at settlement reflects the ability of this mytilid to delay metamorphosis until encountering favorable settlement sites. Under laboratory conditions, pediveliger stages of *B. solisianus* have been shown to last 11 days and reach average lengths of 273 μm (Monteiro-Ribas et al., 2006). Thus, a PII size of 400 μm suggests that *B. solisanus* pediveligers in nature may remain adrift for a few weeks, delaying metamorphosis. Understanding how, why, and when individuals disperse from their place of origin is a central question in ecology. At the most basic level, the causes and outcomes of dispersal are driven by the interactions of individuals with their environment (Bowler and Benton, 2005; Nanninga and Berumen, 2014). Nanninga and Berumen (2014) classified such interactions as “context-dependent” (i.e., external environmental factors that trigger specific dispersal behaviors) and “condition-dependent” effects (i.e., the condition or the phenotype of an individual that affect dispersal). Context and condition effects may be reciprocal and affect each other during dispersal, for instance when short-term environmental stochasticity affects individual condition (e.g., energy reserves) and causes variation in behavioral strategies during larval dispersal (Nanninga and Berumen, 2014). Therefore, we suggest that in the meso-oligotrophic waters of the SSC (Flores et al., 2015; Leal et al., 2018) short-term environmental stochasticity in the form of pulsed rainfall events and resulting organic-rich plumes modulate larval nutritional status and trigger specific dispersal behaviors in *B. solisianus*.

Further, our results indicate that most sampled secondary settlers were short-term drifters, and that drifting is commonplace in this mytilid population. We demonstrate the high mobility of settlers after first settlement, which adds to the growing evidence that the transition between the planktonic and benthic phase is not as abrupt as generally thought (Martel, 1993; Le Corre et al., 2013; Fôret et al., 2018; Leal et al., 2019). By distinguishing between primary and secondary settlement, we found that secondary settlers (drifting juveniles) represented a high proportion of *B. solisianus* settlers. Secondary settlers accounted for 37 % to a prevalence of 85 % of nearshore settlers. Secondary dispersal is considered to have less impact on large-scale connectivity when compared to primary settlement, remaining largely local (Le Corre et al., 2013). The variability of size at settlement and metamorphosis, directly impacting dispersal of pre-metamorphic larval stages, is likely to have large-scale effects on population connectivity (e.g., Burgess et al., 2014), and was remarkable in the *B. solisianus* population.

2.6.4 Ecological implications

Ecosystems are closely linked to one another. Agricultural and forestry practices, fragmentation, and desertification affect not only terrestrial habitats, but the productivity, food webs, and community structure of nearby rivers, estuaries, and oceans (Polis et al., 1997). Inputs of river-born nitrates, raw untreated human effluents, and other urban and industrial inputs impact local ecosystems through the classic "paradox of enrichment", where rich subsidies result in poorer diversity or other declines in abundance (Palumbi, 2003). Nutrient-driven shifts of habitats from perennial (i.e., canopy-forming algae) to opportunistic species (i.e., turf-forming algae) have already been described for the warm temperate coastline of Brazil (Gorman et al., 2020). Projected increases in storm intensity, duration, and possibly frequency, in combination with enhanced nutrient and sediment inputs related to human activities will expose estuarine and coastal habitats more often, and for longer time periods, to eutrophication (Herbeck et al., 2011). Population dynamics of rocky shore communities that respond to such nutritional pulses, e.g., the subtropical mytilid *B. solisianus*, may be disrupted upon nearshore eutrophication and deserve further attention.

2.7 CONCLUSION

We provide novel knowledge on fatty acid signatures of bivalve larvae and pelagic trophic resources entering benthic food webs in subtropical Brazilian shores. We suggest that precipitation-driven pulses of organic matter mediate variation in energy sources available for suspension-feeding *B. solisianus* larvae and postlarvae, and influence the dispersal and settlement dynamics of this coastal foundation species. This demonstrates how ecosystems are closely linked to one another, and sets the stage for research on the effects of eutrophication on the structure of benthic communities at the land–ocean interface.

2.8 ACKNOWLEDGEMENTS

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2.9 SUPPLEMENTARY MATERIAL

Table 5. Fatty acid composition (% ± SE) of suspended matter (TSM) collected in subtropical nearshore waters. Fatty acids accounting for less than 1 % of the Mass of Total Fatty Acids (MTFA) were removed. Fatty Acid Classes: Saturated (SFA), Monounsaturated (MUFA), Polyunsaturated (PUFA) and Essential (EFA; comprising ARA, EPA and DHA). *n* stands for the number of samples analysed.

Fatty Acid	**Month**	
	March *n* = 65	October *n* = 54
14:0 Myristic	5.4 ± 0.2	5.5 ± 0.3
15:0 Pentadecanoic	0.6 ± 0.1	1.1 ± 0.0
16:0 Palmitic	28.8 ± 0.3	26.4 ± 0.4
18:0 Stearic	12.3 ± 0.4	17.0 ± 0.7
20:0 Arachidic	2.3 ± 0.1	1.4 ± 0.1
22:0 Behenic	0.8 ± 0.1	1.5 ± 0.1
24:0 Lignoceric	2.7 ± 0.1	1.8 ± 0.1
SFA	**53 %**	**55 %**
16:1 Palmitoleic	11.8 ± 0.2	10.4 ± 0.4
18:1ω9 Elaidic & Oleic	12.3 ± 0.3	11.1 ± 0.3
24:1ω9 Nervonic	0.0 ± 0.0	1.7 ± 0.1
MUFA	**24 %**	**23 %**
18:2ω6 Linolelaidic & Linoleic	5.1 ± 0.1	3.8 ± 0.2
18:3ω6 γ-Linolenic	1.2 ± 0.0	1.0 ± 0.1
18:3ω3 α-Linolenic	4.2 ± 0.1	3.1 ± 0.1
20:4ω6 Arachidonic (ARA)	2.6 ± 0.1	2.5 ± 0.1
20:5ω3 Eicosapentaenoic (EPA)	4.5 ± 0.1	6.5 ± 0.2
22:6ω3 Docosahexaenoic (DHA)	3.2 ± 0.1	3.5 ± 0.2
PUFA	**21 %**	**20 %**
EFA	**10 %**	**12 %**
MTFA (µg/mg TSM)	1.0 ± 0.1	0.9 ± 0.1

Table 6. Fatty acid composition (% ± SE) of the neutral (energetic) and polar (structural) fractions of *Brachidontes solisianus* pediveliger larvae collected in subtropical nearshore waters. Fatty acids accounting for less than 1 % of the Mass of Total Fatty Acids (MTFA) were removed. Fatty Acid Classes: Saturated (SFA), Monounsaturated (MUFA), Polyunsaturated (PUFA) and Essential (EFA; comprising ARA, EPA and DHA). *n* stands for the number of larvae analysed.

Fatty Acid	**March** | *n* = 570		**October** | *n* = 350	
	Neutral	*Polar*	*Neutral*	*Polar*
14:0 Myristic	4.1 ± 0.8	1.9 ± 0.3	3.4 ± 0.8	2.7 ± 0.3
15:0 Pentadecanoic	1.4 ± 0.1	1.1 ± 0.2	1.2 ± 0.1	1.2 ± 0.1
16:0 Palmitic	28.3 ± 0.6	34.0 ± 2.1	26.6 ± 0.3	12.2 ± 11.5
17:0 Heptadecanoic	0.9 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.0
18:0 Stearic	28.0 ± 5.0	37.0 ± 4.6	26.7 ± 5.9	35.9 ± 18.3
20:0 Arachidic	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
22:0 Behenic	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.0
24:0 Lignoceric	1.2 ± 0.1	0.7 ± 0.1	1.2 ± 0.2	0.8 ± 0.0
SFA	**65 %**	**77 %**	**62 %**	**55 %**
16:1 Palmitoleic	8.3 ± 1.0	2.3 ± 0.6	8.3 ± 1.4	3.4 ± 0.4
17:1 Cis-10-heptadecenoic	0.9 ± 0.2	3.3 ± 1.0	1.3 ± 0.2	5.8 ± 2.2
18:1ω9 Elaidic & Oleic	10.9 ± 1.1	5.0 ± 0.9	9.6 ± 2.3	5.7 ± 0.2
20:1ω9 Cis-11-eicosenoic	1.2 ± 0.3	1.5 ± 0.5	1.5 ± 0.4	3.4 ± 0.7
MUFA	**21 %**	**12 %**	**21 %**	**18 %**
18:2ω6 Linolelaidic & Linoleic	3.2 ± 0.4	2.2 ± 0.5	3.4 ± 0.1	3.1 ± 0.4
18:3ω6 γ-Linolenic	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
18:3ω3 α-Linolenic	1.4 ± 0.3	0.9 ± 0.3	1.2 ± 0.0	1.1 ± 0.1
20:3ω6 Eicosatrienoic	0.5 ± 0.1	1.2 ± 0.3	0.6 ± 0.1	1.9 ± 0.6
20:4ω6 Arachidonic (ARA)	1.0 ± 0.2	1.0 ± 0.3	1.5 ± 0.1	2.6 ± 0.3
20:5ω3 Eicosapentaenoic (EPA)	2.1 ± 0.5	1.1 ± 0.4	3.0 ± 0.4	3.8 ± 0.5
22:6ω3 Docosahexaenoic (DHA)	3.3 ± 1.0	3.1 ± 1.1	3.4 ± 0.2	11.1 ± 0.9
PUFA	**12 %**	**10 %**	**14 %**	**24 %**
EFA	**6 %**	**5 %**	**8 %**	**18 %**
MTFA (μg/larva)	0.05 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01

ARTICLE 3

DRIFTING IN THE CARIBBEAN: HINTS FROM THE INTERTIDAL BIVALVE *ISOGNOMON ALATUS*

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3.1 ABSTRACT

Settlement of most marine benthic invertebrates is intrinsically linked to the end of the pelagic life, but for a few, the transition to the benthos can be less well-defined. Early metamorphosed postlarvae of temperate and subtropical bivalves are known to delay final settlement, i.e., undergo secondary migrations, until detecting specific habitat cues. Here, we test the hypothesis that postlarval drifting occurs as well in tropical systems, being mediated by the nutritional quality of nearshore waters. We monitored early stages of the flat tree oyster *Isognomon alatus* on the Caribbean coast of Panama, and observed successive numerical dominance of late-stage larvae (pediveligers) and postlarvae (juvenile drifters) of this isognomonid in the water column. Of all the pelagic individuals collected, 19 % corresponded to juvenile drifters, suggesting that conditions favoring juvenile drifting were present at the time of sampling and/or juvenile drifting may be commonplace for this species. During 23 consecutive sampling days, we detected a single settlement peak of long-term drifters that followed a decrease in sea-surface salinity. The shell morphology of juveniles revealed that first settlement for *I. alatus* takes place after theoretical competence, when prodissoconch II exceeds c.a. 300 µm. A shift in nearshore trophic conditions was detected after settlement, with particulate matter dominated by tracers of microalgae and vascular plant matter shifting to tracers for detritic matter and bacterial load. Essential fatty acids comprised 28 % of the seston, yet, were minimally accumulated by *I. alatus* pediveligers (2 % of energetic reserves). We highlight the importance of accounting for juvenile drifters of *I. alatus* on settlement estimates, as the spatial distribution of late larval stages and/or early settlers might not reflect their final distribution. This information is key to manage, exploit and/or conserve natural stocks.

Keywords: Larval ecophysiology; Shell morphometry; Fatty acids; Mangroves; Caribbean

3.2 RÉSUMÉ

La fixation de la plupart des invertébrés benthiques marins est intrinsèquement liée à la fin de la vie pélagique, mais pour quelques-uns, la transition vers le benthos peut être moins bien définie. On sait que les postlarves de bivalves tempérés et subtropicaux retardent leur établissement final par des processus de migrations secondaires, jusqu'à la détection de signaux spécifiques de leur habitat. Dans cette étude, nous testons l'hypothèse que la dérive postlarvaire a également lieu dans les systèmes tropicaux sous l'influence de la qualité nutritionnelle des eaux côtières. Nous avons échantillonné les premiers stades de vie de l'huître plate *Isognomon alatus* sur la côte caraïbe du Panama, et nous avons observé une dominance successive des larves de stade avancé (pédiveligéres) et des postlarves (juvéniles dérivants) de cet isognomonidé dans la colonne d'eau. De tous les individus pélagiques collectés, 19 % correspondaient à des juvéniles dérivants, ce qui suggère que des conditions favorisant la dérive juvénile étaient présentes au moment de l'échantillonnage et/ou la dérive juvénile peut être courante pour cette espèce. Au cours de 23 jours d'échantillonnage consécutifs, nous avons détecté un seul pic de fixation qui a suivi une diminution de la salinité de l'eau de surface. L'étude morphologique de la coquille des juvéniles a révélé que le premier établissement de *I. alatus* a lieu après la compétence théorique, lorsque la prodissoconch II dépasse c.a. 300 µm. Un changement dans les conditions trophiques côtières a été détecté après la fixation, avec une dominance passant des particules dominées par les traceurs de microalgues et de la matière végétale vasculaire vers les traceurs de la matière détritique et bactérienne. Les acides gras essentiels constituaient 28 % du seston, mais étaient peu accumulés par les larves pediveligères (2 % des réserves énergétiques). Nous soulignons l'importance de considérer les juvéniles dérivants d'*I. alatus* dans les estimations de fixation, car la distribution spatiale des stades larvaires tardifs et/ou des premières postlarves fixées pourrait ne pas refléter leur distribution finale. Ces informations sont essentielles pour gérer, exploiter et/ou conserver les populations naturelles.

Mots clés : Écophysiologie larvaire; Morphométrie de la coquille; Acides gras; Mangroves; Caraïbes

3.3 INTRODUCTION

The life-cycle of most marine benthic invertebrates involves a pelagic larval stage that develops and grows in the water column until reaching competency and permanently attaching to the substrate. However, a few bivalve species deviate from this model by passing through an intermediate stage, the pelagic postlarva or drifting juvenile (e.g., Sigurdsson et al., 1976; Baker and Mann, 1997; Olivier and Retière, 1998; Forêt et al., 2018a). This stage follows larval settlement, where the pediveliger begins to metamorphose into juvenile, with major changes in both form and function (e.g., transition from velum to gill as the feeding organ; Yonge et al., 1926) but delays in its final attachment. For these species, settlement occurs in a two-phase process (Sigurdsson et al., 1976; Ackerman et al., 1994): 1) settlement of pediveligers and subsequent metamorphosis (i.e., primary or first settlement), and 2) re-location to new areas. Although pelagic post-metamorphic individuals and post-settlement movements were documented many decades ago (e.g., juvenile mussels crawling at a pace of 7 cm per night; Shevchenko, 1949), the ecological significance of this intermediate stage remains unclear.

As early as 1928, Nelson (1928) observed post-metamorphic mussels (*Mytilus edulis*) in the water column, referring to them as “dissoconch-stage” juveniles; a malacological term denoting shell secretion after first settlement. Several mechanisms have been proposed for this phenomenon (e.g., re-suspension through active swimming or sediment scour, Williams and Porter, 1971; drifting through byssal threads, Sigurdsson et al., 1976; active migration mediated by trophic triggers, Forêt et al., 2018b). Regardless of the underlying mechanism, pelagic juveniles may play an important role in population dynamics as long as they endure secondary dispersal (or secondary migrations; *sensu* Günther, 1992), and thus enhance connectivity among benthic populations. Postlarval drifting was remarkable over coarse-sediment habitats of a mega-tidal area (Chausey Archipelago, France), spanning 25 bivalve taxa, and was considered a possible cause for the low abundance of benthic recruits in the area (Forêt et al., 2018a). Another noteworthy example is the case of the clam *Macoma balthica* in the Wadden Sea, whose postlarval drifters were found 15 km away from initial settlement sites (Armonies and Hellwig-Armonies, 1992). As such, initial settlement may be a poor indicator of recruitment strength at spatial scales smaller than a few km.

While drifting, juveniles increase their chance to find adequate habitat at the expense of longer exposure to pelagic predators and the risk of being transported too far from potential adult habitats. Such an extended pelagic phase may, however, be adaptive in oligotrophic systems, provided that individuals are capable to detect water-borne cues indicative of environmental trophic quality and to change their behavior accordingly, favoring a more demersal habit and definite settlement. Forêt et al., (2018b) suggested that bivalve drifters may specifically respond to peak abundances of nanoeukaryotes in the water column. Similarly, postlarval drifting in a meso-oligotrophic subtropical system is possibly ceased upon contact to trophic cues of terrestrial origin, as suggested for pelagic stages of the intertidal mussel *Brachidontes solisianus* (Leal et al., 2018). Indeed, bottom-up processes in tropical, oligotrophic systems have been shown to explain variability in the settlement and distribution of other benthic invertebrates (e.g., Caribbean sponges, Lesser, 2006; mussel *Mytella falcata*, Marques-Silva et al., 2006). In Northern Brazil, Marques-Silva et al., (2006) found that during the wet season, erosion of mangrove sediments caused by heavy rainfall elevated the nutrient concentration in coastal waters and likely favored the settlement of encrusting benthic invertebrates.

Settlement stimuli can thus greatly impact post-metamorphic events (e.g., postlarval drifting) and shape population dynamics of many marine species with an indirect life-cycle (Rodriguez et al., 1993). However, the multitude of environmental factors that larval stages are exposed to in the field (e.g., changes in salinity, temperature and trophic conditions over fine-temporal scales) is rarely considered in experimental studies (Pineda et al., 2009). Behavioral repertoires known so far may therefore be a subset of the responses that these pelagic stages are capable of to maximize their chance to settle in favorable habitats.

In the present study, we investigated juvenile drifting and its mediation through the trophic status of nearshore waters (i.e., fatty acid composition of particulate matter) in the bivalve *Isognomon alatus*, a key intertidal species from an extensive rocky-shore in Bocas del Toro Archipelago, Panama. We advanced that drifting in this tropical, oligotrophic system would be similar or more pronounced compared to the temperate and subtropical ones examined so far (Forêt et al., 2018a; Leal et al., 2018). To test this hypothesis, we monitored the supply of pelagic stages and settlement rate, together with morphological (shell characteristics) and physiological (nutritional status) parameters of sampled individuals, as well as the main nearshore oceanographic and trophic conditions. Scarce information is available on the settlement dynamics of Caribbean *I. alatus* populations and, to our knowledge, the present study is the first to report juvenile drifting in this isognomonid.

3.4 MATERIAL AND METHODS

3.4.1 Study area and biological model

Populations of the flat tree oyster *Isognomon alatus* (Gmelin, 1791) were sampled at Paunch beach, Isla Colon (9°22'25"N, 82°14'19"W), part of the Bocas del Toro Archipelago in the Caribbean coast of Panama (see map, Fig. 18; Ocean Data View, 2019). Sampling was carried out continuously for a period of 23 days during April 2017. This coincides with the onset of recruitment for *I. alatus* reported for another island in the Caribbean (Jamaica; Siung, 1980). Samples were initially processed at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station in Panama (STRI) and later analyzed at the Institute of Ocean Sciences of Rimouski in Canada (ISMER/UQAR). Permits to collect and export were issued by the Panamanian MiAmbiente (SE/AO-9-16; SEX/A-46-17).

Located on the exposed coastline of Isla Colon, Paunch beach comprises an extended rocky platform exposed to a micro-mixed, semidiurnal tidal regime (reported mean tide range of 24 cm, Kaufmann and Thompson 2005). *Isognomon alatus* dominates the upper-midlittoral shore, forming clustered assemblages over steep microhabitat gradients (as reported in other locations; Wilk and Bieler, 2009), and is mainly found in crevices in this area (Inês Leal, pers. obs.). With an exclusively tropical distribution, *I. alatus* occurs from

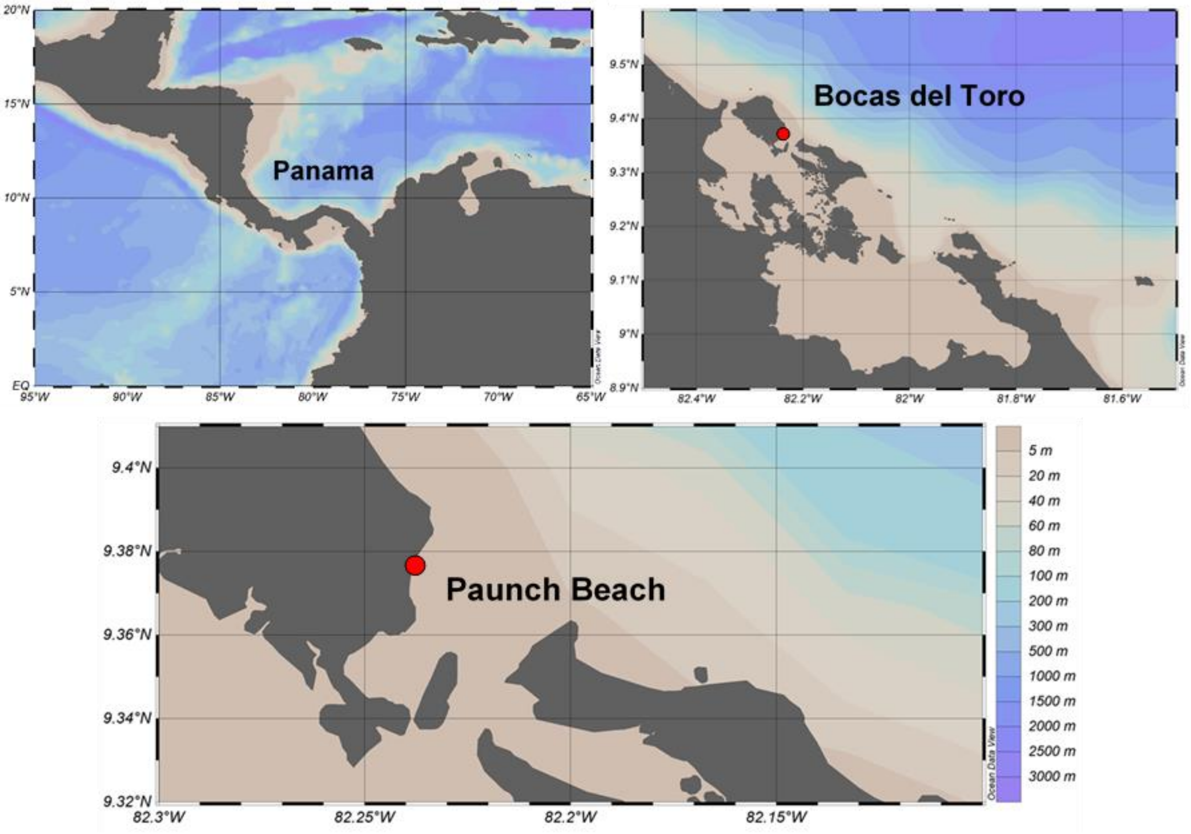

Figure 18. Map of the study area, a rocky platform situated in the Caribbean coast of Panama (Paunch Beach, Bocas del Toro Archipelago; represented by the red circle). Color scale represents depth (meters).

central Florida to Bermuda, the Bahamas, Caribbean Central America and as far south as Brazil (Siung, 1980; Mikkelsen and Bieler 2008). Despite its wide and abundant distribution in the Caribbean, very little is known about this species. It is considered an economically interesting species to cultivate in Caribbean islands, as an alternative to the mangrove oyster *Crassostrea rhizophorae* (Siung, 1980). The ability to tolerate widely fluctuating conditions, makes it possible to cultivate *I. alatus* in many coastal areas. Low salinity at the beginning of the rainy season appears to be the trigger mechanism for spawning and establishes the onset of the recruitment period (Siung, 1980). In Bocas del Toro, surface water salinity (typically ≈ 30–34) has been reported to decrease to as low as 20 after heavy rainfall (annual averages exceeding 3 m; Collin et al., 2009), presumably forming low-salinity, nutrient-rich plumes that float on top of warmer bottom water (Kaufmann and Thompson, 2005).

3.4.2 Supply of pelagic stages, settlement rate and oceanographic conditions

Pelagic stages, settlers and oceanographic variables were sampled daily. We collected early-stage individuals of *I. alatus*, both pelagic and benthic, to understand the drifting dynamics of the Caribbean population (see Fig. 19 for schematic representation of *I. alatus* life-cycle). We identified pediveligers by the presence of the ciliated velum, well-defined foot and visible eyespot, and postlarval stages by the presence of a retracted velum, fully-developed differentiated gills and dissoconch shell. We further divided postlarval stages into 1) planktonic juveniles ("drifters", collected in water column; *sensu,* Sigurdsson et al., 1976), and 2) benthic, settled juveniles ("settlers", collected on artificial collectors). Larval and postlarval densities in the water column (ind·m^{-3}) were estimated by sampling the nearshore sub-surface waters at each diurnal low tide. A 150 µm plankton net (30 cm of net mouth diameter) was manually towed just below the surface, for a 100-m long-shore transect. A subset of collected pediveligers (*n* = 20 per day) were 1) photographed alive to estimate larval shell length (µm), 2) individually removed with scientific round tip tweezers (stainless steel) to ensure that the PII shell would remain intact, and 3) preserved in dichloromethane-methanol (2:1, v/v) at -80 °C to later estimate their energetic content (see below). One dichloromethane-methanol vial containing 20 pediveligers per day was used to estimate the energetic status of daily larval cohorts (µg·larva^{-1}). Postlarval specimens in the plankton were preserved in 70 % ethanol for later measurements of shell morphology (see below).

Settlement rates were estimated by counting the number of individuals in replicate (*n* = 10) spat artificial collectors (multifilament nylon pads, SOS ® Products; Menge et al., 1994). Collectors were haphazardly attached over a horizontal 100–meter stretch of the upper-midlittoral zone, parallel to the shore. Artificial collectors were placed under running seawater for 1-week prior sampling to allow for biofilm colonization. At the peak of each diurnal low tide, collectors were removed and replaced. Settlers were extracted with a soft jet of seawater into a 100 µm mesh sieve, counted and preserved in 70 % ethanol for later measurements of shell morphology. Individuals were identified to species according to Siung (1980) and Wilk and Bieler (2009).

At each sampling, spot measurements of sea-surface salinity were also obtained directly from nearshore waters (EcoScan SALT 6 Plus Salinity Meter with electrode). Daily rainfall data (mm) for the sampling period were provided by the STRI Physical Monitoring Program (Meteorological Data).

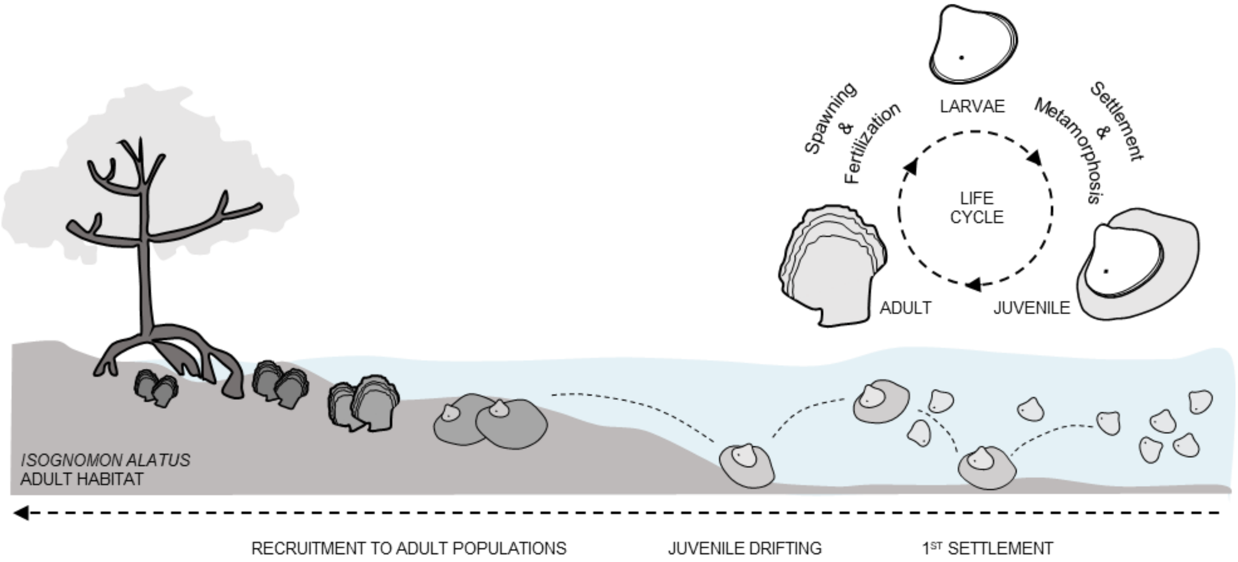

Figure 19. Schematic representation of *I. alatus* life-cycle and proposed settlement process, that involves the drifting of juveniles as observed for the Caribbean population sampled.

3.4.3 Shell morphometry

The singular shell development of *I. alatus* makes this bivalve an excellent candidate for testing the value of shell morphology as a tool to document larval and early benthic ecology (Martel et al., 2014). *Isognomon alatus* larval shell has conspicuous, regularly spaced commarginal growth lines, known as lirae (Fig. 20A), and the prodissoconch II (PII) is sharply demarcated from the early dissoconch by a subtriangular, metamorphic line (Fig. 20B). Its homogeneous surface texture contrasts the fragile, prismatonacreous microstructure of the dissoconch shell, in agreement with previous shell observations of other specimens (*I. ephippium*, Jackson, 1890; *I. alatus*, Siung, 1980; *I. spathulatus*, Temkin and Printrakoon, 2016). As described above, juvenile drifters (Fig. 20B) were collected by a plankton net and juvenile settlers (Fig. 20C) by artificial collectors.

Morphometric analyses were undertaken to estimate PII and dissoconch shell lengths (μm) in *I. alatus* individuals. PII shell is secreted from when the veliger larva begins to feed in the plankton until the pediveliger metamorphoses into a postlarva, thus indicating the size at metamorphosis, which we measured as the longest distance across the anteroposterior axis, ending at the PII–dissoconch boundary (Martel et al., 1995, 2014). Dissoconch shell is secreted after settlement and metamorphosis, when the postlarva is crawling over the benthic habitat or re-suspended in the water column (i.e., "drifting"). Pediveligers were photographed alive after collection under a Nikon microscope (100 ×) and shell lengths measured using the software Fiji, an open-source platform for scientific image analysis (Schindelin et al., 2012). Postlarval specimens were preserved in 70 % ethanol after collection and PII and dissoconch shell lengths measured with a wide-range zoom lens, high-resolution digital microscope (Keyence VHX-2000E, 1 μm resolution, HDR images).

3.4.4 Trophic status of the water column and larval physiological condition

To characterize the trophic status of the nearshore pelagic environment, we estimated the Total Particulate Matter (TPM), a proxy of food availability for bivalve feeding larvae and postlarvae ($mg \cdot L^{-1}$). 6 replicate seawater samples of 2 L per day were collected during low tide, filtered through a 100 μm mesh sieve, and then through Whatman® glass microfiber filters (GF/F 25 mm, pore size 0.7 μm) pre-burnt and weighted. To the best of our knowledge, no information is available on the particle size of *I. alatus* food intake. Generally, bivalves (e.g., *Mytilus edulis*) retain particles lower than 25 μm (Raby et al. 1997). However, as variation could be possible between species, particularly those from tropical environments that are subjected to oligotrophic conditions (poor abundance of food particles), we chose a mesh size for TPM characteristics of 100 μm, which also represents the minimal approximate size of the velum for a pediveliger of *I. alatus* (Inês Leal, pers. obs.). Three glass microfiber filters were rinsed with 0.3-M Ammonium formate to dissolve residual salts (Aminot and Chaussepied, 1983), and subsequently heated at 70 ^{o}C for 24h and weighted for estimation of TPM. Three other glass microfiber filters were stored at -80 °C, in dichloromethane-methanol vials (2:1, v/v) for estimation of Fatty Acid (FA) composition of seston, a proxy of quality and composition of the planktonic food. FAs were extracted through direct transesterification (Lepage and Roy, 1984) and analyzed by Gas Chromatography–Mass Spectrometry (GC-MS), to determine the mass of total fatty acids (MTFA; µg·mg^{-1} TPM) and its composition (% FA), following the protocol described in Toupoint et al. (2012a). We used a full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled to a multichannel gas chromatograph "Trace GC ultra" (Thermo Scientific) equipped with an autosampler (model Triplus), a PTV injector and a mass detector model ITQ900 (Thermo Scientific). An Omegawax 250 (Supelco) capillary column was used for separation with high purity helium as a carrier gas. Data were treated using Xcalibur v.2.1 software (Thermo Scientific) and FAs identified and quantified with standards Supelco 37. FAs were further discriminated into saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids.

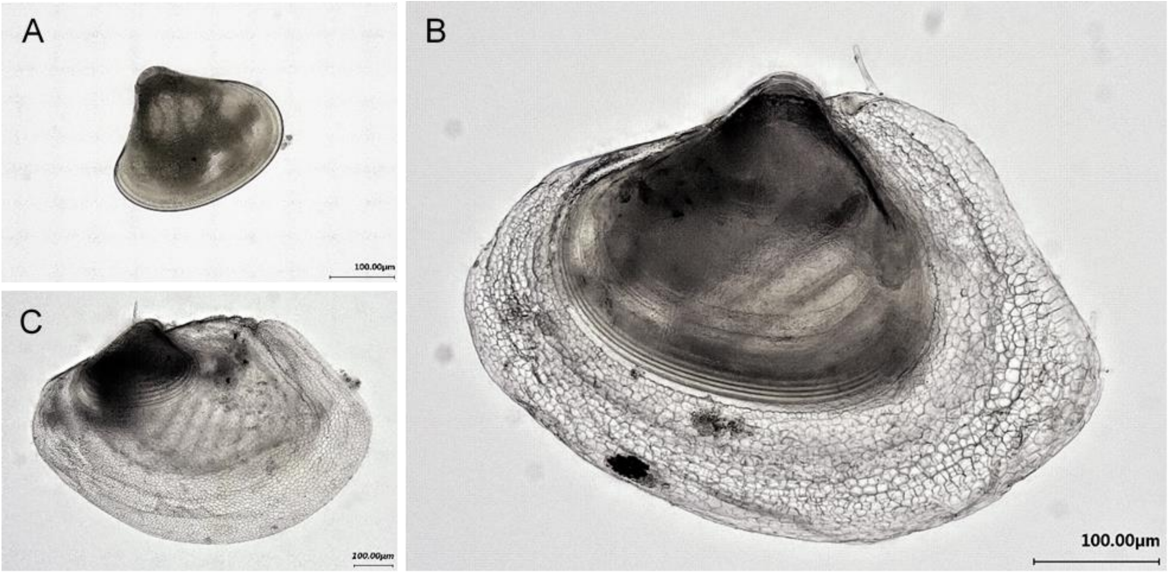

Figure 20. Identified *I. alatus* early stages. A. Pediveliger; B. Juvenile drifter; C. Juvenile settler. A clear demarcation line divides prodissoconch II and dissoconch shells, used for morphometric measurements (Keyence Digital Microscope; 500 ×). Scale bars correspond to 100 µm.

To characterize the energetic status of daily larval cohorts, pediveligers collected from the nearshore subtidal waters of Paunch beach were pooled for lipid analysis (n = 20 pediveligers per day). The accumulation and composition of fatty acids can be used as physiological indicators of larval quality (Tremblay et al., 2007b). Lipids were extracted by a modified Folch procedure (Folch et al., 1957) and the neutral fraction (including triglycerides, free FA, and sterols) was isolated from the polar fraction (mainly phospholipids) (Marty et al., 1992). Neutral fatty acids make up most of the energetic reserves, being correlated to larval performance (Pernet and Tremblay, 2004; Tremblay et al., 2007b), and therefore comprise the representative fraction of larval energetic status. FAs were extracted and analyzed with the GC-MS, as previously described, to determine the total energetic content in individual larvae (sum of all neutral fatty acids identified; $\mu g \cdot larva^{-1}$) and its composition (% FA; further discriminated into SFAs, MUFAs, PUFAs, EFAs), following the protocol described in Freuchet et al., (2015).

3.4.5 Statistical analysis

To describe the shell morphometry of *I. alatus* during early development, size-frequency distributions of pediveligers (n = 180), drifters (n = 55) and settlers (n = 27) were plotted, using total length in pediveligers and PII shell length in juveniles. Larval shell lengths were compared among development stages through a one-way analysis of variance (ANOVA) and subsequent *post-hoc* pairwise multiple comparisons, after normality (Shapiro-Wilk test; $p > 0.05$) and homoscedasticity (Levene’s test; $p > 0.05$) assumption checks. To identify differences in dissoconch shell lengths between juvenile stages (drifters *vs*. settlers), a Mann-Whitney *U* Test was carried out, as data was not normally distributed (Shapiro-Wilk test; $p < 0.05$). For both routines, the maximum sample size that could be met across developmental stages was used to allow for a balanced design (n = 27, randomly selected once). The drift ratio (Total shell length/PII) was calculated for all *I. alatus* juveniles (n = 82), and used as a proxy for juvenile drifting behaviour in the population (as previously published for a subtropical mytilid; Leal et al., 2018). For example, a juvenile with a ratio of less than or equal to 1, would have just settled and metamorphosed, with no post-metamorphic drift.

Permutational multivariate analyses of variance (PERMANOVAs) were carried out to investigate the variation in FA composition (% total lipid content; FAs computed as variables) between 1) daily dietary FAs available for the seston-feeding larvae and postlarvae (trophic status of nearshore Caribbean waters), 2) dietary FAs available during the pre- and post-settlement event (potential trophic stimulus), and 3) dietary FAs and FAs present in the neutral fraction of pediveligers (nutrient accumulation). Euclidian distances and 9,999 permutations were used to calculate pseudo-*F* ratios. Principal component analyses (PCA) were carried out to visualize the variation in FA composition between samples (dietary FAs and larval FAs; nutrient accumulation) and sampling events (pre- and post-settlement; potential trophic stimulus). PCA biplots (see Gabriel, 1971), displaying information both on samples (points) and variables (vectors), were used to visualize the 5 variables that contributed the most for the variation within the datasets.

A linear regression between salinity and larval supply (dependent variable; n = 23 sampling days) was carried out to determine if the onset of recruitment was related to a reduction in salinity, as concluded by Siung (1980). Because consecutive daily samples were collected, a temporal autocorrelation (i.e., serial correlation) was likely in all series involved. The Durbin-Watson statistic was used to test for autocorrelation at lag 1 in the residuals from the regression analysis. Because no autocorrelation was detected (see below), no adjustment of the model was necessary. To identify possible effect(s) of trophic conditions (TPM, SFAs, MUFAs, PUFAs) on the drift ratio of *I. alatus* juveniles, we carried out a forward stepwise regression model using daily averages collected over the sampling period (n = 14 days where juvenile drifters and settlers were observed and collected). We used the R software (version 3.6.1; R Core Team, 2019) and package ggplot2 (Wickham, 2016) to conduct statistical analyses and as graphical tools.

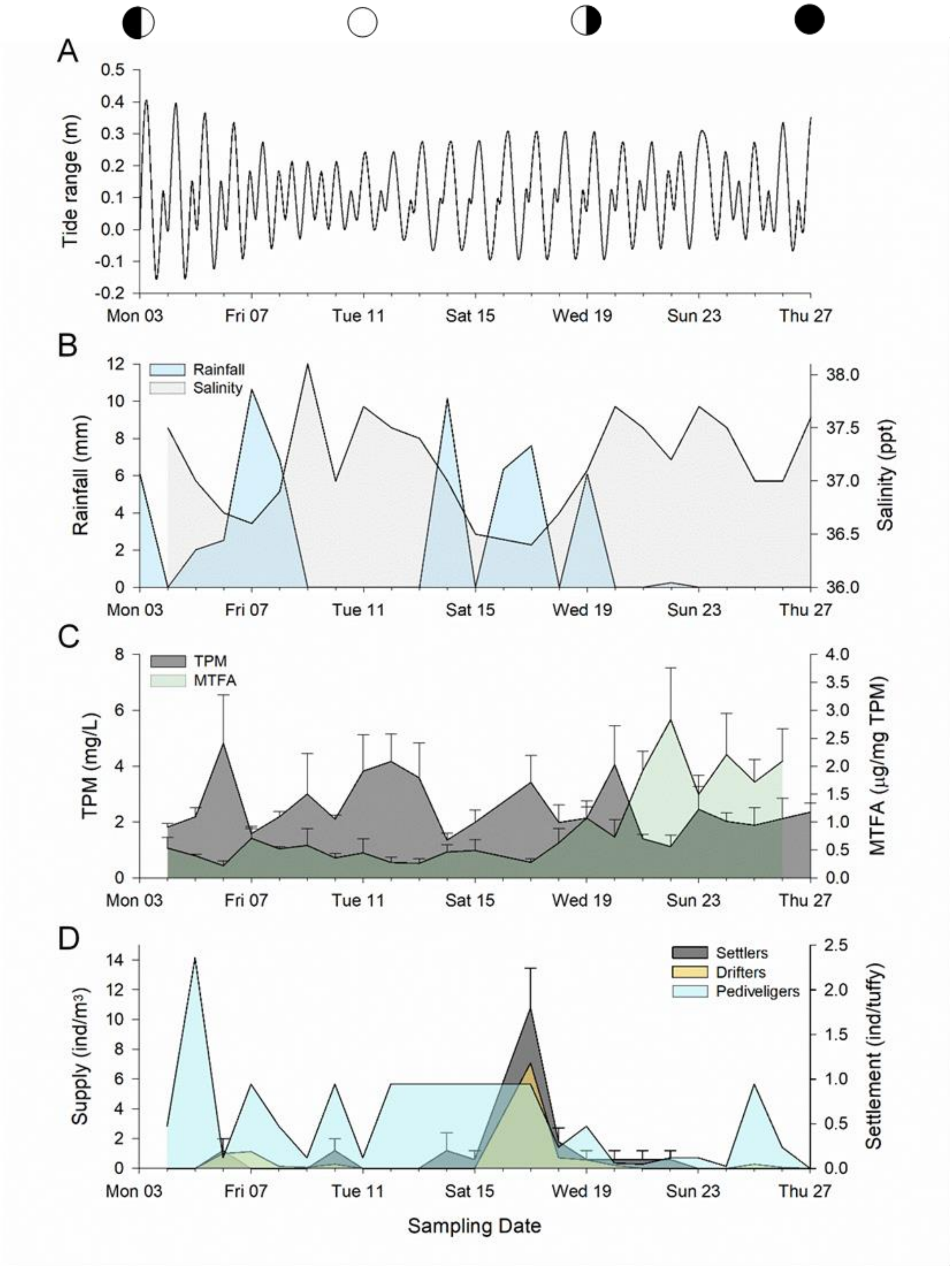

Figure 21. Time series plots describing A. Tide range; B. Rainfall and salinity; C. Total Particulate Matter (TPM) and Mass of Total Fatty Acids (MTFA); D. Density of the different *I. alatus* stages at Paunch beach.

3.5 RESULTS

3.5.1 Environmental conditions

Time series plots of the environmental conditions present during sampling are given on Figure 21. Tides ranged from 12 to 40 cm in a mixed semi-diurnal pattern (Fig. 21A). Rainfall ranged from 0 to 11mm, with 2 observed rainfall events lasting for 4 days (one from the 5th to 8th April and the second from the 14th to the 17th April; Fig. 21B). Salinity varied from 36 to 38, reaching its minimum on the 17th April (Fig. 21B). Food quantity (TPM) oscillated from 1 to 5 mg/L, and its quality (MTFA) from 0.2 to 3 μg/mg TPM (Fig. 21C). There was a successive dominance of pediveligers and juvenile drifters in the plankton (Fig. 21D), with 19 % of all planktonic *I. alatus* specimens collected corresponding to juvenile drifters. A single 2-day settlement peak was observed on the 17-18th April, that accounted for 65 % of all settlers sampled. This settlement pulse coincided with a peak of juvenile drifters (Fig. 21D), that accounted for 54 % of all drifters sampled. Note that lower salinities were related to higher concentrations of pediveligers per cubic meter ($p = 0.037$; $F = 4.96$; $R^2 = 0.19$; $DW = 2.14$).

3.5.2 Evidence for post-metamorphic drifting

Larval shell lengths of the different developmental stages were significantly different ($p < 0.001$; Table 7). Tukey test revealed size differences between pediveligers' total length (Mean ± SE, 272 ± 6 μm) and juveniles' PII size (drifters 321 ± 4 μm, settlers 323 ± 5 μm), yet no differences were found between PII measurements of drifters and settlers (see size distribution plots, Fig. 22A). Regarding post-metamorphic size (dissoconch shell length), no significant differences were observed between drifters (292 ± 33 μm) and settlers (311 ± 22 μm) ($p = 0.35$; $U = 310.0$). Yet, two modal sizes were observed, allowing us to differentiate between 2 size-groups (Fig. 22B). This bimodal size distribution may result from two different and discrete spawning events. Importantly, the older (longer-sized) cohort was the one that settled the most. The largest specimen sampled in this study was a drifter measuring 1,031 µm total shell length and 702 µm dissoconch.

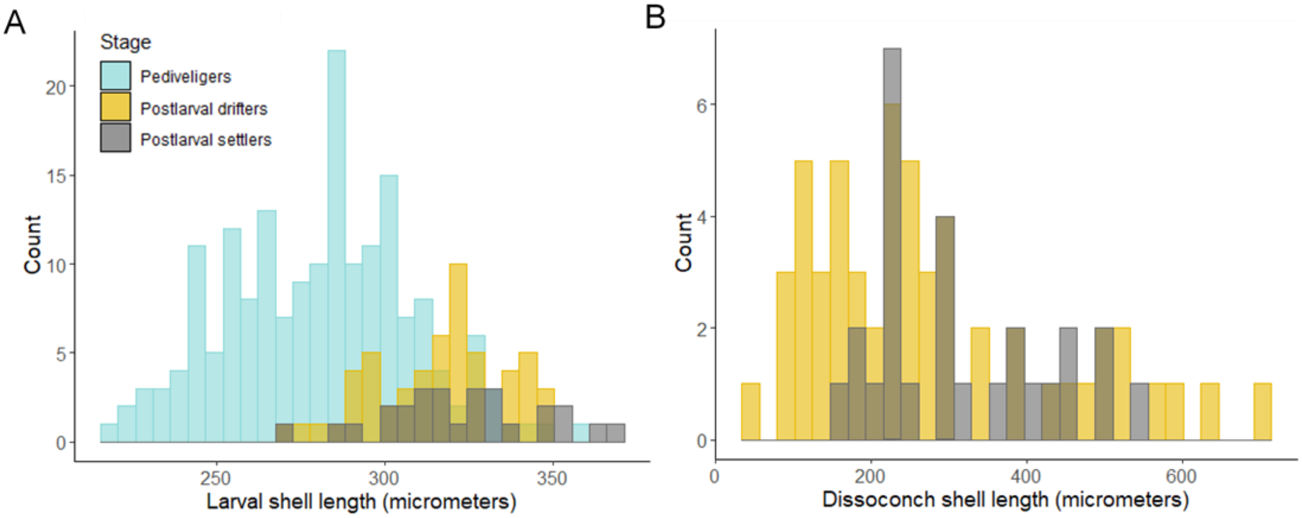

Figure 22. Shell-size distribution plots for *I. alatus* pediveligers (*n* = 180), drifters (*n* = 55) and settlers (*n* = 27). A. Larval shell (total length in pediveligers and PII length for juveniles); B. Dissoconch shell (post-metamorphic size).

Table 7. Analysis of variance result table concerning the larval shell length, i.e., size at metamorphosis (µm; dependent variable) across different developmental stages (pediveligers *vs*. drifters *vs*. settlers).

Source of Variation	***df***	***SS***	***MS***	***F-Statistic***	***p***
Larval shell					
Developmental Stage	2	45261.232	22630.616	39.52	**<0.001**
Residual	78	44668.383	572.672		
Total	80	89929.615			

The distribution of the drift ratio observed for juveniles of *I. alatus* collected daily in Paunch beach, either pelagic (drifters) or benthic (settlers) are plotted in Fig. 23. Ratios averaged 1.9, indicating that the bulk of juveniles collected had a pelagic post-metamorphic development. Drift ratio reached values as high as 3.1, indicating that part of the population was enduring long-term drifting. No apparent effect of trophic parameters on juvenile drifting ratio was detected in the regression analysis ($p > 0.05$).

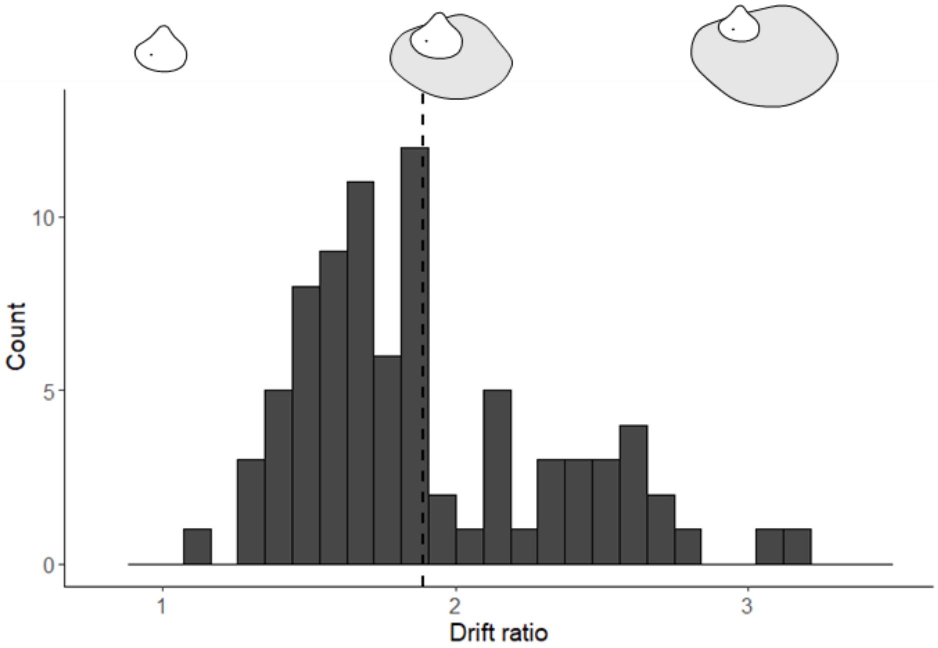

Figure 23. Frequency distribution of the drift ratio observed for juveniles of *I. alatus* collected in Paunch beach. Dashed line corresponds to the mean. Idents represent individuals with drifting ratios of 1 (no drift), 2 (short-term drifters) and 3 (long-term drifters).

3.5.3 Larval energetics and seston composition

There was a significant temporal variability in coastal trophic conditions (% FA composition; PERMANOVA, pseudo-$F_{day} = 2.81$, $p = 0.001$), suggesting that the nutritional status of nearshore waters in Paunch beach follows a rather erratic pattern. Yet, when comparing the FA composition of coastal TPM during the pre- and post-settlement event, there was a significant difference among periods (pseudo-*F* = 17.34, *p* = 0.001). The PCA biplot visually separated both periods, with PCA components cumulatively explaining 68.3 % of the variation in the dataset, and the first component explaining as much as 52.4 % of this variation (Fig. 24A). The PUFAs 18:3ω3 and 18:2ω6, tracers for mangrove detritus in tropical environments (Kelly and Scheibling, 2012), alone explained 10.5 % and 9.6 % of the variation in the first component, respectively. The SFAs 16:0 and 20:0 were also major contributors for the first component, explaining 10.5 % and 10.2 % of the variation, respectively. This PCA biplot clearly distinguishes the FA composition of seston during the pre- and post-settlement periods, showing that some temporal consistency might be found, depending on the observed scale (days, weeks or months). Note that the seston FA composition during the settlement peak coincided with that of the pre-settlement period, dominated by PUFAs.

Moreover, significant differences were found between FA composition of seston and larvae (pseudo-*F* = 21.31, *p* = 0.001), suggesting that although certain dietary FAs might be present in the water column, nutrient accumulation by pediveligers does not readily reflect such availability. A detailed description of FA profiles of seston and neutral lipids of larvae is given in Table 8. SFAs were the main group of fatty acids present in both samples (43 % in seston, 60 % in larvae), followed by PUFAs and MUFAs. Note that while seston was composed by 28 % EFA, larvae accumulated only 2 % in their neutral lipid reserves. This was visually confirmed by the PCA biplot comparing larval and sestonic samples (Fig. 24B). PCA components cumulatively explained 71.7 % of the variation in the dataset. We highlight the contribution of the EFAs, 20:4ω6, 20:5ω3 and 22:6ω3, together explaining 29.1 % of the variation in the first component. For the second component, we outline the contribution of SFAs, of which 16:0 alone contributed for 8.4 % of the variation in this component. This PCA biplot visually highlights that while EFAs and PUFAs were present in the coastal TPM collected, pediveligers mainly retained SFAs in their neutral lipid fraction.

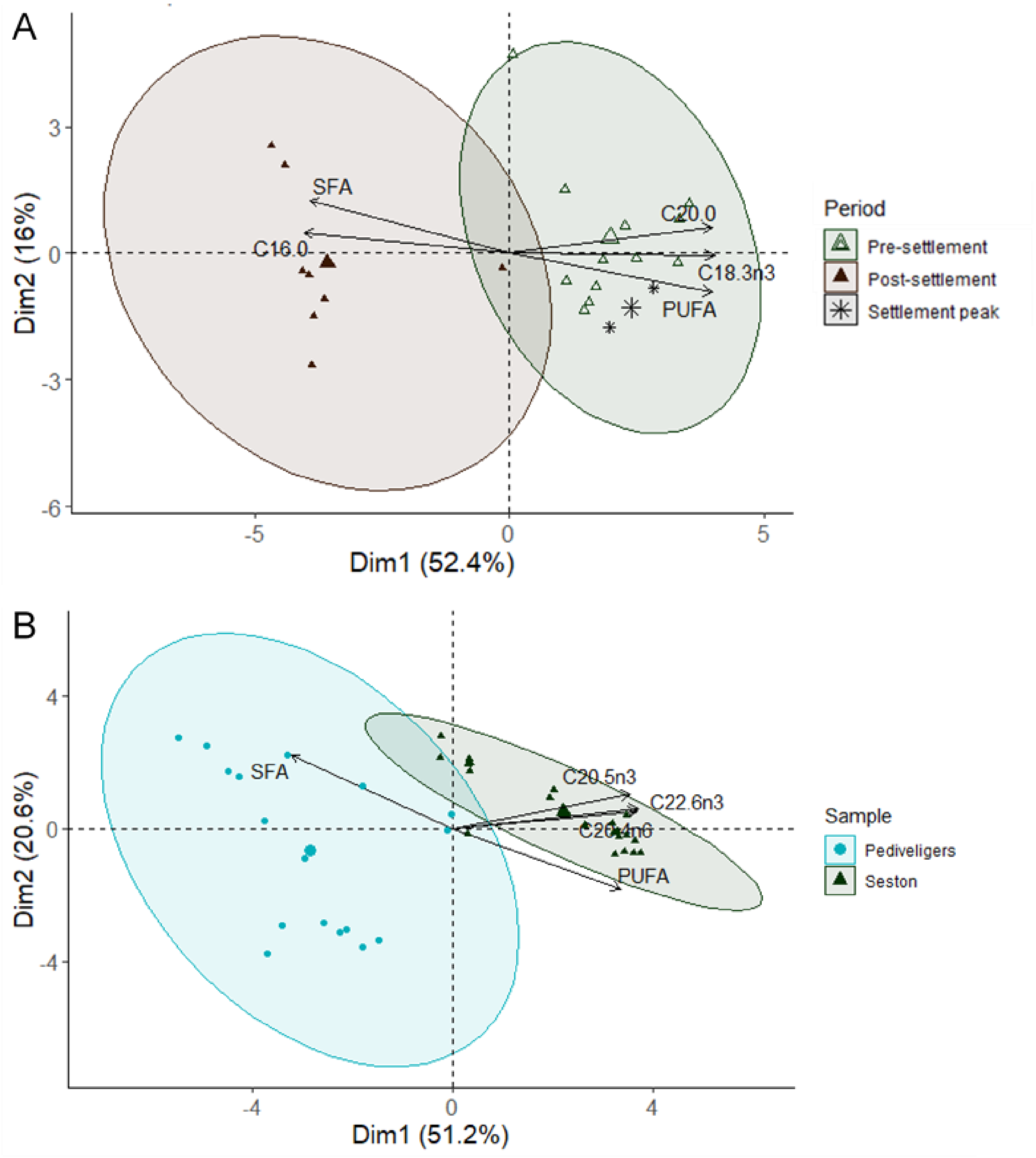

Figure 24. Principal Component Analysis (PCA) biplot of the fatty acid composition (% FA; averages of 3 daily replicate samples) for A. Total particulate matter (TPM) during the pre-settlement event (*n* = 36 samples), post-settlement event (*n* = 24 samples), and settlement event (*n* = 6 samples); B. Neutral fraction of *I. alatus* pediveligers (*n* = 17 samples of 20 larvae) together with the pooled TPM samples (*n* = 66 samples). PCA ellipses show 95 % confidence intervals. SFA: Saturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Table 8. Fatty acid composition (FA % ± SE) of seston (particulate matter) and pediveligers collected from nearshore waters. FAs accounting for less than 1 % of total FAs were removed. Abbreviations: SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; EFA, Essential Fatty Acids; MTFA, Mass of Total Fatty Acids.

	Seston	**Larvae**
Fatty acid	Total lipids	Neutral lipids
C14:0 **Myristic**	4 ± 0.1	4 ± 0.4
C15:0 **Pentadecanoic**	2 ± 0.0	1 ± 0.2
C16:0 **Palmitic**	23 ± 0.8	27 ± 2.1
C17:0 **Heptadecanoic**	2 ± 0.0	–
C18:0 **Stearic**	10 ± 0.5	27 ± 3.3
C20:0 **Arachidic**	2 ± 0.1	1 ± 0.2
Σ SFA	**43 %**	**60 %**
C16:1 **Palmitoleic**	7 ± 0.2	3 ± 0.7
C18:1ω9 **Elaidic**	5 ± 0.2	–
C18:1ω9 **Oleic**	10 ± 0.4	15 ± 1.8
Σ MUFA	**22 %**	**18 %**
C18:2ω6 **Linolelaidic**	5 ± 0.1	17 ± 2.5
C18:3ω3 **Alpha-Linolenic**	2 ± 0.1	2 ± 0.2
C20:4ω6 **Arachidonic**	9 ± 0.5	–
C20:5ω3 **Eicosapentaenoic**	11 ± 0.3	2 ± 0.8
C22:6ω3 **Docosahexaenoic**	8 ± 0.3	–
Σ PUFA	**35 %**	**21 %**
Σ EFA	**28 %**	**2 %**
MTFA	**0.95 µg·mg TPM^{-1}**	**0.02 µg·larva^{-1}**

3.6 DISCUSSION

The present study comprises the first documented evidence of juvenile drifting in the Caribbean flat tree-oyster, *I. alatus*. We observed successive dominance of late-stage larvae (pediveligers) and postlarvae (drifters) of this isognomonid in the water column, suggesting that conditions favoring postlarval drifting were present at the time of samplings, and/or postlarval drifting may be an integral part of the life cycle for this species (as suggested for other bivalve taxa; Baker and Mann, 1997). This result validates the first part of our hypothesis suggesting that juvenile drifting occurs in bivalves from tropical habitats.

Susceptibility to passive resuspension for *I. alatus* likely depends on individual's density and drag, the last being definitely larger in juveniles with greater dissoconch shell. This might explain why the largest pelagic juvenile found in this study was a drifter (1,031 µm). The length of the dissoconch shell (secreted after metamorphosis) of recently settled (1 day) juveniles (154 µm to 539 µm) indicates that settled individuals were previously drifting. Moreover, our results show that modal sizes of juvenile drifters and settlers overlap in this species. Flat shell morphologies have been associated with lower sinking speeds (e.g., scallop *Pecten maximus*, Forêt et al., 2018a). Similarly, *I. alatus* shell morphology (i.e., flat, thin shells and large umbonal angle) may give postlarval individuals a gliding shape that favors drifting in the water column.

The second part of our hypothesis suggesting that secondary migrations were mediated by the trophic status of nearshore waters (as demonstrated for subtropical and temperate systems; Leal et al., 2018; Forêt et al., 2018b) is not confirmed for *I. alatus*. Yet, we provide additional *in situ* evidence that low salinities (driven by heavy rainfall; Siung, 1980) may mediate settlement dynamics in this region by triggering early settlement stages (pediveligers). Salinity has been considered a major spawning trigger for other tropical bivalves (e.g., pearl oysters *Pinctada maxima*, Knuckey, 1995; *Pinctada imbricata*, Urban, 2000). Knuckey (1995) observed that during monsoon rains, particulate matter increased and salinity decreased due to run-off, and the nutrients washed into the sea favored spat settlement. In Bocas del Toro, heavy rainfalls at the onset of the rainy season may produce low-salinity plumes that flow on top of the typically warm Caribbean waters (Kaufmann and Thompson, 2005). Such nutrient input from terrestrial runoff may constitute an energetic subsidy to nearshore benthic communities (Gorman et al., 2018). Indeed, we detected a shift in trophic conditions in coastal waters, with organic matter dominated by tracers of microalgae and vascular plant matter shifting to an SFA-dominated composition (tracers for detritic matter and bacterial load) over a fine-temporal scale. It is possible that such shifts in nutritional quality of nearshore waters at Paunch beach were driven by heavy rainfall, but such effect(s) remain to be tested. Further studies should aim for longer-term samplings, where seasonal patterns (e.g., rainy *versus* dry season) may become evident.

Our results of the nutritional quality of nearshore Caribbean waters are indicative of an oligotrophic system (MTFA; ≈ 1 μg·mg TPM^{-1}), contrasting with values reported for meso-oligotrophic environments (e.g., subtropical Brazilian coast; Leal et al., 2018). Essential FAs (20:4ω6, 20:5ω3 and 22:6ω3) composed 28 % of seston, indicating an important phytoplanktonic food source for filter feeders (e.g., Perez et al., 2013; Toupoint et al., 2012a). These FAs are biosynthesized by marine microalgae (Bergé and Barnathan, 2005) and used in ecological studies as phytoplanktonic trophic markers (Dalsgaard et al., 2003). In our study, similar levels of FA markers of diatoms (11 % of EPA, 20:5ω3) and dinoflagellates (8 % of DHA, 22:6ω3; Budge and Parrish, 1998) were observed in the seston, indicating that these two groups were present and available (but low-concentrated) for feeding larvae and juveniles.

Following feeding, FA markers can be recognized in the lipid profile of their consumers (Dalsgaard et al., 2003, Bergé and Barnathan, 2005). Although microalgae constitute a major source of essential FAs needed for survival and growth of bivalve larvae (Pernet and Tremblay, 2004), very low levels of these EFAs were observed in *I. alatus* pediveligers (2 % of neutral reserves). However, only the neutral fraction (energetic lipid reserves) of pediveligers was analyzed, which means that the level of EFAs transferred to the membranes (polar lipids), for example, could not be estimated. We observed that larvae largely assimilated and accumulated SFAs (60 % of neutral reserves). This pattern might be explained by 1) the high metabolic demand of this larval stage, which may require the transfer of assimilated EFAs to tissue development during metamorphosis (as opposed to energy storage), and/or 2) the β-oxidation of SFAs releases energy more efficiently than that of EFAs (Langdon and Waldock, 1981), which may be advantageous under high energetic demands (e.g., changing osmoregulatory conditions; Cabrol et al., 2015). Importantly, our results demonstrate that the presence of dietary FAs in the water column does not necessarily reflect FA accumulation in late-stage *I. alatus* larvae. FA profiles of the neutral lipid fraction in pediveligers showed the ingestion of particles from terrestrial inputs and macrophyte residues, with two FA markers of these sources (18:2ω6 and 18:3ω3; Budge and Parrish, 1998) constituting nearly 20 % of the larval lipid content.

Analyses of shell development of juveniles showed that the bulk of settlement for *I. alatus* takes place when prodissoconch II exceeds 300 μm, after theoretical competence (the size of pediveligers in the water column), suggesting the presence of metamorphosis delay (Martel et al., 2014). Later dissoconch shell built-up may assist drifting and advection, necessarily increasing dispersal potential. However, no trophic cues capable to deter these wanderers were detected. It is possible that 1) in this tropical system, individuals get more competent with age, i.e., drifters settle as they age, and/or 2) *Isognomon*, a tropical taxon that is more adapted to oligotrophy, compared to other subtropical (e.g., *Brachidontes solisianus*; Leal et al., 2018) and temperate bivalves (e.g., Forêt et al., 2018a), does not respond to trophic triggers. These findings point to the need of accounting for juvenile drifters in *I. alatus* settlement estimates, as the spatial distribution of late larval stages and/or early settlers might not reflect their final distribution. Knowing the size of bivalves at settlement is crucial for managing and exploiting natural stocks. Indices of spatfall (i.e., settlement and attachment) provide managers the possibility of predicting major fluctuations in recruitment (Sause et al., 1987). In the particular case of *I. alatus*, the findings of this study contribute to a better assessment of its natural supply for commercial production. Although we advance knowledge on the settlement dynamics of the flat tree oyster, many questions remain unanswered. We hope this work sets the stage for future studies to better understand dispersal mechanisms in tropical systems, the scale of dispersal and the ecological significance of drifting juveniles.

3.7 ACKNOWLEDGEMENTS

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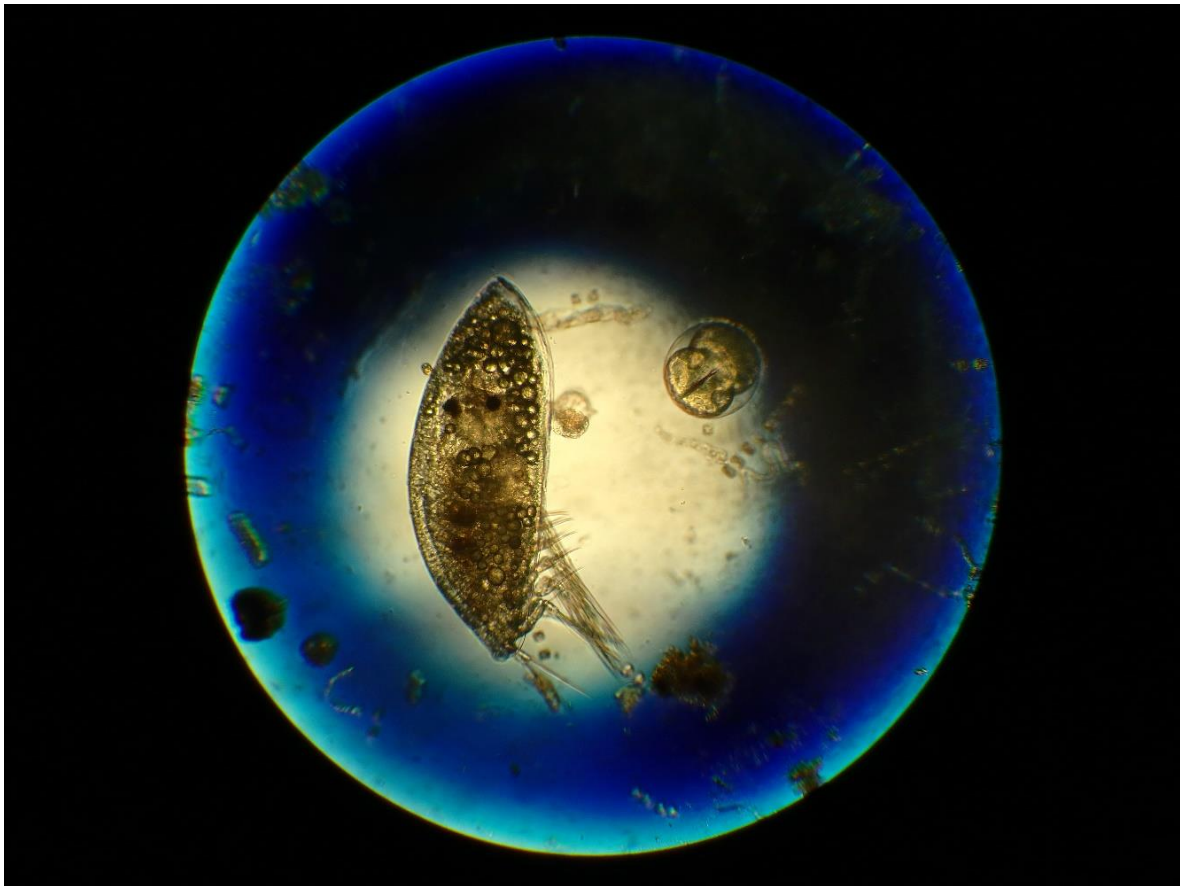
CHAPTER 2

LECITHOTROPHIC BARNACLES



“Energy reserves in the Arctic”: Micro-imaging Honourable mention by the Royal Society Publishing Photography Competition 2018. Caption: “Invertebrate larvae face many challenges before settling on hard ground. They must rely on their energy reserves to sustain the dramatic process of metamorphosis (…) Here, I highlight the fascinating larval ecology of these invertebrates, that still have secrets to yield.”

ARTICLE 4

LIPID ALLOCATION IN LATE-STAGE BARNACLE LARVAE FROM SUBTROPICAL AND TEMPERATE WATERS

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IN PRESS IN MARINE ECOLOGY PROGRESS SERIES

[RESEARCH ARTICLE]

4.1 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 *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 (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 ± 0.4 mg Chl-*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 comparable for both chthamalids, with cyprids equally storing TAG reserves (≥ 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.

Keywords: Supply-side Ecology, Settlement dynamics, Larval physiology, Lipids, Energetics

4.2 RÉSUMÉ

La transition de la plupart des larves d’invertébrés marins d’une vie pélagique à une vie benthique nécessite une énergie suffisante pour soutenir la métamorphose, et peut être compromise par une limitation de la nourriture au début de l’ontogénie. Nous avons réalisé une étude comparative entre une espèce subtropicale et tempérée pour mieux comprendre la physiologie larvaire des espèces fondatrices côtières exposée à des régimes contrastés de productivité primaire : *Chthamalus bisinuatus* dans le sud-est du Brésil, sous un régime méso-oligotrophique, et *Chthamalus montagui* en sud-ouest de l’Angleterre, sous un régime hautement productif. Nous avons utilisé un indice basé sur la composition lipidique, le ratio des triacylglycérols (TAG) sur les phospholipides (PL) (lipides énergétiques / structuraux), pour caractériser l’allocation lipidique dans les tissus larvaires, et nous avons anticipé l’épuisement des réserves de TAG chez les cypridés provenant de zones moins productives. Malgré les niveaux considérablement différents de productivité primaire entre les eaux subtropicales (1,31 ± 0,4 mg Chl-*a* / m^3) et tempérées (3,09 ± 1,2 mg Chl-*a* / m^3), les ratios TAG / PL et le succès de fixation étaient comparables pour *C. bisinuatus* et *C. montagui*. L’allocation physiologique des lipides de chaque cohorte quotidienne était aussi comparable pour les deux chthamalides avec des réserves de TAG $\geq$ 50 % de la teneur en lipides totaux. Cela indique un seuil énergétique au-dessus duquel les nauplii ne peuvent pas se développer en cypride et / ou une sélection pour l’accumulation de lipides dans des conditions trophiques pauvres. Nous soulignons les défis liés à la relation directe des estimations de la productivité primaire avec l’approvisionnement alimentaire et l’état physiologique des larves, car des concentrations plus faibles de Chl-*a* n’indiquent pas nécessairement une limitation alimentaire pour les nauplii. Nous proposons un modèle conceptuel pour clarifier le processus d’allocation des lipides (énergétiques aux lipides structuraux) dans les tissus larvaires.

Mots clés : Théorie «supply-side ecology», Dynamique de fixation, Physiologie larvaire, Réserves lipidiques, Energétique

4.3 INTRODUCTION

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 1982, Roughgarden et al. 1985, Rodriguez 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. 2008, Kasten et al. 2019a) patterns of pelagic food supply as indicated by chlorophyll-*a* concentration in coastal waters (Hawkins & Hartnoll 1982, Menge 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 chlorophyll-*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, more evidence has emerged 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 & Emlet 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 of temperature) were addressed by classic works 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 Laptikhovsky 2006, for a new look at Thorson's and Rass's rules). Further, a decreasing 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., planktonic 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 raise baseline oligotrophic conditions to eutrophic ones during short periods, 1 to 2 days; Kasten & Flores 2013) plus nutrient-rich estuarine plumes (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 synthetic process of 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 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 late-stage 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 & 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, specialized for substrate exploration and settlement. We assessed lipid allocation in cyprid larvae by using a ratio of energetic to structural lipids (triacylglycerol 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 lower TAG/PL and settlement success of *C. bisinuatus* compared to *C. montagui* 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 been developed to place our work into a wider context.

4.4 MATERIAL AND METHODS

4.4.1 Study sites and species

Late-stage (cyprid) larvae of two chthamalid barnacle species were sampled from two different latitudes (subtropical and temperate) in the Atlantic: *Chthamalus bisinuatus*, on the 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 25). 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 two sites per region (separated by 3–6 km), during March 2015 in São Sebastião and August 2015 in Plymouth Sound. These dates correspond to late summer/early autumn months in either region. 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 Kingdom (MBA, Plymouth, British Isles), and later analysed at the Institute of Ocean Sciences 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 (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 saturated (close to 100 % cover) *Chthamalus* zone in the mid-high littoral (e.g., Kasten and 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 chlorophyll-*a* pattern reaching maximum concentrations in winter (0.36 ± 0.12 mg/m^3; Ciotti et al. 2010). During late summer, organic detritus dominate nearshore suspended matter composition over the SSC (Leal et al. 2018).

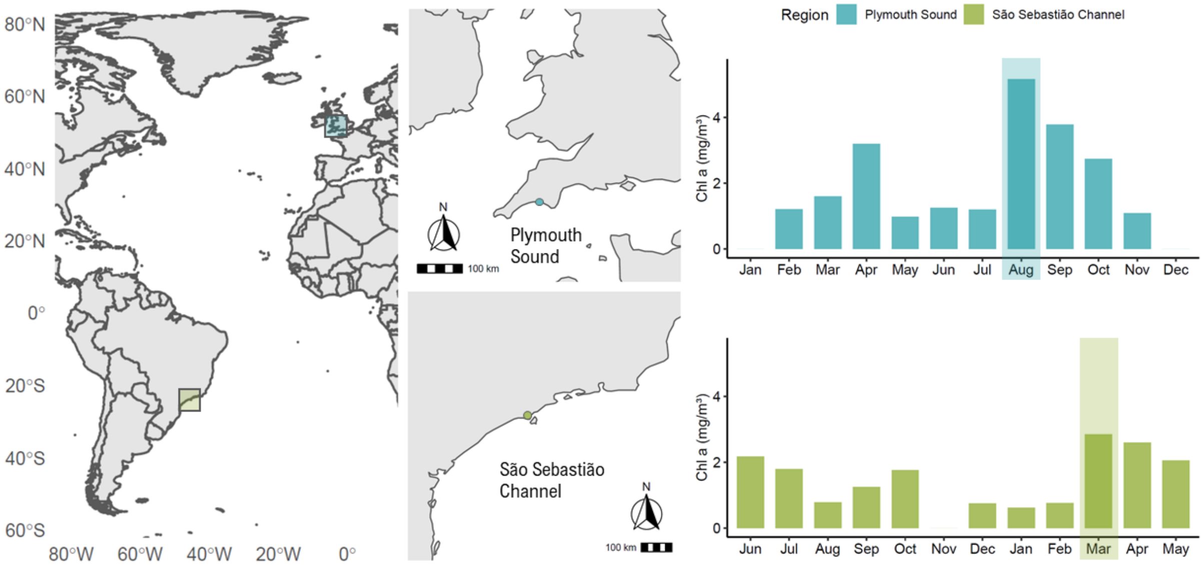

Figure 25. Map displaying sampled locations: southwest of the British Isles (Plymouth Sound) and southeast Brazil (São Sebastião Channel). Monthly estimates of surface chlorophyll-*a* concentration (Chl a; mg/m^3), acquired from MODIS-Aqua NASA Ocean Color database using Level-3 mapped images of 4 km 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.

In the English Channel, the rocky shores of Mount Batten (50°21'28"N, 4°7'41"W) and Plymouth Hoe (50°21'47"N, 4°8'17"W) were sampled. These shores are located in Plymouth Sound (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 two yearly productivity peaks (spring bloom of diatoms followed by summer dominance of flagellates; Rodriguez et al. 2000). In contrast with other cold-temperate acorn barnacle species such as *Semibalanus balanoides*, in which release of single larval brood is synchronized to match the predictable spring phytoplankton bloom of diatoms (e.g., Barnes 1956, Crisp & Spencer 1958, Hawkins & Hartnoll 1982), flagellates appear to be the type of phytoplankton which best promotes successful development of the planktonic larvae of *Chthamalus* (Moyse 1963; Burrows et al. 1992). Chlorophyll-*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 higher during the characteristic spring and autumn phytoplankton blooms (reaching nearly 3 mg/m^3 from August through September; Skákala et al. 2018).

4.4.2 Field sampling

Sampling was conducted consecutively over a 4-day period in Brazil (03-March to 06-March-2015) and in the British Isles (19-Aug to 22-Aug-2015). Two replicate plankton tows (10 m apart; mesh 160 µm; 50-100 m tow length at sub-surface) were collected daily at each of the 2 sites in the São Sebastião Channel and Plymouth Sound, 100-200 metres offshore, to estimate larval supply (cyprids/m^3) and larval morpho-physiological traits. A minimum of 20 cyprids per tow sample were collected for morphometric measures (see below 4.4.4) and lipid analysis (see below 4.4.5). 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 (over 1500 chthamalid cyprids sampled in the PS area; Jenkins 2005). The cyprid carapace length was therefore used to distinguish between both temperate *Chthamalus* spp. (Power et al. 1999). All temperate cyprids analysed were well below 540 µm (see results), the reported length threshold for *C. montagui* (Powers et al. 1999, Jenkins 2005). Daily settlement rates (individuals/plate/day) were determined by random deployment of 10 settlement plates (10cm × 10cm; covered with rough tape 3M™ Safety-Walk) over the 4-day sampling period at each site. Plates were replaced every 24 hours and surroundings were cleared for potential predators. Settlement success was estimated by calculating settlement/supply ratios, and the size of the recently metamorphosed juveniles (rostro-carinal diameter) was measured (see below 4.4.4).

4.4.3 Remote estimation of primary productivity

The trophic conditions at each sampling region were characterized using remote estimates of chlorophyll-*a* concentration (Chl-*a*; mg/m^3), a commonly used proxy of food supply (similarly to 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/l3/). Level-3 mapped images of 4 km resolution were processed using the software SeaDAS (Version 7.5.3). Local estimates were averaged for one specific cell (4 km × 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 where remote sensing data was available for the São Sebastião Channel (23°56'15''S, 45°26'15''W; dates: 23-02-15, 25-02-15, 27-02-15, 03-03-15, 04-03-15) and Plymouth Sound (50°16'15''N, 4°11'15''W; dates: 06-08-15, 08-08-15, 17-08-15, 18-08-15, 23-08-15). The extracted dates spanned the pelagic larval development (from nauplius to cyprid) of both species (12 days for *C. bisinuatus*, Barbosa et al. 2016; 14-17 days for *C. montagui*, Burrows et al. 1999).

4.4.4 Morphometric analysis

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 length; µm) and (2) lipid droplet cover (droplets 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 (Figure 26), 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 (Guerra et al. *unpublished*). 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 ×) in lateral view for later measurements through image analysis. Similarly, juveniles settled on the artificial plates (1-day 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).

Figure 26. Live *Chthamalus bisinuatus* cyprid larvae in lateral view. Conspicuous lipid droplets are visible in the anterior part of the carapace, the main larval energy storage.

4.4.5 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 an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan) developed in a four-solvent system (Parrish 1999). Three main lipid classes were identified:

Triacylglycerols (TAG), phospholipids (PL) and acetone mobile polar lipids (AMPL). TAG are storage lipids that make up most of the energetic reserves, PL are the main components of cell membranes, and AMPL are a group constituted mainly from glycolipids, pigments, and degradation products of PL (Parrish 1999). Storage lipids are generally associated with energy supply, while structural lipids are important for the 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).

4.4.5.1 The TAG/PL ratio

Given the size-dependency associated with TAG (Fraser 1989), the ratio of triacylglycerol to phospholipid (TAG/PL ratio) was used to characterize lipid allocation in larval tissues, that is, standardized TAG measures by a lipid class related to body size, PL. The ratio of storage to membrane lipids has been found to be 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 less than 0.2. For lobster larvae, Harding & Fraser (1999) considered a condition index less than 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) have shown that the ratio of storage to membrane lipids in *C. 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.

4.4.6 Statistical analysis

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. 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 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 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 two-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 morpho-physiological larval traits, a Pearson correlation matrix was computed for the variables cyprid 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.

4.5 RESULTS

4.5.1 Local conditions: trophic regime, supply, and settlement

Monthly estimates of pelagic primary production (Chl-*a*) for 2015 (Fig. 25) revealed that sampling was carried out during a peak of Chl-*a* for both the São Sebastião Channel (SSC) (March, 2.85 mg/m^3) and Plymouth Sound (PS) (August, 5.17 mg/m^3). Throughout the sampling period, daily estimates averaged 1.31 ± 0.4 mg/m^3 for the SSC, contrasting with the higher Chl-*a* concentration of PS, 3.09 ± 1.2 mg/m^3 (Fig. 27A). Larval supply (Fig. 27B) was around three 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 daily settlement rates (Fig. 27C), with *Chthamalus montagui* settling at higher rates (~11 settlers/plate/day) compared to its subtropical congener *Chthamalus bisinuatus* (~ 4 settlers/plate/day). However, settlement/supply ratios (Fig. 27D) were similar, with no differences being found between regions, nor between sites within regions ($p > 0.05$; Table 9). 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. Within-region variability (coefficient of variation; % CV) was, however, 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.

Table 9. Nested analyses of variance (ANOVA) summary table for regional/species differences (São Sebastião Channel/*C. bisinuatus versus* Plymouth Sound/*C. montagui*) in supply to settlement patterns and in lipid allocation in cyprid tissues given by the TAG/PL ratio.

	Settlement/Supply ratio					**TAG/PL ratio**				
Source	*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		

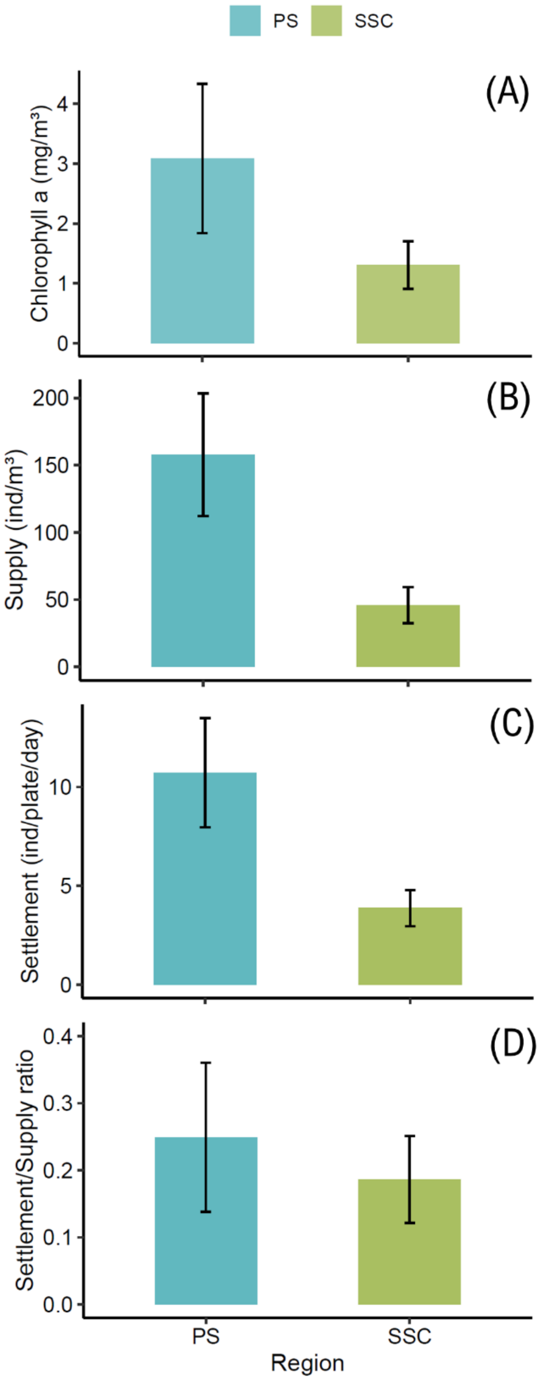

Figure 27. Daily parameters found at each sampling location, (A) Chlorophyll-*a* concentration, i.e., estimate of primary productivity, (B) Supply, i.e., cyprid abundance, (C) Settlement rate, and (D) Settlement/Supply ratio, i.e., a measure of settlement success. Error bars correspond to mean ± SE. SSC stands for São Sebastião Channel (*C. bisinuatus*) and PS for Plymouth Sound in the English Channel (*C. montagui*).

4.5.2 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. 28A) and juvenile stage ($K_{d\ 148,\ 442} = 7.50$, $p < 0.0001$; Fig. 28C), 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. 28B), with *C. bisinuatus* having greater covers than the temperate *C. montagui*. *C. bisinuatus* averaged 224 ± 28 µm in cyprids' carapace length, 24 ± 8 % in lipid droplet cover, and 226 ± 23 µm in juvenile size (Mean ± SD). *Chthamalus montagui* averaged 290 ± 70 µm in cyprids' carapace length, 14 ± 7 % in lipid droplet cover, and 283 ± 41 µm in juvenile size.

A detailed description of the lipid classes found in the larval tissue of both chthamalids is given in Table 10. Total lipid content averaged 50 µg/cyprid for *C. bisinuatus*, and 48.5 µg/cyprid for *C. montagui*. Triacylglycerols (TAG) and phospholipids (PL) were the major lipid classes of both species, constituting more than 90 % of the total larval 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 9), indicating that larvae similarly allocated lipids to energy storage and cellular structure, and that larvae had previously accumulated TAG reserves during their naupliar development. There was, however, considerable intra-specific variation (% CV) in this condition index (30 % in *C. bisinuatus* and 32 % in *C. montagui*), which might explain the nearly significant effect of site in the ANOVA comparisons ($p = 0.05$; Table 9).

The relationship between the TAG/PL ratio and other larval traits shows that cyprids with similar physiological status may vary in size (Fig. 29A), lipid droplet cover (Fig. 29B), and total lipid content (Fig. 29C); yet, some patterns emerged. The highest ratios (TAG/PL ≥ 1.5) found in *C. montagui* cyprids comprised small-sized cohorts (< 300 µm) with lipid droplets covering a greater area of the body (≥ 20 % cover). This was confirmed by the positive relationship between TAG/PL and lipid droplet cover ($p = 0.01$, $r = 0.90$; Table 11). 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 11). No significant relationships were detected among *C. bisinuatus* larval traits (Table

11). *Chthamalus 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 (≥ 20 % cover).

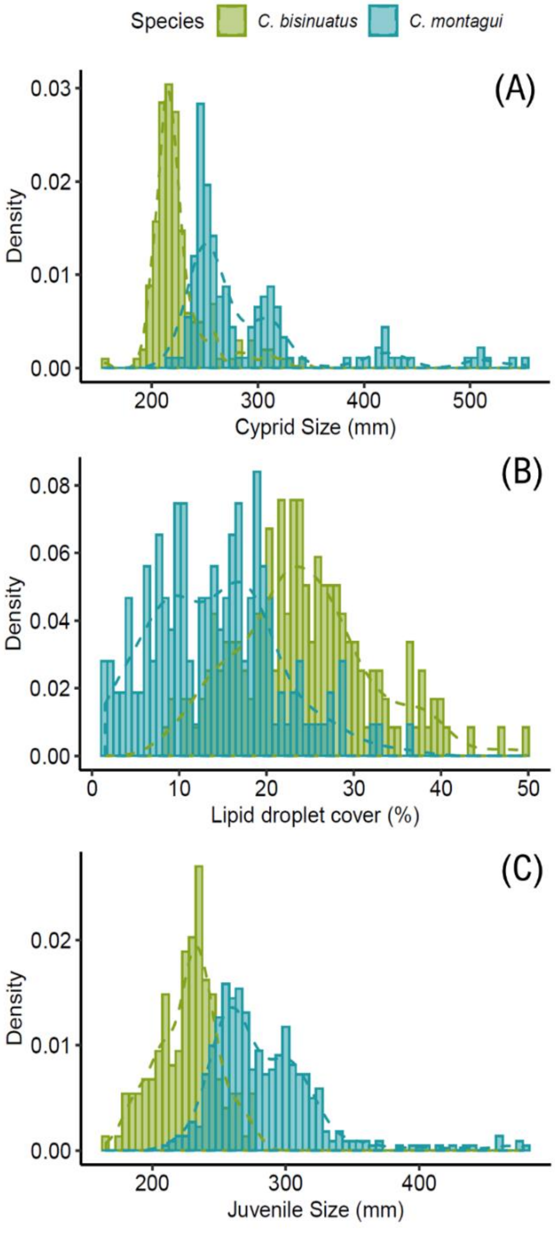

Figure 28. Histograms showing the distributions with density curves for *C. bisinuatus* (subtropical) and *C. montagui* (temperate): cyprid larvae (A) carapace length and (B) lipid droplet cover, and early (1-day old) juveniles (C) rostro-carinal diameter.

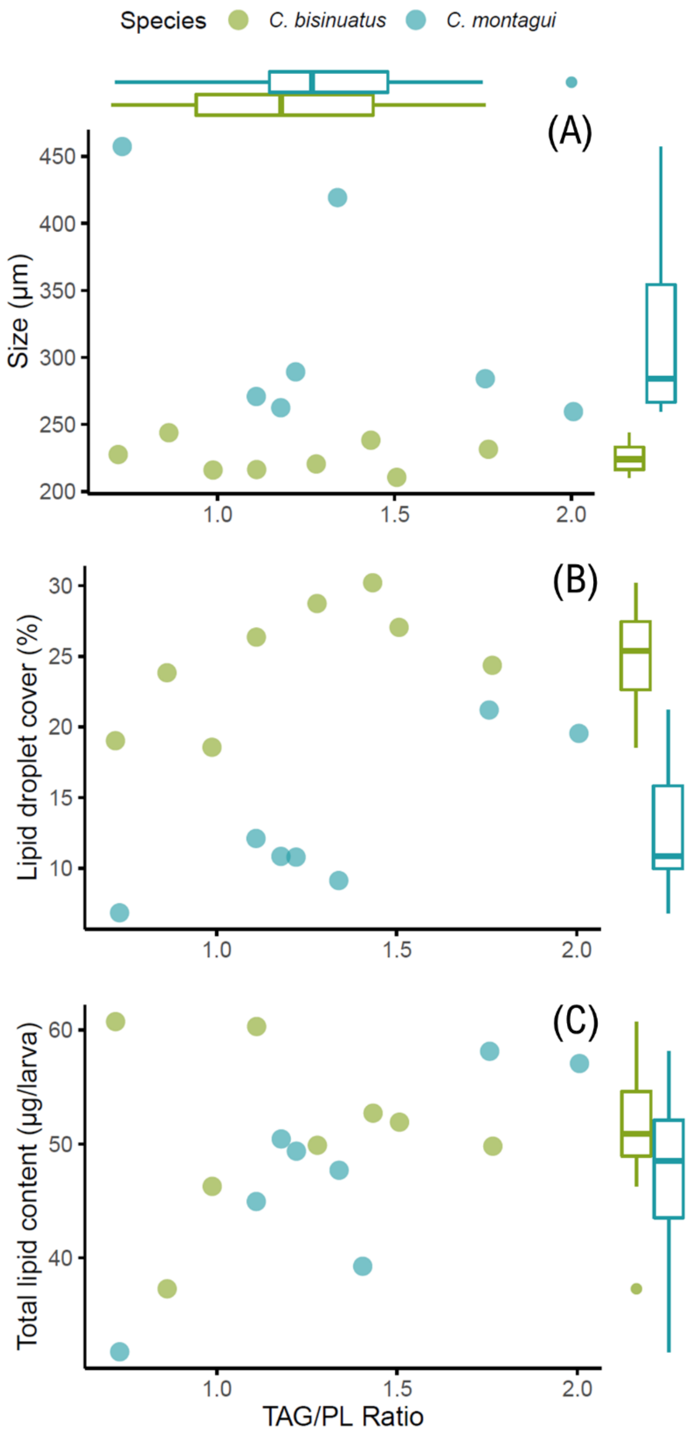

Figure 29. Series of scatterplots with marginal boxplots showing the TAG/PL of daily cyprid cohorts (daily average of two replicate plankton tows per site) plotted against other morpho-physiological traits: (A) size, (B) lipid droplet cover, and (C) total lipid content.

Table 10. Lipid class composition of the cyprids collected from the subtropical (*C. bisinuatus*) and temperate (*C. montagui*) nearshore waters. Lipid classes (% ± SE), divided in TAG (triacylglycerol), PL (phospholipids) and AMPL (acetone mobile polar lipids), constituted the larval total lipid content (µg/larva ± SE). The TAG/PL Ratio, i.e., the ratio of storage to membrane lipids, is provided for both species.

Lipid class (%)	***Chthamalus bisinuatus***	***Chthamalus montagui***
TAG	49.8 ± 1.8	52.8 ± 2.1
PL	41.3 ± 1.4	41.6 ± 1.9
AMPL	8.8 ± 0.9	5.6 ± 0.4
∑ Lipid content (µg/larva)	50.0 ± 1.9	48.5 ± 2.3
TAG/PL Ratio	**1.21**	**1.27**

Table 11. Pearson correlation coefficients for cyprid morpho-physiological traits. Each sample (*n*) corresponds to the daily average of two replicate plankton tows, representing daily cohorts. Significance denoted as '***' 0.001; '**' 0.01; '*' 0.05; '**.**' 0.1.

		Chthamalus bisinuatus			***Chthamalus montagui***		
Variable		Size	Droplet cover	Total lipid	Size	Droplet cover	Total lipid
TAG/PL	*r*	-0.10	0.60	0.00	-0.55	0.90	0.82
	p-value	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-value		0.85	0.29		0.12	0.05 **.**
	n		8	8		7	7
Droplet cover	*r*			0.04			0.84
	p-value			0.93			0.02 *
	n			8			7

4.6 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 *Chthamalus bisinuatus* and *Chthamalus montagui*. We highlight the challenges 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-physiological traits when assessing lipid allocation (energetic to structural lipids) in the tissues of cyprid larvae.

4.6.1 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 cyprids of both *Chthamalus* species here tested. 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 and Walker (1975) in 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).

Total lipids and TAG are predictors of settlement success, post-metamorphic growth, and survival in other species (e.g., *Balanus 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, Freites 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. 30). We found similar ratios for both chthamalid barnacle species, with a TAG/PL ratio above 1 (1.2 *C. bisinuatus*, 1.3 *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. 30). For instance, focusing 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 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 a balanid barnacle (*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 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, Giménez et al. 2017, Hentschel and Emlet 2000), the physiological condition of both chthamalids here described likely reflects local selection for larval phenotypes that match the requirements for juvenile survival (see 4.6.3 discussion below).

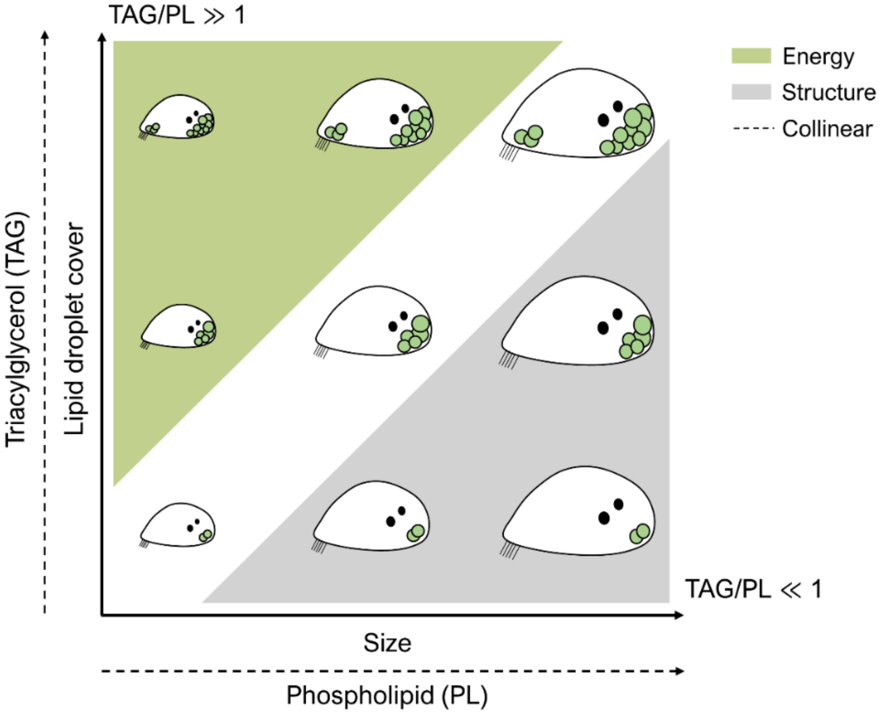

Figure 30. Conceptual model for the integration of morpho-physiological traits when assessing lipid allocation (energy, structure) in late-stage barnacle larvae. The triacylglycerol (TAG) to phospholipid (PL) ratio can be used as an index of physiological condition. For instance, larvae with a TAG/PL = 1 (non-shaded area of the scheme) resemble in physiological condition, although having different sizes and 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.

4.6.2 Effects of food supply

Our findings show that lower Chl-*a* concentrations do not necessarily indicate food limitation for barnacle nauplii (see below 4.6.3), 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 below 4.6.3). 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, herbivorous zooplankton are, however, rarely limited by food (Strathmann 1996), and barnacle nauplii are unlikely to be 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 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 by Bemal and Anil (2019). The authors found that picoplankton (< 2.0 μm), 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 (Bemal & Anil 2019). Even species adapted to more productive regimes, such as the nauplii of *Chthamalus montagui* and *Balanus perforatus*, have been grown successfully when fed on small cells (< 5 μm) of *Micromonas pusilla* and *Isochrysis galbana* (Stone 1989). Vargas et al. (2006) also reports that nauplii of two barnacle species, *Jehlius cirratus* and *Notobalanus flosculus* from central Chile, primarily graze on picophytoplankton and small nanophytoplankton (< 5 μm) when incubated in natural sea water. 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 possible therefore 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 (Leal et al. *in prep*). In *C. bisinuatus* for instance, a large proportion of energetic reserves contain trophic tracers for terrestrial detritus and macrophytes (Leal et al. *in prep*). Triacylglycerols, the main constituent of larval reserves, are tri-esters consisting of a glycerol 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., 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 (neutral) lipid droplets (Hentschel & Emlet 2000). Considering the duration of pelagic larval development from nauplius to cyprid of both *Chthamalus* species – 12 days for *C. bisinuatus* (Barbosa et al. 2016), and 14-17 days for *C. montagui* (Burrows et al. 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 short periods (1 to 2 days) 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 % to 14 % size increase; Kasten et al. 2019b), which may compensate for the low food supply in these subtropical shores.

4.6.3 Implications

4.6.3.1 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 essential at crowded shores, Kasten et al. 2019b). It 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). In 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 on 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 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 replication using a nested design, if wanting to draw wider conclusions about latitudinal patterns. Ideally, more than one species should be used in each locality, with several localities in both 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 longer larval life with greater dispersal (Burrows et al. 1999, Pannacciulli et al. 1997) and is more prevalent on exposed headlands (Crisp, Southward & Southward 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.

4.6.3.2 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 ± 0.4 mg/m^3 in the SSC, southeast Brazil, *vs.* 3.09 ± 1.2 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 Supplementary Material Fig. 31), 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, e.g., surf-zone hydrodynamics 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, are often overlooked. 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, compromising 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).

4.7 CONCLUSION

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 two 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.

4.8 ACKNOWLEDGEMENTS

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4.9 SUPPLEMENTARY MATERIAL

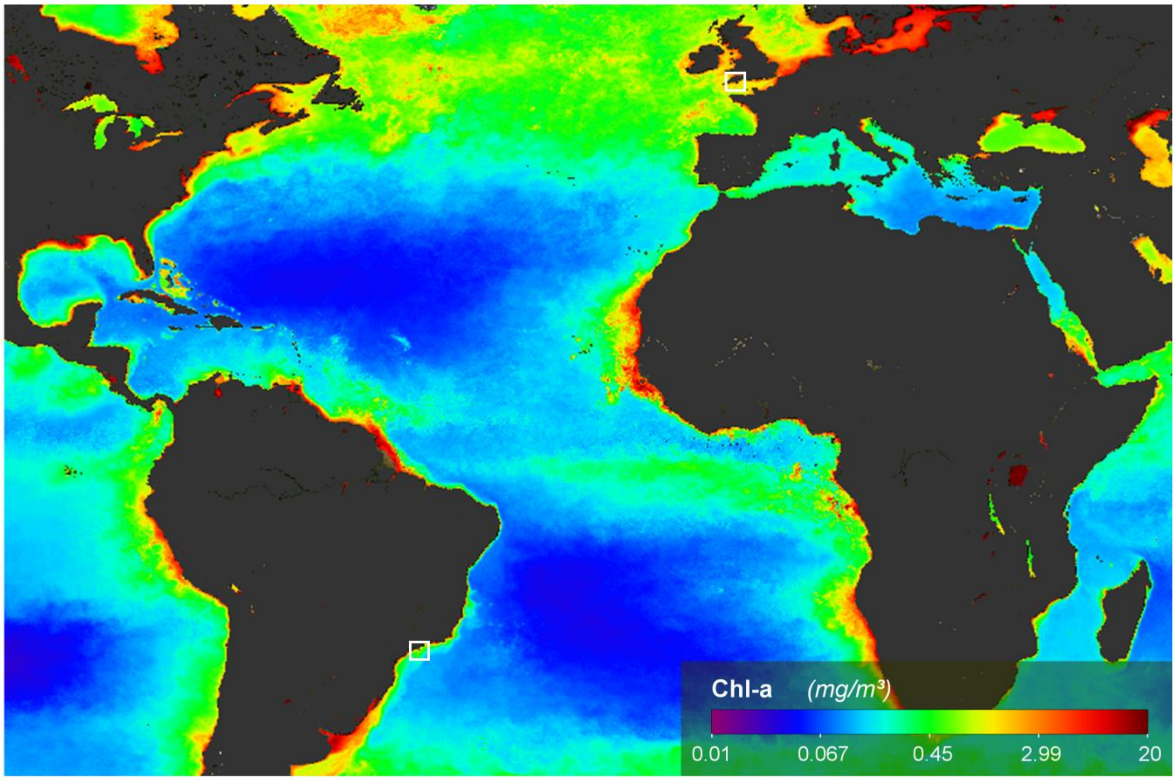

Figure 31. Surface chlorophyll-a concentration (Chl-a; mg/m^3) map, produced using data from MODIS-Aqua NASA Ocean Color database using Level-3 mapped images of 4 km resolution, showing the latitudinal patterns of pelagic Chl-a during 2015. Sampled coasts indicated (São Sebastião Channel, Brazil, and the English Channel, British Isles).

ARTICLE 5

PHYSIOLOGICAL LIPID REMODELING AS A FUNCTION OF LATITUDE IN LATE-STAGE BARNACLE LARVAE

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TO BE SUBMITTED TO ECOLOGY

[RESEARCH ARTICLE]

5.1 ABSTRACT

The physiological needs of cyprid larvae during the critical pelago-benthic transition are different according to the environment they inhabit. We investigated how the physiology of late-stage barnacle larvae varied from tropical to arctic shores. Using an integrative and comparative approach, we measured the fatty acid levels present in nearshore particulate matter and cyprid larvae from *Chthamalus* spp. and *Semibalanus* sp., and identified (1) the dietary sources available for filter-feeders, and (2) the capacity of larvae to remodel lipids in their membranes. We found that available dietary fatty acids in tropical waters derived mainly from detrital particles as opposed to living microalgae in more productive arctic waters. Cyprid larvae actively regulated the fatty acids incorporated into membrane lipids by metabolizing the readily-available reserves in their lipid droplets. The essential docosahexaenoic acid (DHA; 22:6ω3), although not abundant in pelagic food (< 4 % of particulate matter across all latitudes) appears to have an important role in lipid remodeling for cyprid larvae. We suggest that cyprid larvae have a high retention of this fatty acid due to (1) dietary deficiencies during naupliar development, and/or (2) importance of this fatty acid in lipid remodeling in response to temperature. We advance the hypothesis that cyprids in warmer and less productive environments are prone to higher physiological stress. We provide novel knowledge on the fatty acid signatures of late-stage barnacle larvae and pelagic food sources entering benthic food webs, and open the door for future research on the importance of larval lipid remodeling in shaping population dynamics of this important group of habitat-forming species.

Keywords: Cyprid larvae; Energetics; Fatty acid biomarkers; Pelago-benthic transition; Ecophysiology; Rocky intertidal

5.2 RÉSUMÉ

Les besoins physiologiques des larves de cypris pendant la transition pélago-benthique sont différents selon l'environnement dans lequel elles vivent. Nous avons étudié comment la physiologie des cypridés variait sur un large gradient latitudinal, des côtes tropicales aux côtes arctiques. En utilisant une approche intégrative et comparative, nous avons mesuré les niveaux d'acides gras présents dans les matières particulaires en suspension des eaux côtières et dans les cypridés de *Chthamalus* spp. et *Semibalanus* sp. Nous avons identifié (1) les sources alimentaires disponibles pour les organismes filtreurs, et (2) la capacité des larves à remodeler les lipides dans leurs membranes. Nous avons constaté que les acides gras alimentaires disponibles dans les eaux tropicales provenaient principalement de particules détritiques par opposition aux microalgues vivantes dans les eaux arctiques plus productives. Les larves de cypris régulent activement les acides gras incorporés dans les lipides membranaires en métabolisant les réserves disponibles dans leurs gouttelettes lipidiques. L'acide docosahexaénoïque (DHA; 22:6ω3), bien que peu abondant dans les aliments pélagiques (< 4 % des matières en suspension sous toutes les latitudes), semble avoir un rôle important dans le remodelage lipidique des cypridés. Nous suggérons que les larves de cypris ont fortement retenu cet acide gras en raison (1) de carences alimentaires au cours du développement naupliaire et/ou (2) de l'importance de cet acide gras dans le remodelage des lipides en réponse aux conditions thermiques. Nous avançons que les cypridés dans des environnements plus chauds et moins productifs sont sujets à un stress physiologique plus élevé. Nous fournissons de nouvelles connaissances sur les signatures en acides gras des larves de balanes et des sources de nourriture pélagique entrant dans les réseaux trophiques benthiques, ce qui ouvre la porte à des futures recherches sur l'importance du remodelage des lipides dans les larves et sur la dynamique des populations de cet important groupe d'espèces.

Mots clés : Cypridés; Marqueurs trophiques; Énergétique; Transition pélago-benthique; Écophysiologie; Intertidale rocheuse

5.3 INTRODUCTION

Across a variety of marine benthic invertebrates, high quality larvae are considered those that survive, grow, and recruit successfully in the wild. For barnacles, habitat-forming species across the world's rocky shores, larval physiological quality plays an important role in modulating recruitment (Rittschof et al., 1984; Pechenik et al., 1993; Miron et al., 2000; Jarrett, 2003), being linked to the optimal settlement window, vertical position in the intertidal, and habitat selection (Miron et al., 1999). The quality of late-stage barnacle larvae (cyprids) is largely determined by energy reserves, stored as lipid droplets during the previous feeding naupliar stages, and age (Holland and Walker, 1975; Harder et al., 2001; Tremblay et al., 2007). Lipid droplets are organelles for cellular neutral lipid storage, transport, and metabolism. Cyprid larvae do not feed (lecithotrophic), relying on these lipid reserves for energy, to build up membranes, and synthesize hormones (Lee and Walker, 1995). These reserves are gradually consumed while the larvae finds a suitable settlement site (Holland and Walker, 1975; Lucas et al., 1979). Depleted energy reserves have been shown to impair substrate exploration behaviour of cyprids (Miron et al. 2000; Tremblay et al., 2007) and reduce early growth and survival of recently-metamorphosed juveniles (Jarrett and Pechenik, 1997; Leal et al., 2020). Larval quality can vary considerably among daily cohorts (e.g., *Balanus Amphitrite*, Miron et al., 2000; *Semibalanus balanoides*, Jarrett, 2003; *Chthamalus bisinuatus*, Leal et al., 2020) attributable to (1) the quantity and quality of planktonic food (Thiyagarajan et al., 2003; Burrows et al. 2010), (2) the duration of delayed metamorphosis while in the plankton (Lucas et al., 1979; Pechenik et al., 1993; Miron et al., 2000), and (3) the maternal investment in offspring (Freuchet et al., 2015; Kasten et al., 2019).

The process of larval settlement and metamorphosis is energetically expensive, and lipids alone account for 55–65 % of the energy used during metamorphosis of cyprid larvae (Waldock and Holland, 1978; Lucas et al., 1979; Miron et al., 2000; Thiyagarajan et al., 2003). Lipids are an ideal storage material because they liberate twice as much energy by an equal mass of carbohydrate or protein (Giese, 1966; Lee et al., 2006), and can be stored as

(1) energy reserves for times of need, accumulated in cells as free globules (e.g., triacylglycerols, wax esters) and (2) structural compounds in the membrane of cells and organelles (e.g., phospholipids, cholesterol) that maintain membrane fluidity in environments with cold or variable temperatures (Giese, 1966; Copeman and Parrish, 2003). Once a lipid is stored, it is then available for catabolism, and the energy released can be captured in the form of high energy compounds such as ATP (O'Connor and Gilbert, 1968). The fatty acid constituents of triacylglycerols and membrane phospholipids are important for larval growth and survival (e.g., Prowse et al., 2009; Falker et al., 2015), and several studies have focused on the importance of polyunsaturated fatty acids (PUFAs), particularly the essential fatty acids (EFAs) eicosapentaenoic acid (20:5ω3, EPA), docosahexaenoic acid (22:6ω3, DHA), and arachidonic acid (20:4ω6, ARA) (e.g., Pernet et al., 2005; Thériault and Pernet, 2007; Gendron et al., 2013; Cabrol et al., 2015). Crustacean larvae lack the ability to synthesize EFAs, and must obtain them from their diet (Lee et al., 2006). DHA is involved in maintaining the structural and functional integrity of biological membranes due to its unique structure, which facilitates rapid conformational changes in membrane proteins (Feller et al., 2002). EPA and ARA are precursors of eicosanoids, a group of highly biologically active hormones (e.g., prostaglandins, leukotrienes) (Smith and Murphy, 2003). When dietary sources are deficient in EFAs, invertebrate larvae are known to selectively incorporate EFAs into membrane phospholipids at the expense of readily-available triacylglycerols in their lipid droplets (Tremblay et al., 2007), through a process known as selective fatty acid retention (Pernet et al., 2005; Thériault and Pernet, 2007; Gendron et al., 2013; Martinez-Silva et al., 2018). If the relative proportion of a given dietary fatty acid in larvae:diet is equal to or less than 1, the larval requirement for that fatty acid is presumably satisfied. If the relative proportion of fatty acid in larvae:diet is higher than 1, then that fatty acid is selectively incorporated by larvae.

Ingested fatty acids are incorporated unmodified into marine invertebrate's storage lipids, and have been extensively used as biomarkers (*sensu* Parrish et al., 2000) in studies of pelagic food webs to identify primary producers and characterize the diets of zooplankton (e.g., Parrish et al., 2000; Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Contrary to pelagic, open-ocean waters, where phytoplankton (diatoms, dinoflagellates) is the major source of primary production, benthic coastal food webs are sustained by a diversity of primary producers that can include macroalgae and vascular plants, in addition to phytoplankton (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Changes in food sources for benthic filter-feeders are generally considered to be greater at higher latitudes as a result of seasonal changes in environmental variables affecting primary production (Lawrence, 1976). Zooplankton groups (e.g., crustaceans and pteropods) found in polar or upwelling biomes have been characterized by pronounced accumulation of large lipid reserves during phytoplanktonic blooms (Hagen and Schnack-Schiel, 1996; Böer et al., 2005). In contrast, zooplankton from tropical biomes, characterized by low concentrations of high-quality fatty acids (e.g., Leal et al., 2018, 2019), and relatively low primary production rates throughout the year, are composed mainly of omnivorous species with small lipid reserves (Lee et al., 2006).

In the present study, we investigated how the physiology of late-stage barnacle larvae is influenced by trophic and thermal environment. By using an integrative and comparative approach, we measured the fatty acid levels in nearshore particulate matter and late-stage barnacle larvae across tropical, subtropical, subarctic and arctic shores, and identified (1) the dietary sources available for benthic filter-feeders, using fatty acid trophic markers, and (2) the capacity of larvae to remodel lipids in their membrane and selectively retain essential fatty acids. We hypothesized that cyprid larvae physiologically regulate their fatty acid composition according to the thermal environment (e.g., larvae in the poles retain PUFAs to increase membrane fluidity) and/or trophic environment (e.g., larvae in the tropics retain EFAs to cope with food deprivation). Although *Semibalanus* and *Chthamalus* supply-side ecology has been extensively studied over the past decades (e.g., Connell, 1961; Lucas et al., 1979; Jarret, 2003; Jenkins, 2005; Kasten and Flores 2013), a comprehensive description of the fatty acid profiles of late-stage larvae is still lacking. The datasets provided by this study create a baseline of trophic data and give insights into food web linkages in the intertidal, knowledge that is particularly sparse for low-latitudes.

5.4 MATERIAL AND METHODS

5.4.1 Study areas and species

We used acorn barnacles as model organisms, sampled across a latitudinal gradient: *Chthamalus proteus* Dando and Southward, 1980, in the northwestern coast of Panama (tropical latitude), *Chthamalus bisinuatus* Pilsbry, 1916, in the southeastern coast of Brazil (subtropical latitude) and *Semibalanus balanoides* (Linnaeus, 1767) in the southeastern coast of Canada (subarctic latitude) and western Greenland (arctic latitude) (Figure 32). These species occupy the same intertidal level, the upper mesolittoral or midlittoral zone, as in Lewis (1964), playing a similar role in the rocky shore community. Services provided by these foundation species include bentho-pelagic coupling and habitat engineering, which are vital for generating and maintaining local species diversity in the intertidal (Bertness and Leonard, 1997; Kordas et al., 2015).

Field work was undertaken at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station (STRI; Panama), Center for Marine Biology of the University of Sao Paulo (CEBIMar/USP; Brazil), Department of Biology of St. Francis Xavier University (StFX; Nova Scotia), and at the Greenland Institute of Natural Resources (GINR; Nuuk), and samples were later analysed at the Institut des Sciences de la Mer de Rimouski (ISMER/UQAR; Québec).

5.4.1.1 Tropical latitude: Bocas del Toro, Panama

In Panama, the rocky platforms of Paunch beach (Site 1, 9°22'25.03"N, 82°14'19.03"W; Site 2, 9°22'20.04"N, 82°14'21.20"W), located on the exposed coastline of Isla Colon in the Bocas del Toro Archipelago, were sampled during March 2017. *Chthamalus proteus* dominates the upper-midlittoral zone, together with the flat-tree oyster *Isognomon alatus* (I. Leal, pers. obs.). Zabin et al. (2007) found adult *C. proteus* barnacles with developing eggs at all times of the year, indicating a year-round larval production. In Isla

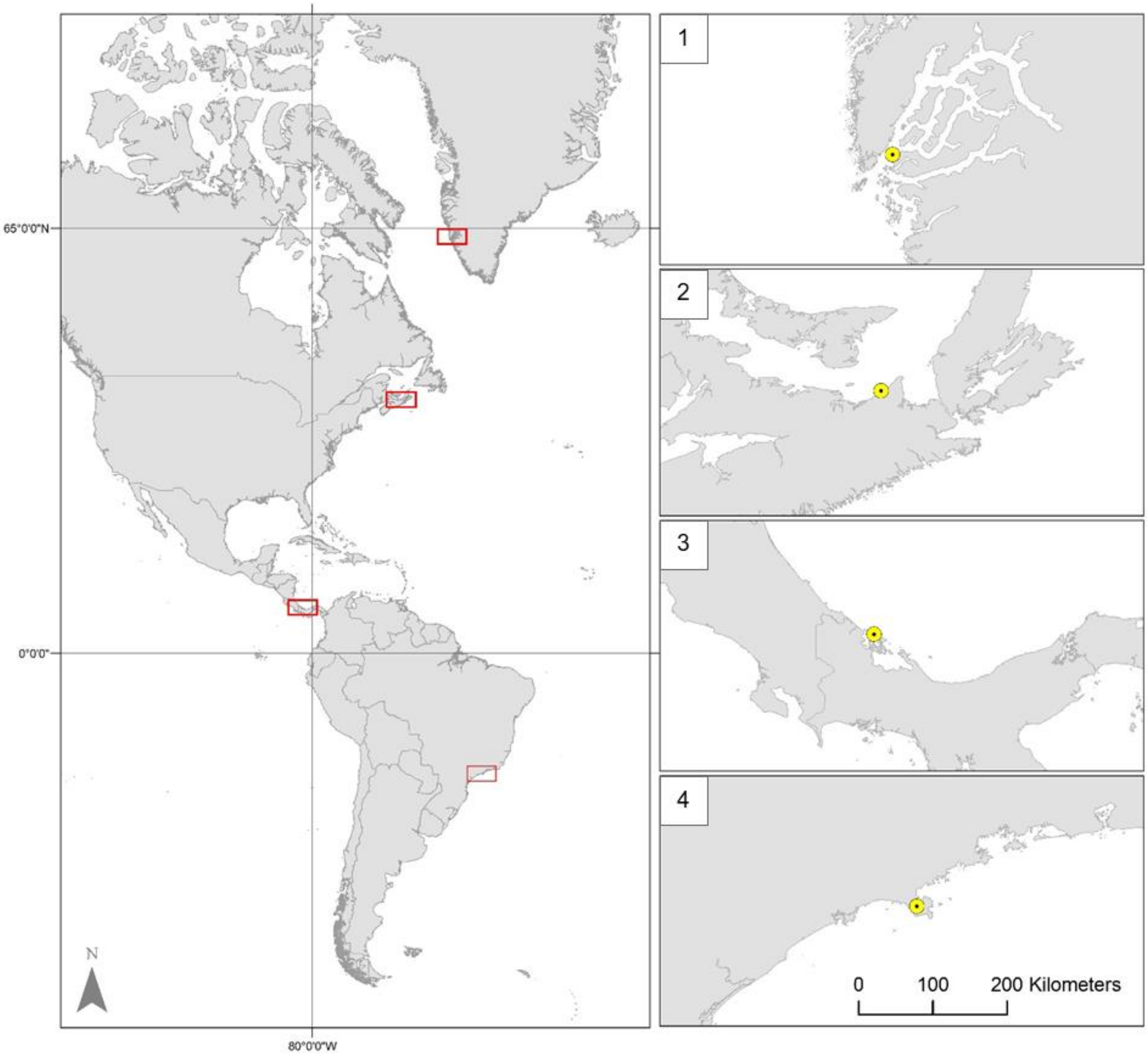

Figure 32. Map of sampling locations, (1) Nuuk, western Greenland (arctic), (2) Antigonish, southeastern Canada (subarctic), (3) Bocas del Toro, northwestern Panama (tropical), and (4) São Sebastião, southeastern Brazil (subtropical). Yellow circle marks the area of the sampled shores within each region, i.e., two shores < 3 km apart.

Colon, the average sea surface temperature of the coldest month of the year, February, 27.9 °C, is not far from that of the warmest month of the year, October, 29.6 °C (Kaufmann and Thompson, 2005). Heavy rainfalls at the onset of the rainy season produce low salinity plumes that flow on top of the typically warm Caribbean waters (Kaufmann and Thompson, 2005), providing a nutrient input from terrestrial runoff that may constitute an energetic subsidy to nearshore benthic communities (Gorman et al., 2019). Indeed, fatty acid profiles of bivalve larvae in Paunch Beach have shown the ingestion of particles from terrestrial inputs and macrophyte residues, with two fatty acid markers of these sources (18:2ω6 and 18:3ω3; Budge and Parrish, 1998) constituting nearly 20 % of the larval lipid content (Leal et al., 2019). Markers for diatoms (20:5ω3) and dinoflagellates (22:6ω3) have also been found in larval neutral reserves, but low-concentrated (< 5 % of lipid content, Leal et al., 2019).

5.4.1.2 Subtropical latitude: São Paulo, Brazil

In Brazil, the rocky shores of Baleeiro Head (Site 1; 23°49'46.44"S, 45°25'24.96"W) and Jarobá (Site 2; 23°49'42.38"S, 45°25'18.55"W) were sampled during October 2016. Facing the São Sebastião Channel (SSC; northern coast of São Paulo State), these shores are characterized by a steep rocky habitat with a clear midlittoral saturated *Chthamalus* zone (e.g., Kasten and Flores 2013) interspersed with patches of the mussel *Brachidontes solisianus*. Populations of *C. bisinuatus* along the São Paulo coastline exhibit a very clear fortnightly rhythm of larval release (Bueno et al., 2010), and have been proved capable of endogenous timing leading to neap-tide release, consistent with a retention strategy, a pattern that is broken down in nature by stochastic environmental stressors, of which food supply appears relevant (Kasten and Flores, 2013). In the study area, average sea surface temperature presents is lowest in July, 21.9 ºC, and highest in February, 28.6 ºC (Valentim et al., 2013). The SSC is principally a meso-oligotrophic system year-round, with high-quality nutrients varying on a daily basis (Leal et al. 2018). SFAs make up most of the dietary lipid content of organic matter (Leal et al., 2018), with fatty acid trophic markers indicating the presence of organic detritus and bacterial production, likely deriving from terrestrial inputs (Leal et al., *unpublished*). Energy content at metamorphosis has been shown to be important for the initial growth of *C. bisinuatus*, with varying quality cohorts being found over the SSC (Leal et al. 2020).

5.4.1.3 Subarctic latitude: Nova Scotia, Canada

In Canada, the rocky shores of Arisaig (Site 1, 45°45'45.31"N, 62°10'18.17"W) and Sea Spray (Site 2, 45°46'18.71"N, 62° 8'47.96"W) were sampled during June 2018. Located on the southern coast of the Gulf of St. Lawrence, the sampled locations have consistent barnacle recruitment (e.g., 12 years of records, Scrosati and Ellrich, 2016). *Semibalanus balanoides* shares its niche with other sessile species, e.g., fucoid algae (*Fucus* spp. and *Ascophyllum nodosum*) and blue mussels *Mytilus edulis* (Ellrich et al., 2015; Scrosati and Ellrich, 2016). After the ice melts in late winter or early spring in this region, settlers appear on the substrate during a limited recruitment season between early May and mid- to late June (Ellrich et al., 2015). Low sea surface temperatures are observed on the Sea Spray coast early every year (below 3 °C in April; Scrosati and Ellrich, 2016). Sea surface temperature and pelagic food supply (chlorophyll *a* concentration) in the study area in April have been suggested to influence intertidal recruitment primarily through a positive influence on pelagic cyprid larvae (Scrosati and Ellrich, 2016). Pelagic food supply has also been suggested to explain variation in intertidal barnacle size along this coastline (Scrosati and Ellrich, 2019). Scarce information is available regarding the fatty acid composition of nearshore food sources for filter-feeders in this area.

5.4.1.4 Arctic latitude: Nuuk, Greenland

In Greenland, the rocky shores of Quassussuup Tungaa (Site 1, 64°11'48.71"N, 51°41'59.76"W; Site 2, 64°11'50.19"N, 51°42'24.02"W), facing the Nuup Kangerlua fjord in the northwestern part of Nuuk, were sampled during June 2016. Brown algae *Ascophyllum nodosum* completely covered the sampled rocky shores, with adult mussels *Mytilus* spp. and *S. balanoides* frequently found attached to the rock underneath (I. Leal, pers. obs.). *Semibalanus balanoides* settlement in the Arctic peaks during mid-summer months, particularly July (Feyling-Hanssen 1953; Kuklinski et al., 2013). Sea surface temperatures typically range from −1.5 °C during winter to 8 °C in late summer in southwest Greenland

(Blicher et al., 2009). Gaillard et al. (2017) identified that the diet of the bivalve *Astarte elliptica* in the nearby sub-Arctic Fjord Kobbefjord involved a mixture of suspended particulate matter, including microalgae and macroalgal (mainly brown algae *Dictyosiphon foeniculaceus* and *F. vesiculosus*) material. Generally, diatoms (particularly the genera *Chaetoceros* and *Thalassiosira*) dominate the microplankton assemblage during the spring bloom in late April/early May in the nearby Godthåbsfjord system and are complemented by haptophytes (e.g., Juul-Pedersen et al., 2015).

5.4.2 Field sampling

Key abiotic (i.e., temperature, salinity, total particulate matter, and mass of total fatty acids [MTFA]) and biotic parameters (i.e., settlement/supply ratio, larval size, and larval MTFA) were measured/collected at the peak of each diurnal low tide, simultaneously at two sites per study area (see above, section 5.4.1), for a 3-week period. Temperature experienced in the intertidal zone was measured using data loggers (HOBO Pendant® Temperature/Light 64 K, contained within waterproof casings). This allowed to characterize the thermal regime experienced by intertidal organisms (e.g., Lathlean and Minchinton, 2011), as these loggers continuously measure air temperature upon emersion and seawater temperature upon submersion over a fine temporal scale (10 min intervals). Spot measurements of sea-surface salinity were obtained directly from nearshore waters using a probe (EcoScan SALT 6 Plus Salinity Meter with an electrode). Nearshore trophic resources available for filter-feeders were estimated by collecting 6 replicate seawater samples of 2 L per day. Samples were first stored in opaque bottles, and after filtered through a 100 µm mesh sieve, and then through Whatman® glass microfiber filters (GF/F 25 mm, pore size 0.7 µm, pre-burnt and weighted). Upon filtration, three glass microfiber filters were rinsed with 0.3-M Ammonium formate to dissolve residual salts (Aminot and Chaussepied, 1983), subsequently heated at 70 °C for 24 h and weighted for estimation of total particulate matter (TPM, mg/L), a proxy of food availability. Three other glass microfiber filters were stored at −80 °C in dichloromethane-methanol vials (2:1, v/v) after filtration, for estimation of Mass of Total Fatty Acids (MTFA;

µg/mg TPM) and its composition (% FA) (see below, section 5.4.3), a proxy of the quality and composition of pelagic food.

A 150 µm plankton net (30 cm of net mouth diameter) was manually towed just below the surface for a 100-m long-shore transect at each shore per day. Collected cyprids were (1) photographed alive in lateral view to estimate larval size (mm; *n* = 90 per study area) using the software Fiji, an open-source platform for scientific image analysis (Schindelin et al., 2012), and (2) preserved in dichloromethane-methanol (2:1, v/v) at −80 °C to later estimate their fatty acid content and composition (see below, section 5.4.3). Settlement plates (10 × 10 cm; *n* = 10) covered with 3 M™ Safety- Walk™ (620 series) to provide surface roughness, a normal feature of rocky intertidal habitats known to facilitate settlement (Letourneaux and Bourget, 1988), were haphazardly distributed over a spatial scale of hundreds of meters, providing substrate for larval settlement in the mid-intertidal zone. Settlement plates were brought to the laboratory to assess daily settlement rates, and new ones reinstalled in the field. Newly settled barnacles were identified using a stereomicroscope.

5.4.3 Lipid analysis

5.4.3.1 Total Particulate Matter

Fatty acids from dietary sources (TPM) were extracted through direct transesterification (Lepage and Roy, 1984) and analysed by Gas Chromatography–Mass Spectrometry (GC-MS), to determine the mass of total fatty acids (MTFA) and its composition (% FA). We used a full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled to a multichannel gas chromatograph “Trace GC ultra” (Thermo Scientific) equipped with an autosampler (model Triplus), a PTV injector and a mass detector model ITQ900 (Thermo Scientific). An Omegawax 250 (Supelco) capillary column was used for separation with high purity helium as a carrier gas. Data were treated using Xcalibur v.2.1 software (Thermo Scientific) and fatty acids identified and quantified with standards Supelco 37. Fatty acids were further discriminated into saturated (SFA; with no double bond), monounsaturated (MUFA; with 1 double bond), polyunsaturated (PUFA; with ≥ 2 double bonds) and essential (EFA; ω3 and ω6) fatty acids. A detailed description of the number of samples processed for lipid analysis can be found in Supplementary Table 15.

5.4.3.2 Cyprid larvae

Cyprids collected from the nearshore waters were pooled for lipid analysis (*n* = 3 pooled weekly samples, per site, in order to have enough biological material for analysis). Lipids were extracted by a modified Folch procedure (Folch et al., 1957) and the neutral fraction (i.e., energetic fraction, including triglycerides, free FA, and sterols) was separated from the polar fraction (i.e., structural fraction, composed mainly of phospholipids) (Marty et al., 1992). It is desirable to know the fractions (i.e., type of lipid) present in the total lipid content of larvae, given their ability to transfer fatty acids, and thus utilize lipids without affecting its levels (Giese 1966). Neutral FAs (energetic reserves) are conservatively assimilated, providing indication of the ingested food (preceding days to weeks), whereas polar FAs reflect long-term uptake of dietary fatty acids under physiological regulation of membrane composition (Delaunay et al., 1993; Freites et al., 2002). Neutral lipids were purified to remove free sterols (Cabrol et al. 2015). On each fraction, fatty acid methyl esters were obtained as described in Lepage and Roy (1984) and analysed with the GC-MS, as above 2.3.1., to determine the total energetic content in individual larvae (sum of all fatty acids identified; µg/larva) and its composition (% FA). A detailed description of the number of larvae processed for lipid analysis can be found in Supplementary Table 16.

5.4.3.3 Larval selective EFA retention

Selective incorporation of EFAs into membrane phospholipids at the expense of reserve, neutral lipids (i.e., the proportion of EFAs in the polar lipids higher than in the neutral lipids and diet; Thériault and Pernet, 2007; Gendron et al., 2013) was tested through a ratio between larval fatty acids and dietary fatty acids, for the essential ARA, EPA and DHA. The absence of selective retention of EFAs in the cell membrane (ratio ‹‹ 1) suggests a better larval condition (Gendron et al., 2013). By assessing the relative proportions of EFAs in neutral and polar fractions of larvae and that of diets we were able to (1) assess the nutritional quality of available dietary fatty acids at each latitude, and (2) indicate whether a given dietary fatty acid is selectively incorporated or used (eliminated) by larvae.

5.4.4 Fatty acid trophic markers

Fatty acid trophic markers (FATM) considered in this study were attributed to six trophic sources according to published research on reliable FATM (Budge and Parrish, 1998; Dalsgaard et al., 2003; Kelly and Scheibling, 2012; Parrish, 2013): (i) bacteria, (ii) detrital matter, (iii) macroalgae, (iv) microeukaryotes, (v) dinoflagellates, (vi) diatoms. Table 12 describes in detail the fatty acids used and their dietary quality.

5.4.5 Statistical analysis

Permutational multivariate analyses of variance (PERMANOVAs) using PRIMER (v. 7.0.13) were used to make latitudinal comparisons (fixed factor, 4 levels: Tropical, Subtropical, Subarctic, Arctic), with sites nested within latitude (random factor, 2 levels: Site 1, Site 2), for the following datasets: (1) FATM (bacteria, detritus, macroalgae, microeukaryotes, dinoflagellates and diatoms) and (2) larvae:diet ratios (selective retention of ARA, EPA, DHA). Analysis were conducted with fatty acids expressed as percentages (% arcsin square-root transformed) using a non-parametric distance-based PERMANOVA based on Bray-Curtis similarities, and 9,999 permutations to calculate pseudo-*F* ratios. Homogeneity was evaluated using the permutation analysis of multivariate dispersion (PERMDISP) (Anderson 2001). To help uncover the nature of the latitudinal differences detected by PERMANOVA, we carried out Principal Component Analyses (PCA) Biplots

(Gabriel, 1971). PCA biplots, displaying information both on samples (points) and variables (vectors), were used to visualize the 5 variables that contributed the most for the variation within the datasets.

We used the R software (version 3.6.1; R Core Team, 2019) and packages ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2019), factoextra (Kassambara and Mundt, 2017) and FactoMineR (Le et al., 2008) to conduct statistical analysis and as graphical tools.

Table 12. List of fatty acid trophic markers used to describe the origin of nearshore particulate matter available as dietary source for filter feeders. SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; EFA: Essential Fatty Acids.

Lipid class	**Fatty acid**	**Trophic marker of**	**Nutritional quality**
SFA	14:0, 16:0, 18:0	Detritus	Degraded matter
	15:0, 17:0	Bacteria	
MUFA	16:1ω7	Diatoms	↓
	18:1ω9	Mangrove detritus, Brown macroalgae	
PUFA	18:2ω6, 18:3ω3, 18:3ω6	Green macroalgae, Vascular plants, Terrestrial matter	
	18:4ω3	Dinoflagellates	
EFA	20:4ω6 (ARA)	Microeukaryotes, Red algae	
	20:5ω3 (EPA)	Diatoms	Nutritionally rich matter
	22:6ω3 (DHA)	Dinoflagellates	

5.5 RESULTS

5.5.1 Nearshore conditions

Nearshore conditions observed at each study location can be found in Figure 33. A decreasing gradient in intertidal temperature with increasing latitude was observed (Fig. 33A). The tropical sites experienced the highest temperatures recorded by *in situ* HOBO loggers (29.8 ± 0.08 ^{o}C) followed by subtropical (24.1 ± 0.05 ^{o}C), subarctic (11.1 ± 0.07 ^{o}C) and arctic ones (7.1 ± 0.10 ^{o}C). Daily thermal range ($\Delta T = T_{max}$-T_{min}, calculated by subtracting the low from the high T ^{o}C on each day, and generating a mean and SD for those ranges) was considerable among all sampled shores ($\Delta T_{Tropical}$ = 14.8 ± 5.5 ^{o}C; $\Delta T_{Subtropical}$ = 8.5 ± 7.1 ^{o}C; $\Delta T_{Subarctic}$ = 6.1 ± 5.7 ^{o}C; ΔT_{Arctic} = 16.1 ± 6.8 ^{o}C). The same latitudinal trend was found for salinity (Fig. 33B), higher in tropical (36.9 ± 0.52 ppt) and subtropical shores (33.0 ± 0.28 ppt), compared to subarctic (29.1 ± 1.91 ppt) and arctic ones (28.6 ± 1.05 ppt). Regarding dietary food sources, no clear latitudinal pattern emerges regarding availability (TPM; Fig. 33C) and lipidic content (MTFA; Fig. 33D). The highest TPM was found in subtropical shores (6.08 ± 1.01 mg/L), and the lowest in the tropical ones (2.6 ± 0.20 mg/L). The dietary sources with higher MTFA were found in the arctic shores (5.3 ± 0.56 µg/mg TPM), and the lowest in subtropical ones (0.8 ± 0.2 µg/mg TPM). Larval supply to settlement ratios revealed the highest larval supplies in the tropical *C. proteus*, and the highest settlement for the arctic *S. balanoides* (Fig. 33E). The cyprid sizes of *C. proteus* and *C. bisinuatus* averaged 0.446 mm and 0.592 mm, respectively (Fig. 33F). *Semibalanus balanoides* cyprids averaged 1.224 mm at the subarctic sites, and 1.61 mm at the arctic ones. Arctic *S. balanoides* cyprids, visible to the naked eye (I. Leal, pers. obs.), reached the largest size (maximum size of 2.003 mm). The fatty acid content of neutral (energetic) reserves (Fig. 33G) was lowest for the chthamalid cyprids in tropical and subtropical sites, 0.1 µg/larva for *C. proteus*, and 0.5 µg/larva for *C. bisinuatus*. Subarctic and arctic *S. balanoides* had almost twice as much neutral reserves, 1.0 µg/larva. Similarly, the fatty acid content of polar lipids (Fig. 33H) was lowest for *C. proteus*, 0.05 µg/larva, and *C. bisinutus*, 0.1 µg/larva, compared to *S. balanoides* from subarctic, 0.5 µg/larva, and arctic shores, 0.3 µg/larva.

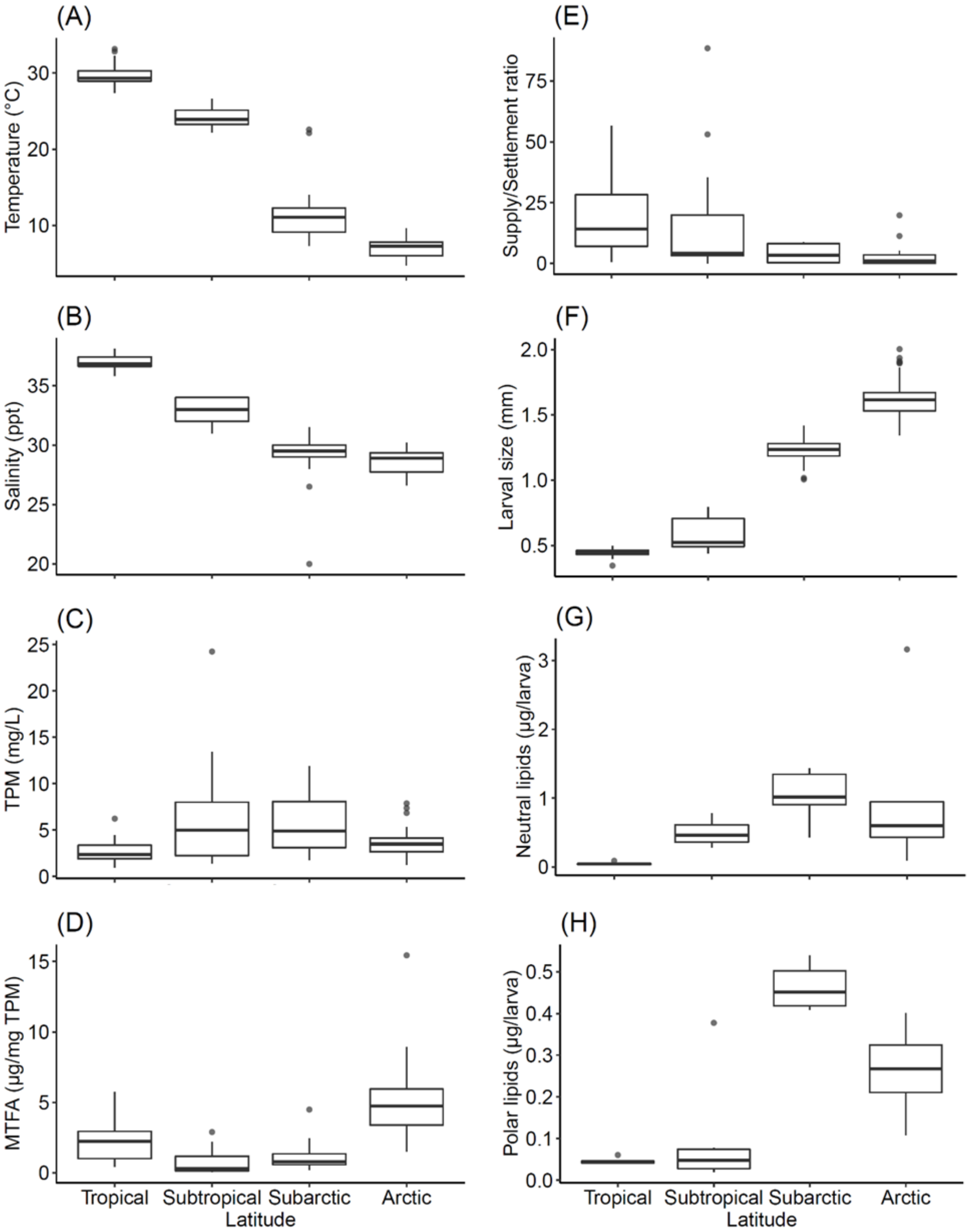

Figure 33. Nearshore abiotic (A–D) and biotic (E–F) conditions found at each sampling location. TPM: Total Particulate Matter; MTFA: Mass of Total Fatty Acids.

5.5.2 Lipid profiles of dietary sources & cyprid larvae

Low-quality, SFAs present in dietary sources showed a latitudinal trend, reaching the highest values at tropical shores (57.3 %), followed by subtropical (55.3 %), subarctic (45.4 %) and arctic ones (38.9 %; Figure 34). Inversely, high-quality, PUFAs reached minimum values at the tropical locations (14.3 %), followed by subtropical (20.4 %) and subarctic (19.9 %), and reached maximum values at the arctic shores (40.1 %). The most abundant EFA present in dietary sources was 20:5ω3 (EPA) across all latitudes, tropical (4.3 %), subtropical (6.5 %), subarctic (5.7 %) and arctic (22.5 %). A more detailed description of the FA composition (% of total FA) of food sources is given in Supplementary Table 15.

The same trends were not obvious regarding cyprid lipidic composition (Figure 34). Energetic, neutral reserves of the chthamalids cyprids at the tropical and subtropical areas were composed mainly of SFAs, 51.9 % for *C. proteus* and 58.2 % for *C. bisinuatus*, of which the fatty acids 16:0 and 18:0 contributed the most. *Semibalanus balanoides* neutral reserves at the subarctic latitude were mainly composed by MUFAs (43.2 %), of which 16:1 and 18:1ω9 contributed the most. The neutral composition of *S. balanoides* inhabiting arctic areas did not differ strongly from that of low-latitude chthamalid cyprids, however, being composed of mainly SFAs (54.4 %). Similar levels of EFAs were found in the neutral fraction of cyprids across all latitudes, comprising 14 % of total lipid content for *C. proteus*, 17.8 % for *C. bisinuatus*, 17.7 % for subarctic *S. balanoides*, and 15.6 % for arctic *S. balanoides*. EFA accumulation in triacylglycerol (neutral) reserves seems to be physiologically regulated to serve as buffer for membrane needs, independently of availability in the food.

The structural, polar fractions of cyprids was more distinctive between low- and high-latitudes. While for chthamalid cyprids SFAs remained the most abundant, comprising 52.2 % for *C. proteus*, and 62.9 % for *C. bisinuatus, S. balanoides* cyprids had a far greater proportion of PUFAs, reaching 43.9 % in subarctic cyprids, and 41.0 % in arctic ones. The EFA 20:5ω3 (EPA) alone constituted 29.0 % of structural fraction for subarctic and 27.1 % for arctic *S. balanoides* cyprids, being the most abundant EFA in this species composition. Increasing levels of EFA and PUFA to maintain the fluidity and function of membrane components is expected given the low temperatures experienced at the subarctic and arctic locations sampled, that reached minimum intertidal temperatures of 6.9 °C, and 1.2 °C, respectively. A detailed description of the fatty acid composition (% of total fatty acids) of cyprid neutral and polar fractions is given in Supplementary Table 16.

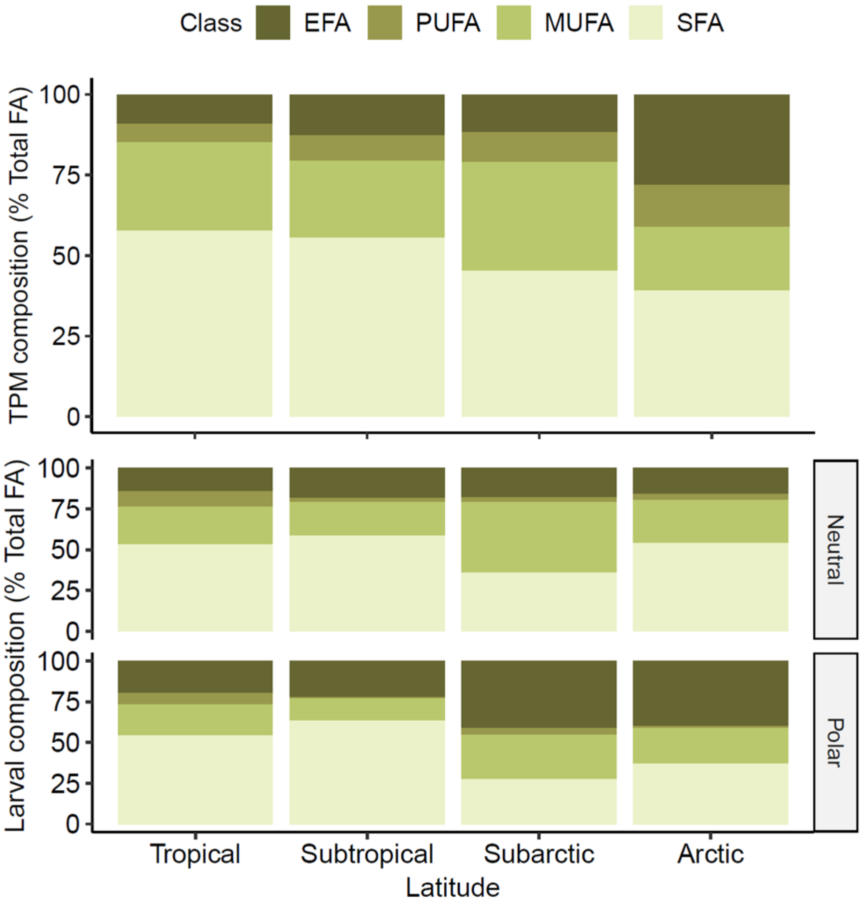

Figure 34. Lipid class composition (%) of nearshore total particulate matter (TPM; upper panel) and larval neutral and polar fractions (bottom panel). SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids. Color gradient depicting a decreasing gradient in nutritional quality of fatty acids.

5.5.2.1 Fatty acid trophic markers

The FATM used to determine the origin of the dietary sources available for filter-feeders is given in Table 12. We observed differences in FATM found in particulate matter across all latitudes (Table 13-a). PCA biplot (Figure 35-A) clearly distinguishes the composition of tropical dietary tracers, with a relative higher proportion of detrital particles compared to living microalgae, and that of arctic ones, with higher percentages of diatom and dinoflagellate markers that reflect higher local primary productivity. Tracers for macroalgae, including macroalgal detritus, green and brown macroalgae, show an overlap in particulate matter origin/composition between subtropical and subarctic latitudes, although significant differences were detected between all latitudes. In Bocas del Toro (tropical), the detrital composition of nearshore matter is likely explained by rainfall typical of this region, that may discharge a large amount of terrestrial detrital particles.

Regarding larval neutral (energetic) fraction, differences in trophic markers were also found across latitudes (Table 13-b; PERMANOVA). PCA biplot (Figure 35-B) show an overlap in the short-term source of the dietary fatty acids assimilated as energy fuel for cyprid larvae, with no differences detected between arctic and subarctic (*S. balanoides*), and arctic and subtropical (*C. bisinuatus*) cyprids. It appears that to build up their energetic reserves during their pelagic (naupliar) development, larvae assimilate dietary fatty acids from various food sources across arctic, subarctic, and subtropical species. Contrarily, regarding larval polar (structural) fraction, tropical and subtropical chthamalid cyprids showed similarities in origin of dietary tracers that significantly differed from that of subarctic and arctic *S. balanoides* (Table 13-b; PERMANOVA). The PCA biplot in Fig. 35C shows a clear separation, diatoms and macroalgae appearing as long-term source of the dietary fatty acids assimilated as membrane building blocks for high-latitudes, and microeukaryotes, detritus and bacteria for low-latitudes.

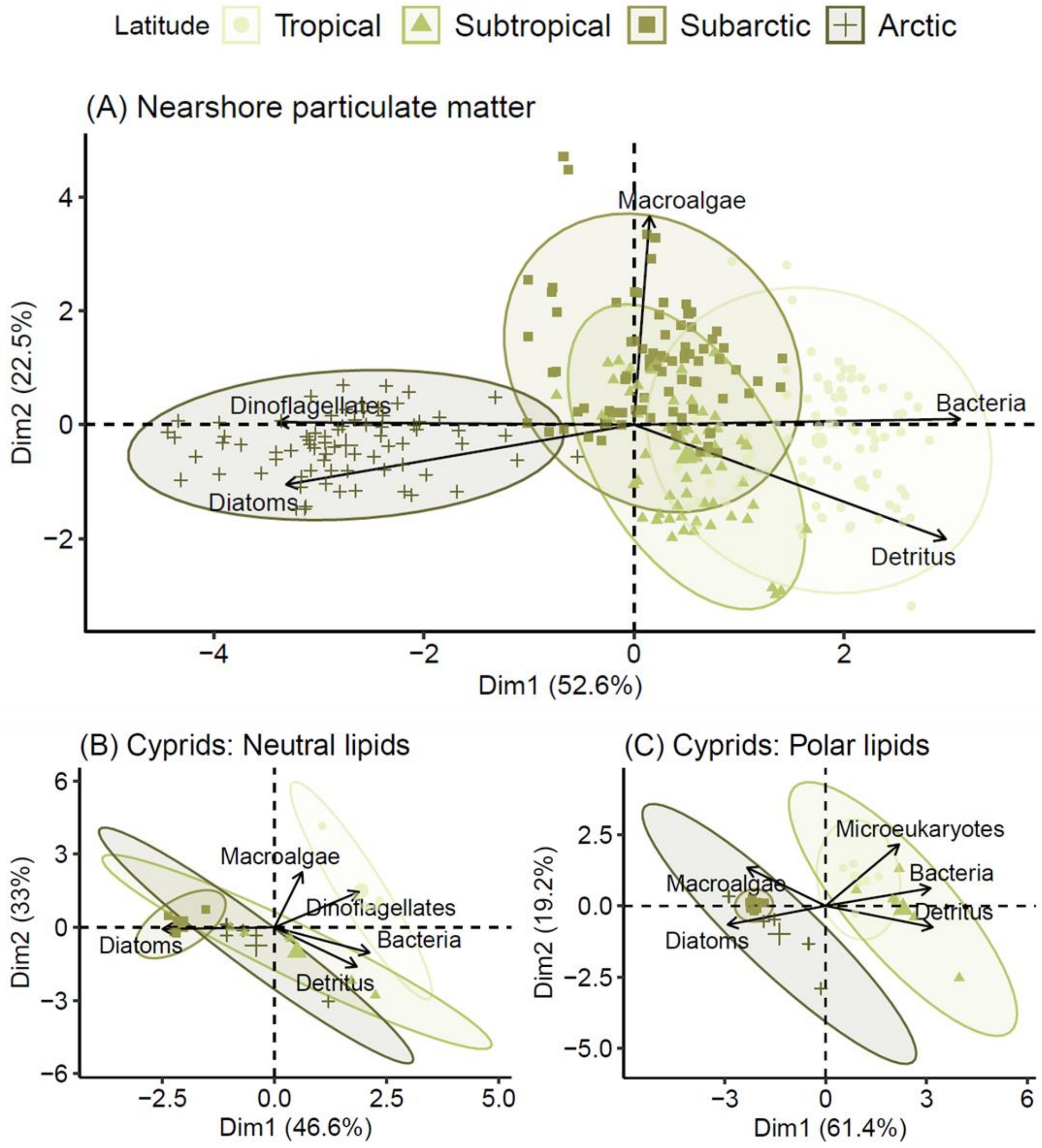

Figure 35. Principal component analysis biplots of the fatty acid trophic markers present in (A) the particulate matter collected from nearshore waters, showing the origin of the food available for filter feeders; (B) the neutral (energetic) fraction of cyprids, showing the short-term source of the dietary fatty acids assimilated as energy fuel; (C) the polar (structural) fraction of cyprids, showing the long-term source of the dietary fatty acids assimilated as membrane building blocks. The biplots show the two dimensions (Dim1, Dim2) which account for the maximum amount of variation in the datasets.

Table 13. PERMANOVA table of results for the latitudinal comparison of trophic markers (FA %) for bacteria, detritus, macroalgae, microeukaryotes, dinoflagellates and diatoms present in (a) particulate matter (TPM), and (b) barnacle cyprid larvae. Significance denoted as ‘*’ < 0.05, ‘**’ < 0.01, ‘***’ < 0.001.

		Trophic markers					**Pair-wise tests**		
	Source	*df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Groups*	*t*	*P(MC)*
(a) TPM							Tropical, Subtropical	3.93	0.007 **
	Latitude	3	1075.0	3584.3	67.99	0.007 **	Tropical, Subarctic	4.90	0.006 **
	Site(Latitude)	4	211.4	52.8	2.15	0.016 *	Tropical, Arctic	13.91	0.000 ***
	Residual	301	7409.1	24.6			Subtropical, Subarctic	3.96	0.006 **
	Total	308	1857.0				Subtropical, Arctic	16.09	0.000 ***
							Subarctic, Arctic	8.78	0.001 ***
(b) Cyprids									
Neutral fraction							Tropical, Subtropical	7.01	0.002 **
	Latitude	3	1812.8	604.3	20.75	0.003 **	Tropical, Subarctic	11.27	0.000 ***
	Site(Latitude)	4	116.3	29.1	0.61	0.723	Tropical, Arctic	3.45	0.011 **
	Residual	15	717.2	47.8			Subtropical, Subarctic	9.42	0.000 ***
	Total	22	2661.3				Subtropical, Arctic	2.19	0.092
							Subarctic, Arctic	2.58	0.068
Polar fraction							Tropical, Subtropical	1.86	0.133
	Latitude	3	2051.0	683.7	19.58	0.005 **	Tropical, Subarctic	11.43	0.000 ***
	Site(Latitude)	4	139.5	34.9	0.70	0.665	Tropical, Arctic	6.82	0.003 **
	Residual	15	744.9	49.7			Subtropical, Subarctic	4.64	0.026 *
	Total	22	2986.9				Subtropical, Arctic	3.62	0.023 *
							Subarctic, Arctic	5.26	0.002 **

Table 14. PERMANOVA table of results for the latitudinal comparison of selective EFA retention (Larvae:Diet ratio) for the fatty acids, ARA, EPA and DHA present cyprids' (a) neutral, energetic fraction, and (b) polar, structural fraction. Significance denoted as '*' < 0.05, '**' < 0.01, '***' < 0.001.

		Selective EFA retention					**Pair-wise tests**		
	Source	*df*	*SS*	*MS*	*Pseudo-F*	*P(perm)*	*Groups*	*t*	*P(MC)*
(a) Neutral fraction							Tropical, Subtropical	17.10	0.000 ***
	Latitude	3	16771.0	5590.4	57.71	0.006 **	Tropical, Subarctic	7.63	0.005 **
	Site(Latitude)	4	387.3	96.8	0.83	0.603	Tropical, Arctic	9.75	0.001 ***
	Residual	15	1744.3	116.3			Subtropical, Subarctic	2.98	0.031 *
	Total	22	19628.0				Subtropical, Arctic	8.36	0.001 ***
							Subarctic, Arctic	6.33	0.005 **
(b) Polar fraction							Tropical, Subtropical	2.48	0.067
	Latitude	3	5053.9	1684.6	10.12	0.005 **	Tropical, Subarctic	4.16	0.015 *
	Site(Latitude)	4	664.6	166.1	0.58	0.834	Tropical, Arctic	3.50	0.015 *
	Residual	15	4290.1	286.0			Subtropical, Subarctic	2.71	0.058
	Total	22	10260.0				Subtropical, Arctic	2.82	0.030 *
							Subarctic, Arctic	3.50	0.013 *

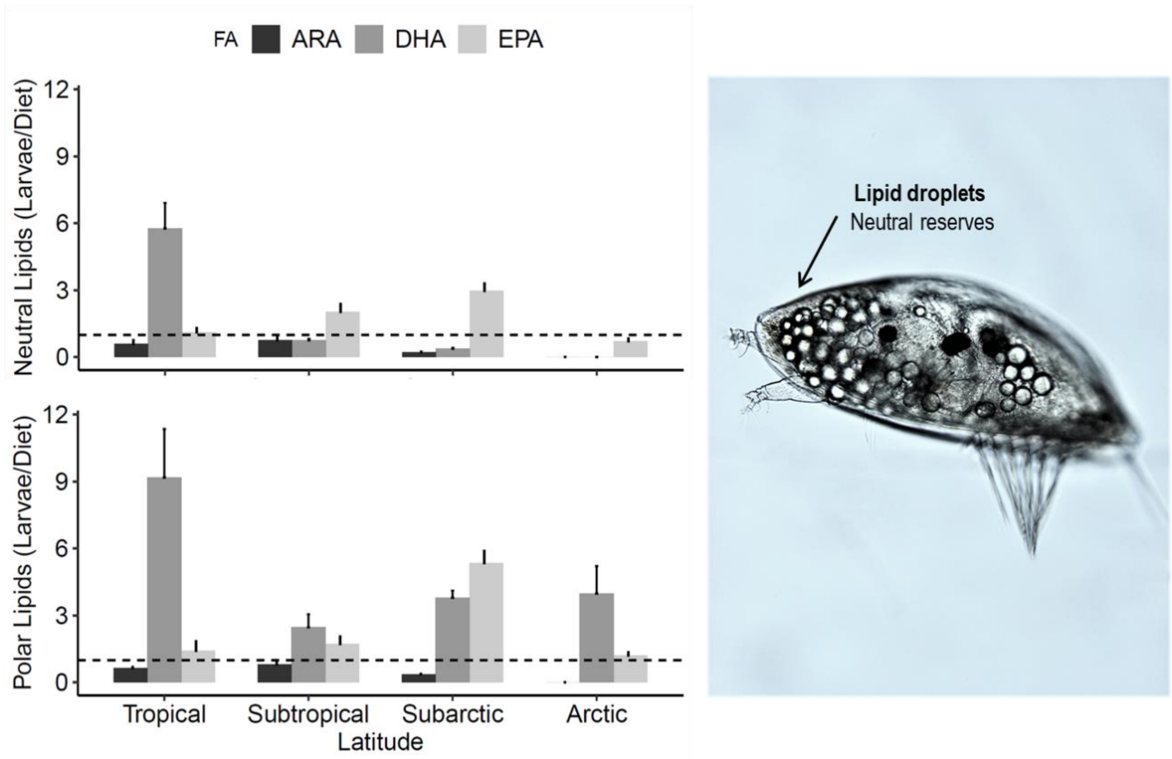

Figure 36. Ratios of EFAs in cyprid larvae to dietary EFAs. The dashed line indicates equal amounts of fatty acids in larvae and their environment. A ratio ≥ 1 indicates that larvae are selectively incorporating fatty acids into energetic reserves (upper panel) and/or cellular structure (bottom panel). The microphotograph (100 ×) shows an arctic *S. balanoides* cyprid in lateral view, with conspicuous lipid droplets, its prime lipid storage. FA: Fatty acid; ARA: Arachidonic acid (20:4ω6); DHA: Docosahexaenoic acid (22:6ω3); EPA: Eicosapentaenoic acid (20:5ω3).

5.5.2.2 Larval selective EFA retention

A deeper look into the EFAs assimilated by cyprid larvae according to dietary availability (larvae:diet ratios, Figure 36), shows a distinctive pattern according to energetic and structural lipids. While the ratio remained below 1 for most of the EFAs in the neutral larval fraction (i.e., EFAs not limited in the short-term source of food), most EFAs were well above a ratio of 1 in the larval polar fraction, showing selective incorporation of EFAs into membrane structural components. This selective incorporation of EFAs (especially DHA and EPA) was common across all species, with no differences detected between the tropical *C.*

proteus, subtropical *C. bisinuatus*, and subarctic *S. balanoides* (PERMANOVA, Table 14-b). The fact that the ratio for DHA for the tropical *C. proteus* was highly above 1 in both larval fractions suggests that this EFA might be limiting in the available pelagic food sources. The same holding true for the ratio for EPA for the subarctic *S. balanoides*, that appears to be limiting as a dietary fatty acid.

5.6 DISCUSSION

The physiological needs of cyprid larvae during the critical pelago-benthic transition are different according to the environment they inhabit. Our results suggest that during their larval (naupliar) development, barnacles use a diverse array of dietary particles to build their neutral (triacylglycerol) reserves, stored in conspicuous lipid droplets (e.g., Tremblay et al., 2007; Thiyagarajan, 2010), that can be rapidly metabolized (Lee et al., 2006). These storage lipids are then selectively allocated by the larva according to the environmental constraints (trophic, thermal) it faces, i.e., physiological lipid remodeling (e.g., Pernet et al., 2006; Gaillard et al., 2015). Although it is arguable that the physiological trends across latitudes may in part be phylogenetically related, as *Semibalanus* and *Chthamalus* are different genus of barnacles, the species here studied are ecological analogs, populating the same intertidal zone (Lewis, 1964), and thus very likely play similar functional roles across sites. One important result of the PERMANOVA comparisons is that no small-scale variation was detected in larval physiology ($p_{\text{Site(Latitude)}} > 0.05$; Tables 13,14), strengthening the importance of cross-latitudinal differences. Below, we argue that the patterns of fatty acid retention found in this study can be related to (1) dietary availability, (2) response to physical variables, and/or (3) energetic or synthetic requirements.

5.6.1 Latitudinal patterns in dietary sources

The share of SFAs in pelagic matter entering benthic food webs was quite important across latitudes, ranging 55-60 % in low-latitudes and 40-45 % in high-latitudes. This likely reflects the land-ocean connectivity typical of intertidal shores, which receive considerable amounts of allochthonous detritus, contributing to strong detrital-based food webs close to shore. Despite the considerable fraction of SFAs, different sources contributed to the bulk of dietary fatty acids available for suspension-feeders over the different latitudes. FATM analysis showed that the dietary composition of pelagic matter in tropical waters had a higher proportion of phytodetritus (14:0, 16:0, 18:0, 18:1ω9 markers; Dalsgaard et al., 2003) compared to living microalgae, and that of arctic ones had higher percentages of diatom (16:1ω7 and 20:5ω3; Dalsgaard et al., 2003) and dinoflagellate (18:4ω3 and 22:6ω3; Dalsgaard et al., 2003) markers, suggesting higher local primary productivity. The pronounced heterotrophic pathways (detrital and bacterial) in low-latitude shores is likely sustained by coastal runoff. Storm-induced perturbations have been shown to cause a rapid export of nutrients and suspended matter to nearshore waters in tropical regions (e.g., Herbeck et al., 2011).

Tracers for macroalgae including macroalgal detritus, green and brown macroalgae, and terrestrial detritus (18:1ω9, 18:2ω6, 18:3ω3 and 18:3ω6; Kelly and Scheibling, 2012), contributed substantially to food webs at both subtropical (19 % of TPM) and subarctic shores (28 % of TPM). These results are in accordance with reports of seaweed canopies comprising the main subtidal vegetated habitats at both subtropical (*Sargassum*, Duarte et al., 2016; Figueiredo et al., 2020) and subpolar sites (*Ascophyllum nodosum*, Beermann et al., 2013). Suspension feeders, presumed to feed mainly on phytodetritus, are also known to incorporate significant amounts of resuspended macroalgal material (Gaillard et al., 2017). Most of the biomass of dominant primary producers, from mangrove leaves to microalgae, enters the food web via detrital pathways rather than being directly grazed (Meziane et al. 1997; Alfaro et al., 2006). We suggest that macroalgal-derived matter (given mainly by the fatty acid 18:1ω9 [7-20 % TPM] across all latitudes) could be a substantial dietary source in benthic food webs, especially in subtropical and subarctic waters.

The composition of the dietary fatty acids described here is, however, probably not wholly representative of the benthic food supply. Temporal/seasonal variability in nearshore trophic conditions, for example, could not be captured and might be significant at some sites. Shifts over fine temporal scales (days) have been reported at both tropical (shifts in matter composition following rainfall events in Bocas del Toro; Leal et al., 2019) and subtropical sampled shores (scattered oceanographic processes [1–2days] leading to localized vertical mixing and surplus production in the SSC; Kasten and Flores, 2013). For the arctic locations, seasonal shifts have been reported (differential pelagic matter composition during spring bloom and post-bloom in Kobbefjord; Gaillard et al., 2017). Future research should therefore aim to capture such temporal variability for a better understanding of dietary sources entering benthic food webs.

5.6.2 Lipid remodeling as a function of temperature

While the fatty acid composition of reserve lipids provides reliable information on food web structure, the lipid composition of membranes can reveal how ectotherms physiologically adjust to their environment (Hazel, 1995; Pernet et al., 2006). We found that SFAs comprised a substantial fraction cyprid reserves, particularly that of the tropical and subtropical *Chthamalus* cyprids, exceeding 50 % of larval energetic and membrane structure content (the fatty acids 16:0 and 18:0 alone accounting for ≥ 40 %). SFAs accumulation has been shown to be important for invertebrate species (e.g., copepods, Cabrol et al., 2015; bivalve larvae, Leal et al., 2019), and suggested to be advantageous under high energetic demands (e.g., demanding osmoregulatory processes) given the efficient oxidation and high energy yield of this class of fatty acids. *Semibalanus* cyprids had a far greater proportion of PUFAs (41-44 % of polar lipids) in their structural component in both subarctic and arctic waters, where intertidal temperatures reached a minimum of 1.2 ^{o}C. This goes in accordance with studies showing that PUFAs are accumulated in higher proportions in polar and temperate marine organisms than those from the tropics (Copeman and Parrish 2003; Colombo et al., 2017; Thyrring et al., 2017). In Gilbert Bay, Labrador, Canada, Copeman and Parrish (2003) found elevated concentrations of PUFAs (~ 48 % total fatty acids) across 16 species of macroinvertebrates exposed to "freezing" temperatures.

The inclusion of phospholipids with higher levels of PUFA in biological membranes in these environments is a process referred to as "homeoviscous adaptation" (Hazel, 1995; Pernet et al., 2006; Parent et al., 2008). Lipid remodeling may therefore vary as a function of latitude, reflecting local adaptation to environmental temperature (Pernet et al., 2006; Parent et al., 2008). Temperature has a strong effect on the physical properties of membrane lipids, which in turn influence the proteins they surround (Hochachka and Somero, 2002). A decrease in temperature usually induces an increase in the overall packing order of membrane phospholipids, that is, a decrease in membrane fluidity, and elevated temperatures decrease membrane order, which may lead to membrane dysfunction (Hazel, 1995). Persistent exposure to temperatures, either above or below those required to maintain optimal fluidity, initiates acclimatory (within the lifetime of an individual) or adaptational (over generations) changes in membrane lipid composition that largely offset the direct effects of temperature on membrane lipid fluidity (Hazel, 1995). We suggest that modification of membrane lipid composition, that is, physiological lipid remodeling, is also a strategy used by *Chthamalus* and *Semibalanus* late-stage barnacle larvae to adjust to the surrounding environment. We advance, however, that physiological lipid remodeling occurs not only as a function of temperature ("homeoviscous adaptation"), but also as a function of the trophic environment, signaling food deprivation ("selective retention").

5.6.3 Larval selective EFA retention

Both *Chthamalus* and *Semibalanus* cyprids contained higher EFA levels than the relative share of lipids in their environment, and the low levels of 18-carbon precursor fatty acids in food resources (≤ 5 % of TPM lipid content) suggests selective retention rather than biosynthesis (e.g., Calado et al., 2005). The essential 20:4ω6 (ARA), 20:5ω3 (EPA), and 22:6ω3 (DHA) increase cellular membrane fluidity and associated enzyme activity (Else and Hulbert, 2003; Hall et al., 2002), facilitate metabolic activity (Else et al., 2004; Hulbert and Else, 1999), and favor the larval osmoregulatory processes necessary to increase larval robustness at the moment of the pelago-benthic transition (hyper-osmoregulation, i.e., extracellular medium more concentrated than the external medium; Charmantier et al., 2001). The selective incorporation of EFAs, notably DHA, into polar lipids was common across all species, and substantial in tropical *C. proteus*.

De novo synthesis of DHA by crustaceans is minimal (Kanazawa et al., 1979) or non-existent (Castell 1983; Nelson et al., 2006). Similar to other invertebrate taxa (e.g., lobsters, Thériault and Pernet, 2007; clams, Parent et al., 2008; scallops, Gagné et al., 2010) we suggest that these long-chain PUFA was selectively incorporated into membrane phospholipids at the expense of reserve lipids. DHA is preferentially retained during food deprivation (Watanabe, 1978; Martinez-Silva et al., 2018), and we argue that cyprid larvae retained this fatty acid due to (1) dietary deficiencies during naupliar development, and/or (2) importance of this fatty acid in lipid remodeling in response to temperature.

The incorporation of DHA in the cell membrane has been widely suggested to increase membrane fluidity, the molecular activity of membrane proteins, and metabolic rate (Hulbert and Else, 2005). Therefore, for *C. proteus* cyprids, DHA retention could allow for an increased metabolic activity of membrane proteins (e.g., Na+/K+-ATPase) and rapid conformational changes in biological membranes (Feller et al., 2002). Elevated temperatures, related with higher metabolism and higher consumption of ATP, constrain the energy that larvae can invest in other physiological functions (e.g., growth, escape from predators). Preferential retention of EFAs in unfavorable conditions is of great importance for maintaining the cellular biochemical competency, but over the long run, a fast metabolism can result in a sharp decline of the DHA reserves and a high mortality (Werbrouck et al., 2016). Our results point to the hypothesis that cyprids living in warmer and less productive environments are prone to higher physiological stress, reflected in the level of DHA retention and supported by the decreasing ARA:EPA ratio in larvae across latitudes (Supplementary Table 16). EPA and ARA are precursors of eicosanoids, which are short-lived hormone-like substances that have opposite effects in inflammation and immune related processes (Werbrouck et al., 2016). ARA-derived eicosanoids promote inflammation while those from EPA are rather anti-inflammatory (e.g., Werbrouck et al., 2016). Knowing that *C. proteus* settlers face increasing levels of thermal stress in Bocas del Toro (Leal et al., 2020), DHA-depleted cyprids may be subjected to high mortalities during the bentho-pelagic transition. Although such hypotheses remain to be tested, we hope to motivate further research into the settlement dynamics of this important group of barnacles.

5.7 CONCLUSION

Our study provides novel data on the fatty acid signatures of late-stage barnacle larvae and pelagic food sources entering the benthic food webs over different latitudes. We show lipid remodeling occurs in late-stage barnacle larvae, varying as a function trophic and thermal constraints. This opens the door for future research on the importance of larval physiology in shaping population dynamics of this important group of habitat-forming species.

5.8 ACKNOWLEDGEMENTS

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5.9 SUPPLEMENTARY MATERIAL

Table 15. Fatty acid composition (FA % ± SE) of total particulate matter (TPM) collected from nearshore waters at each location. FAs accounting for less than 1 % of total FAs were removed. Abbreviations: SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; EFA, Essential Fatty Acids; ARA, Arachidonic acid; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; MTFA, Mass of Total Fatty Acids. *n* stands for the samples analysed.

Fatty acid	**Panama (Tropical)**	**Brazil (Subtropical)**	**Canada (Subarctic)**	**Greenland (Arctic)**
	n = 88	*n = 66*	*n = 80*	*n = 74*
14:0	6.5 ± 0.3	5.5 ± 0.3	6.0 ± 0.2	11.3 ± 0.3
15:0	1.6 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	0.7 ± 0.0
16:0	31.5 ± 0.3	26.4 ± 0.4	24.9 ± 0.3	19.1 ± 0.3
17:0	0.9 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
18:0	12.6 ± 0.5	17.0 ± 0.7	9.4 ± 0.4	4.5 ± 0.2
20:0	1.3 ± 0.0	1.4 ± 0.1	1.4 ± 0.0	0.8 ± 0.0
22:0	1.0 ± 0.0	1.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.0
24:0	2.0 ± 0.0	1.8 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
∑SFA	**57.3 %**	**55.3 %**	**45.4 %**	**38.9 %**
16:1	10.8 ± 0.4	10.4 ± 0.4	12.5 ± 0.4	11.4 ± 0.3
18:1ω9	15.0 ± 0.7	11.1 ± 0.3	19.3 ± 0.6	7.3 ± 0.2
24:1ω9	1.2 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	0.0 ± 0.0
∑MUFA	**27.0 %**	**23.2 %**	**32.5 %**	**18.7 %**
18:2ω6	3.0 ± 0.1	3.8 ± 0.2	5.3 ± 0.2	2.9 ± 0.1
18:3ω3	1.5 ± 0.1	3.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
18:3ω6	0.8 ± 0.0	1.0 ± 0.1	0.8 ± 0.0	1.5 ± 0.1
18:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.3 ± 0.1
20:4ω6 (ARA)	3.1 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	1.7 ± 0.0
20:5ω3 (EPA)	4.3 ± 0.1	6.5 ± 0.2	5.7 ± 0.1	22.5 ± 0.2
22:6ω3 (DHA)	1.6 ± 0.2	3.5 ± 0.2	3.0 ± 0.2	3.8 ± 0.6
∑PUFA	**14.3 %**	**20.4 %**	**19.9 %**	**40.1 %**
∑EFA	**9.0 %**	**12.5 %**	**11.4 %**	**28.0 %**
MTFA (µg/mg TPM)	**2.2 ± 0.2**	**0.8 ± 0.1**	**1.1 ± 0.1**	**5.3 ± 0.6**

Table 16. Fatty acid composition (FA % ± SE) of the neutral lipids, i.e., energetic reserves, and polar lipids, i.e., cell structural components, of late-stage barnacle larvae (cyprids) collected in the field. FAs accounting for less than 1 % of total FAs were removed. Abbreviations: SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; EFA, Essential Fatty Acids; ARA, Arachidonic acid; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; MTFA, Mass of Total Fatty Acids. ARA/EPA: ratio indicative of physiological stress. *n* stands for the total number of cyprids sampled for lipid analysis.

[see next page]

Fatty acid	***C. proteus* (Tropical)**		***C. bisinuatus* (Subtropical)**		***S. balanoides* (Subarctic)**		***S. balanoides* (Arctic)**	
	n = 486		*n = 245*		*n = 146*		*n = 160*	
	Neutral	Polar	Neutral	Polar	Neutral	Polar	Neutral	Polar
12:0	0.0 ± 0.0	0.3 ± 0.2	0.8 ± 0.3	0.5 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	0.7 ± 0.3	0.2 ± 0.1
14:0	5.0 ± 0.6	2.2 ± 0.2	14.3 ± 1.5	4.4 ± 0.8	14.5 ± 1.1	1.5 ± 0.2	7.7 ± 1.5	1.5 ± 0.1
15:0	1.3 ± 0.3	1.1 ± 0.0	1.4 ± 0.2	1.2 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
16:0	31.9 ± 1.8	25.0 ± 0.7	26.9 ± 2.6	28.2 ± 2.8	18.2 ± 0.4	17.6 ± 0.2	29.1 ± 2.9	19.8 ± 1.6
17:0	0.6 ± 0.1	0.8 ± 0.0	0.9 ± 0.2	1.8 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.2	0.3 ± 0.1
18:0	11.8 ± 1.2	21.4 ±1.1	13.1 ± 4.0	25.0 ± 3.6	2.6 ± 0.3	7.5 ± 0.8	15.8 ± 4.7	15.1 ± 2.8
20:0	1.4 ± 0.4	1.4 ± 0.2	0.8 ± 0.2	1.8 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
∑SFA	**51.9 %**	**52.2 %**	**58.2 %**	**62.9 %**	**36.0 %**	**27.2 %**	**54.4 %**	**37.4 %**
14:1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.2	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0
16:1	8.5 ± 0.4	4.0 ± 1.2	14.4 ± 2.5	3.1 ± 0.9	29.1 ± 0.9	3.8 ± 0.2	15.7 ± 2.3	3.8 ± 0.3
18:1ω9	11.0 ± 0.8	10.5 ± 1.1	5.9 ± 0.3	9.6 ± 1.0	10.5 ± 0.4	18.2 ± 0.3	8.6 ± 1.3	12.0 ± 0.9
20:1ω9	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.3	0.6 ± 0.1	1.6 ± 0.1	1.0 ± 0.4	5.3 ± 3.1
24:1ω9	3.0 ± 0.4	3.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.1	3.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
∑MUFA	**22.6 %**	**18.5 %**	**20.4 %**	**13.4 %**	**43.2 %**	**26.9 %**	**25.6 %**	**21.2 %**
18:2ω6	5.7 ± 0.8	5.2 ± 0.5	1.8 ± 0.1	0.7 ± 0.5	1.6 ± 0.4	2.7 ± 0.7	1.0 ± 0.2	0.6 ± 0.2
18:3ω3	2.8 ± 0.3	1.3 ± 0.1	0.8 ± 0.2	0.2 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
18:4ω3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.4	0.7 ± 0.2
20:4ω6 (ARA)	1.8 ± 0.6	1.9 ± 0.1	1.9 ± 0.4	2.0 ± 0.3	0.6 ± 0.1	0.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:5ω3 (EPA)	4.0 ± 0.4	4.4 ± 0.4	13.2 ± 2.6	11.2 ± 2.0	16.0 ± 0.6	29.0 ± 1.1	15.6 ± 2.7	27.1 ± 2.8
22:6ω3 (DHA)	8.2 ± 0.6	12.9 ± 1.7	2.6 ± 0.3	8.7 ± 1.7	1.1 ± 0.1	10.9 ± 0.2	0.0 ± 0.0	12.6 ± 3.4
ARA/EPA	**2.8**	**2.3**	**5.3**	**7.3**	**31.7**	**34.2**	**-**	**-**
∑PUFA	**22.6 %**	**25.7 %**	**20.3 %**	**22.9 %**	**19.7 %**	**43.9 %**	**19.4 %**	**41.0 %**
∑EFA	**14.0 %**	**19.2 %**	**17.8 %**	**21.9 %**	**17.7 %**	**40.7 %**	**15.6 %**	**39.7 %**
MTFA (µg/larva)	**0.05 ± 0.0**	**0.05 ± 0.0**	**0.5 ± 0.1**	**0.1 ± 0.1**	**1.0 ± 0.2**	**0.5 ± 0.0**	**1.0 ± 0.5**	**0.3 ± 0.0**

ARTICLE 6

RESPONSE OF TROPICAL AND SUBTROPICAL CHTHAMALID BARNACLES TO INCREASING SUBSTRATE TEMPERATURES

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6.1 ABSTRACT

Marine invertebrates inhabiting low-latitude shores are exposed to both extremely hot and highly variable conditions. Further changes in habitat temperature may pose a risk to these populations. In this study, we examined the early life stage response of foundation species from subtropical and tropical latitudes to changes in the thermal habitat. We manipulated the color of settling surfaces for barnacle species that occupy the same ecological niche, *Chthamalus bisinuatus* (southeastern coast of Brazil), and *Chthamalus proteus* (northwestern coast of Panama). Using an *in situ* experimental approach, we assessed: 1) the combined effects of temperature and other abiotic parameters, and 2) the significance of larval and early juvenile traits in determining post-settlement performance for the first three days of benthic life. We found that the biological outcomes differed for the two species, according to the local thermal regime experienced. *C. bisinuatus* juveniles responded positively to higher temperatures, through a positive interaction between larval energetic condition, post-metamorphic size and temperature on their initial growth. Conversely, *C. proteus* juveniles grew slower at higher temperatures, although a positive effect of size at settlement was found. Continuous air- and sea-surface temperature measurements revealed that the tropical chthamalids experienced temperatures ≈6°C higher than their subtropical congeners, as well as harsher thermal conditions during aerial exposure. This suggests that manipulated temperatures likely exceeded the tolerance of the Caribbean population, already living on the edge of their thermal threshold. Our findings contribute to the understanding of the factors underpinning the critical post-settlement events that drive patterns of abundance and distribution of barnacles in low-latitude systems.

Keywords: Benthic invertebrates; Larval energetics; Post-settlement performance; *In situ* experiment; Substrate temperature; Tropics

6.2 RÉSUMÉ

Les invertébrés marins vivant sur les côtes des basses latitudes sont exposés à des conditions à la fois extrêmement chaudes et très variables. Les changements croissants de la température de l'habitat peuvent poser un risque pour ces populations. Dans cette étude, nous avons examiné la réponse des premiers stades de vie des espèces fondatrices des latitudes subtropicales et tropicales aux changements de l'habitat thermique. Nous avons manipulé la couleur des surfaces de fixation pour les espèces de balanes qui occupent la même niche écologique, *Chthamalus bisinuatus* (côte sud-est du Brésil) et *Chthamalus proteus* (côte nord-ouest du Panama). En utilisant une approche expérimentale *in situ*, nous avons évalué : 1) les effets combinés de la température et d'autres paramètres abiotiques, et 2) l'importance des traits larvaires et des jeunes juvéniles dans la détermination des performances post-fixation pour les trois premiers jours de la vie benthique. Nous avons constaté que les résultats biologiques différaient pour les deux espèces, selon le régime thermique local expérimenté. Les juvéniles de *C. bisinuatus* ont répondu positivement à des températures plus élevées, grâce à une interaction positive entre l'état énergétique des larves, la taille post-métamorphique et la température lors de leur croissance initiale. À l'inverse, les juvéniles de *C. proteus* croissaient plus lentement à des températures plus élevées, bien qu'un effet positif de la taille au moment de la colonisation ait été observé. Des mesures continues de la température de l'air et de la surface de la mer ont révélé que les chthamalidés tropicaux ont subi des températures ≈ 6 °C plus élevés que leurs congénères subtropicaux, ainsi que des conditions thermiques plus difficiles pendant l'exposition l'émersion. Cela suggère que la manipulation thermique des substrats de fixation a probablement permis de dépasser la tolérance de la population des Caraïbes, vivant déjà à la limite de leur seuil thermique. Nos résultats contribuent à la compréhension des facteurs sous-jacents aux événements critiques post-fixation qui déterminent les modèles d'abondance et de distribution des balanes dans les systèmes de basse latitude.

Mots clés : Invertébrés benthiques; Énergie larvaire; Performance post-règlement; Expérience *in situ*; Température du substrat; Tropiques

6.3 INTRODUCTION

Temperature exerts a strong selective pressure upon marine populations distributed over various spatial scales, from small cm-scales to broad latitudinal scales (e.g., Bertness and Gaines, 1993; Rayssac et al., 2010; Peck, 2011). Marine invertebrates inhabiting tropical shores are exposed to both extremely hot and highly thermally variable conditions, and most are already living close to their thermal tolerance limit (Helmuth and Hofmann, 2001; Nguyen et al., 2011; Kasten and Flores, 2013). Their limited ability to acclimatize, and/or fully-expressed acclimatory response in nature, means that these organisms are at especially high risk from further changes in habitat temperature (Thompson et al., 2002; Nguyen et al., 2011; Marshall et al., 2018). However, marine intertidal ecology has built its foundation on research conducted in colder temperate habitats, leaving tropical or subtropical habitats largely under-studied, where processes determining species' abundance and distribution may be very different (Bertness and Leonard, 1997; Hawkins et al., 2008; O'Riordan et al., 2010).

Barnacles are some of the most important foundation species across temperate, subtropical and tropical rocky shores and have long been recognized as indicators of environmental change (Southward and Crisp, 1954; Southward et al., 1995; Bertness and Leonard, 1997; Hawkins et al., 2003). As early as the 1950s, shifts in *Chthamalus* sp. distribution across the British Isles were linked to a general rise in air- and seawater temperature (Southward and Crisp, 1954). The effects of environmental change were suggested to be apparent in barnacle species within a much shorter time compared to other sessile invertebrates (Southward and Crisp, 1954). The ability to tolerate the harsh thermal conditions that prevail in the intertidal depends on the tolerance of early settlement stages, which are thought to be the most sensitive to temperature (Foster, 1969; Gosselin and Qian, 1997; Hunt and Scheibling, 1997; Pineda et al., 2009; Collin and Chan, 2016). Early tolerance depends on both genetic factors and past embryonic and larval experiences (Emlet and Sadro, 2006; Rayssac et al., 2010; Freuchet et al., 2015). Growing evidence suggests that variability in the physiological condition of settling larvae can give rise to cohort-specific differences in the fitness of juveniles and adults (Jarrett and Pechenik, 1997; Olivier et al., 2000; Emlet and Sadro, 2006; Tremblay et al., 2007b; Thiyagarajan, 2010).

The food supply for barnacle nauplii directly affects the condition of competent cyprids (Tremblay et al., 2007b). Well-fed nauplii develop into larger cyprids that grow faster and have a greater chance of survival (Emlet and Sadro, 2006). Trophic carry-over effects may endure through the successive juvenile stages (Jarrett and Pechenik, 1997; Miron et al., 2000; Thiyagarajan et al., 2003; Pechenik, 2006) and might be a critical energetic pathway linking benthic and pelagic habitats (Pechenik et al., 1998; Tremblay et al., 2007b). At low latitudes, the food supply for suspension feeders frequently falls below critical levels, limiting their growth and reproduction (Ciotti et al., 2010; Kasten and Flores, 2013; Freuchet et al., 2015; Barbosa et al., 2016). Restricted resource allocation to offspring in response to critical habitat temperatures has already been reported in subtropical chthamalid barnacles (Freuchet et al., 2015).

Micro-spatial thermal conditions are strongly dependent on the substratum and incidence of solar radiation, so much so that intertidal sites display unique “thermal signatures” (Helmuth 2002). Patterns of spatial and temporal microclimatic variation between and within intertidal zones has been shown to have important physiological, behavioral and demographic consequences for intertidal organisms (Gunderson et al. 2019). For barnacles, the first moments after settling as the tide recedes may constitute a bottleneck for survival in the intertidal zone. Selective pressures for traits such as habitat selection, energy reserves at settlement, time of settlement relative to the tidal cycle, are most intense during the first day of benthic life (Gosselin and Qian, 1996). Thermal differences in the micro-habitat during this critical period may therefore highly impact low-latitude species, especially those inhabiting the upper intertidal and/or already living on the edge of their thermal threshold.

In this study, we investigated the biological response of *Chthamalus* species from tropical and subtropical latitudes to changes in their thermal habitat. We manipulated the color of settling surfaces for barnacle cyprids directly in the field and assessed 1) the combined effects of higher substrate temperatures and other abiotic parameters on the survival and growth of early settlers, and 2) the role of larval condition (energetic status) and post-metamorphic size on juvenile performance, for the first three days of benthic life. We hypothesized that elevated temperatures would have detrimental effects on the post-settlement performance of young chthamalids, and anticipated that higher larval condition and greater post-metamorphic size would moderate the effect of warm temperatures on the initial growth of juveniles. Scarce information is available on the recruitment dynamics of Caribbean chthamalids and to our knowledge, the present study is the first attempt to investigate the effects of *in situ* increased temperatures on the early stages of this important group of barnacles.

6.4 MATERIAL AND METHODS

6.4.1 Study areas & Species

Two chthamalid barnacle species were sampled from low-latitude locations: *Chthamalus bisinuatus* (Pilsbry, 1916) on the southeastern coast of Brazil (Baleeiro Head, São Sebastião; 23°49'41"S, 45°25'22"W), and *Chthamalus proteus* Dando and Southward, 1980 on the northwestern coast of Panama (Paunch Beach, Isla Colon; 9°22'25"N, 82°14'19"W). These species are considered ecological equivalents, as they occupy the same ecological niche (the upper midlittoral zone), forming largely monospecific bands, and therefore playing a similar role in the ecosystem. Bueno et al. (2010) reported that natural populations of *C. bisinuatus* along the São Paulo coastline exhibit a very clear fortnightly rhythm of larval release. Zabin et al. (2007) found adult *C. proteus* barnacles with developing eggs at all times of the year, indicating a year-round production of propagules.

Located on the exposed coast of the São Sebastião Channel (northern coast of São Paulo State), Baleeiro Head is characterized by a steep rocky shore with a clear midlittoral saturated (100 % cover) *Chthamalus* zone (e.g., Kasten and Flores, 2013; Barbosa et al., 2016). Average monthly sea surface temperature presents the lowest values in July, and the highest in February (Valentim et al., 2013). On the exposed coastal side of Isla Colon (Bocas del Toro Archipelago), Paunch beach comprises an extended carbonate rocky platform, with *C. proteus* dominating the upper-midlittoral (I. Leal, pers. obs.). Temperatures increase in Isla Colon from February to June, followed by a dip from July to September (Kaufmann and Thompson, 2005). Both study locations have microtidal coasts that are subjected to a mixed semi-diurnal tidal regime, i.e., a tidal cycle with two unequal high tides and two unequal low tides. Nevertheless, the timing of low tide plays a key role in determining the exposure of intertidal organisms to potentially stressful conditions. Extreme temperatures perceived by intertidal organisms during aerial exposure is assumed to have a great effect on the survival and physiology of the post-larval stages of these organisms (Finke et al., 2007), and for this reason air temperature and tidal regimes throughout the present study are addressed below (Results section 3.1).

Sampling was carried out continuously for a period of 23 days, during November (2016) in Brazil, where samples were processed at the Center for Marine Biology of the University of São Paulo (CEBIMar/USP), and April (2017) in Panama, where samples were processed at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station (STRI; UNARGEN Research Permit SE/AO-9-16). Data sets provided by the Meteorology Station of CEBIMar/USP and the Physical Monitoring Program of STRI were used to characterize the daily average air temperature at both sampling locations for the years of 2016 and 2017, respectively (Supplementary Material, Fig. 43).

6.4.2 The natural thermal habitat

Natural rock quadrats (10 × 10 cm), initially cleared of all invertebrates using a chisel, were established directly in the field 1 to 2 m apart, at the mid-littoral heights that comprised moderate to high adult densities in both study locations. On 4 sampling dates (i.e., weekly throughout the sampling period), the temperature of 10 quadrats was measured during low tide using an infra-red (IR) thermal camera (FLIR C2, Compact Thermal Imaging System).

One image was taken per quadrat, at a height of 50 cm above the surface, providing an IR image with a spatial resolution <1 mm^2 per pixel. Thermal measurements (average temperature of 10 × 10 cm) were made using the software FLIR Tools, that permits analysis of imported IR images and exportation of data frames giving the temperature per pixel. This allowed us to establish a baseline for natural substrate temperature, and later compare it with the temperature of the artificial substrates used in the present field experiment.

6.4.3 *In situ* manipulation of substrate temperature

The manipulation of substrate temperature using artificial surfaces for settlement was carried out directly in the field, following the method described by Lathlean and Minchinton (2012). Settlement plates (10 × 10 cm) covered with black and white tapes (3M™ Safety-Walk™ 600 series, #610 and #620, respectively) were used (*n* = 10 black plates; *n* = 10 white plates). Plates were pre-conditioned for 2 weeks in a flow-through seawater system to minimize chemical differences, by allowing natural biofilms to accumulate on the artificial surfaces (Qian *et al.*, 2003; Lathlean and Minchinton, 2012). Plates were haphazardly distributed over a spatial scale of hundreds of meters, interspersed among natural rock quadrats (see above, 6.4.2), providing substrate for larval settlement in the *Chthamalus* (midlittoral) zone.

The IR temperature of plates was measured daily (once per low tide), following the same protocol described to obtain the IR temperature of natural rock quadrats. Simultaneously, variability in temperature experienced in the intertidal zone was measured using *in situ* data loggers (HOBO Pendant® Temperature/Light 64K, contained within waterproof casings). This allowed us to characterize the thermal regime experienced by intertidal organisms (Lathlean and Minchinton, 2011), as these loggers continuously measure air temperature upon emersion and seawater temperature upon submersion over a fine temporal scale (10 min intervals), therefore detecting acute changes in intertidal temperatures.

During each daily low tide, settlement plates were brought to the laboratory to assess the biological response of early barnacle settlers, and reinstalled in the field within the same hour of removal. Newly settled individuals were identified using a stereomicroscope and tracked by manually mapping their positions on a transparent sheet. Each settler was tracked for the daily estimation of post-settlement mortality (individuals·plate^{-1}) and growth (rostro-carinal diameter; % increment·d^{-1}). Growth estimates were obtained by photographing each settler individually as shown in Supplementary Material (Fig. 44) and by subsequently analyzing the images using the software Fiji, an open-source platform for scientific image analysis (Schindelin et al., 2012). A total of 211 *C. bisinuatus* settlers, and 452 *C. proteus* settlers were measured throughout the present study.

6.4.4 Environmental monitoring

Key environmental parameters were measured at each diurnal low tide. Spot measurements of sea-surface salinity were obtained directly from nearshore waters using a probe (EcoScan SALT 6 Plus Salinity Meter with electrode; ppt). Total Particulate Matter (TPM), a proxy of food availability, was estimated by collecting three replicate seawater samples of 2 L per day. Samples were filtered through a 100 µm mesh sieve, and then through Whatman® glass microfiber filters (GF/F 25 mm, pore size 0.7 µm, pre-burnt and weighted). After filtration, the three glass microfiber filters were heated to 70 ^{o}C for 24h and weighed for final estimation of TPM (mg·L^{-1}). Cypris larvae were collected using a 150 µm plankton net (30 cm mouth diameter) towed manually at sub-surface, in order to estimate larval physiological condition (lipid droplet cover; see below).

6.4.5 Larval physiological condition

Lipid physiological indices for invertebrate larvae can be determined by coloration of neutral lipids with the dye Nile red, followed by quantification using image analysis to calculate lipid surface relative to total larval surface (Castell and Mann, 1994; Hentschel and Emlet, 2000; Kheder et al., 2010). In cypris larvae, the transparent carapace makes it easy to visualize the conspicuous lipid droplets (triacylglycerol, TAG), the main larval energetic reserves (Tremblay et al., 2007b). TAG content can be expressed relative to DNA content, to compensate the size-dependency of TAG (Miron et al., 2000). Different ratios of TAG/DNA are reflected in the oil droplet volume of cyprids, so that greater volumes indicate better larval physiological condition (Thiyagarajan, 2010). Recent evidence using *C. bisinuatus* cyprids shows that the visual estimation of lipid droplet cover can be used as a proxy of larval quality, given the strong correlation between visual lipid area and stained lipid area (Guerra et al., unpublished). Based on this evidence, lipid droplet covers (%) were estimated for all of the chthamalid cyprids collected. Cyprids were photographed alive under a Nikon microscope (100 ×) in lateral view and morphometric analyses were done using the software Fiji (see Supplementary Material, Fig. 45). The relative area of lipid droplets from the total cyprid area (droplets/body), was estimated for a total of 530 individuals, throughout the 23 days of samplings for both sites. On average, 12 cyprids were collected per day for estimation of physiological quality, the number ranging from 1 to 20 individuals, according to larval supply.

6.4.6 Statistical analyses

6.4.6.1 Temperature of natural versus artificial substrates

To visually illustrate the thermal variability in natural rock substrate (10 × 10 cm; scattered along a 100 m-stretch of the rocky platforms sampled), color maps were built for the 4 sampling dates. Given the inherent local differences in air and sea-surface temperatures of the two tropical regimes (≈ 6 ^{o}C; see Results), comparisons of the effects of the different colored plates on substrate temperature were carried out separately for each location. IR temperature of natural and artificial substrates were compared through one-way ANOVAs on ranks (non-parametric data; Shapiro-Wilk Test, $p < 0.05$), and subsequent pairwise comparisons separately for each location.

6.4.6.2 *Effects of the in situ thermal manipulation*

Although belonging to the same genus, these barnacle species may undergo different developmental physiologies during early post-settlement ontogeny, and therefore direct comparisons of growth or survival may be inappropriate. Therefore, the effects of the different colored plates (*in situ* experiment) on the post-settlement performance of juveniles were carried out separately for each species. Because our data sets correspond to repeated time series (non-independent), we used generalised least-squares (GLS) estimation procedure to fit the linear models to predict post-settlement responses (Zuur et al., 2009; Pinheiro et al., 2019). A residual auto-correlation structure was added with respect to measurement day (correlation = corAR1(form =~ Day | Tape)). A fixed-effects term was given by the categorical variable "Tape" (black or white). Effects of substrate color and IR temperature on the survival of settlers were compared, as peak substrate temperatures during aerial exposure are likely the most influential on survival patterns. As growth is likely related to conditions experienced during submergence, the possible combined effect(s) of local abiotic conditions (see Table 17 for predictor variables) on juveniles' growth was tested. Separate models for growth were run according to the day since settlement (1-day old, 2-days old, 3 days-old juveniles).

Following the model validation process suggested by Zuur et al. (2009, 2010), we carried out a stepwise backwards selection process, and accordingly dropped the least significant term ($p > 0.05$), refitted the model, assessed the quality of the chosen model using Akaike information criterion (AIC; Burnham and Anderson, 2004), and continued this process until all terms were significant. We assessed collinearity by carrying out pairwise scatterplots comparing covariates and exploring correlation coefficients. The easiest way suggested to solve collinearity is by dropping collinear covariates, which can have a bigger impact on *P*-values than dropping non-significant covariates (Zuur et al., 2010). Following this, we have detected that the four temperature-related variables were highly collinear (as would be expected), and dropped from our analysis IR temperature of plates, maximum temperature and average temperature; thermal fluctuation was the predictor variable retained. Yet, whenever two covariates *X* and *Z* are collinear, and *Z* is used in the statistical analysis, it is important to recognize that it might well be *X* that is driving the system (Zuur et al., 2009).

We used the R software (version 3.6.1; R Core Team, 2019) and packages ggplot2 (Wickham, 2016), viridis (Garnier, 2018) and nlme (Pinheiro et al., 2019) to conduct statistical analyses and as graphical tools.

6.5 RESULTS

6.5.1 Two contrasting regimes: Brazil and Panama

A detailed daily description of the temperature and tidal variation during the sampling period is given in Fig. 37. The close interaction of local climate with tidal cycle is clear from this figure. On average, air and sea-surface temperature recorded from the intertidal zone in Brazil was 25.4 ^{o}C, compared to the 31.1 ^{o}C recorded in Panama. Fig. 37 also illustrates the number of days of hot aerial exposure during low tides. Whereas in Brazil maximum temperatures coincided with the peak of low tide for 4 sampling dates (≈32.1 ^{o}C), for Panama, this occurred over 7 sampling dates (≈41.5 ^{o}C). Looking further into the tidal and thermal regimes at each location, we can see that daytime aerial exposure in Brazil was apparently ameliorated by higher low tides (Fig. 38A; potentially attenuating sea-spray effect), compared to Panama (Fig. 38B), where chthamalids experienced higher temperatures. This means that early *C. bisinuatus* settlers were not under the same physiological stress as *C. proteus* settlers upon aerial exposure.

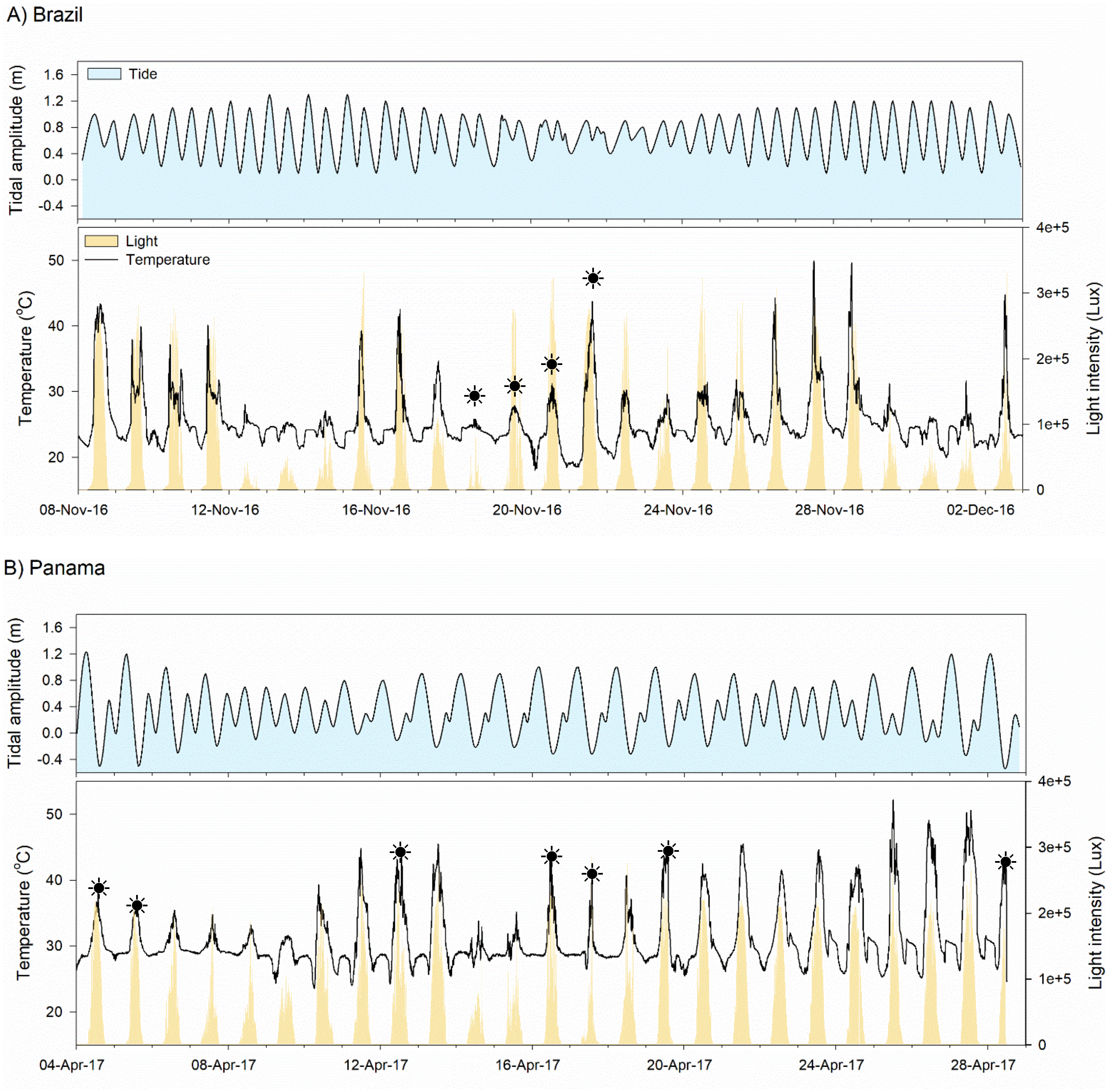


Figure 37. Continuous air and sea-surface temperature (black line) and light intensity (yellow fill) recorded by *in situ* submersible data loggers in the mid-littoral zone. Upper panels refer to the tidal amplitude (blue fill) throughout the 24 sampling days in A) Brazil (subtropical site), and B) Panama (tropical site). ☀ Icon indicates the days that maximum temperatures recorded coincided with the peak of low tide.

Table 17. Predictor terms used to predict post-settlement performance. Ranges of values of each predictor variable over the sampling period is provided.

Predictor	***Type***	***Description***	***C. bisinuatus Brazil***	***C. proteus Panama***
Tape	Categorical	Color of plate surface	White, Black	White, Black
IR temperature	Continuous	IR temperature of plates	White: 20.2 – 31.0°C Black: 23.6 – 39.6°C	White: 26.3 – 36.4°C Black: 29.8 – 53.9°C
Average temperature	Continuous	HOBO measurements of air and sea-surface temperature	23.7 – 28.5°C	28.7 – 35.3°C
Maximum temperature	Continuous	HOBO measurements of maximum temperature	25.3 – 49.9°C	31.6 – 52.0°C
ΔT°C	Continuous	Temperature fluctuation within each day (T_{max} - T_{min})	4.0 – 27.1°C	5.2 – 26.7°C
Salinity	Continuous	Spot measurements	30.3 – 34.9 ppt	36.6 – 37.7 ppt
TPM	Continuous	Total particulate matter (food availability)	1.3 – 4.1 mg/L	1.1 – 4.8 mg/L
Larval condition	Continuous	Lipid droplet cover per daily cyprid cohort (Lag -1)	11.5 – 29.1 %	14.8 – 25.5 %
Size at settlement	Continuous	Size of settled and metamorphosed individuals (Juvenile stage) at D_0	0.387 – 0.602 mm	0.375 – 0.575 mm

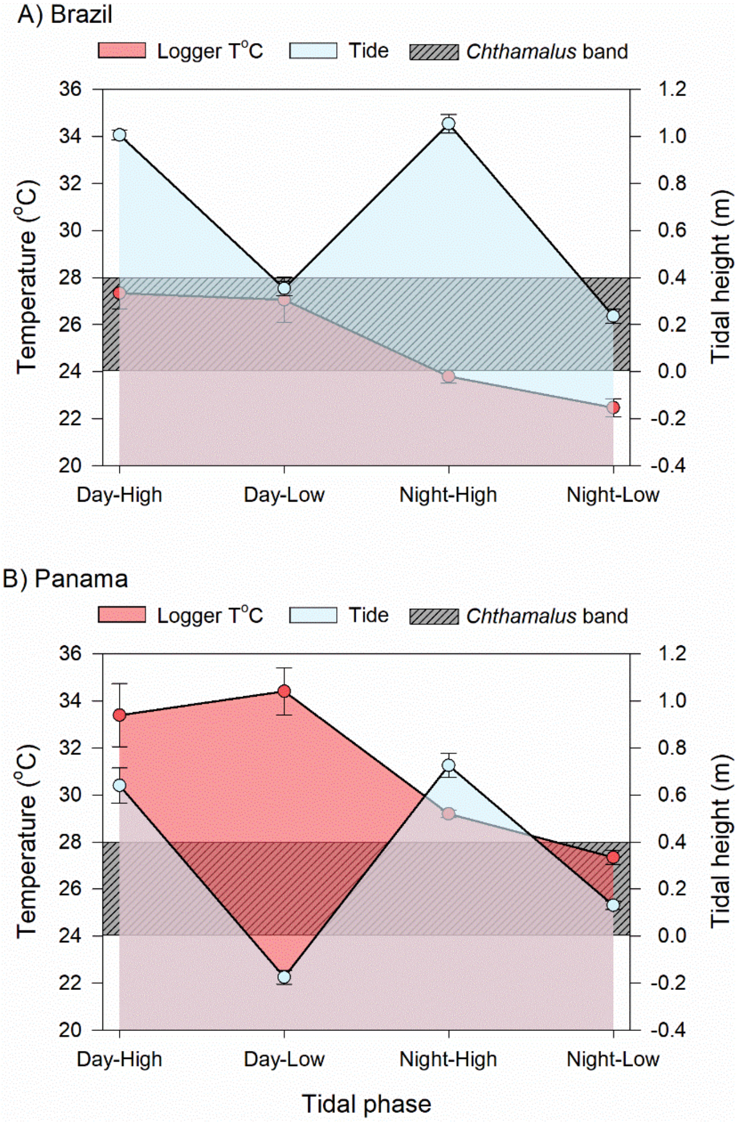
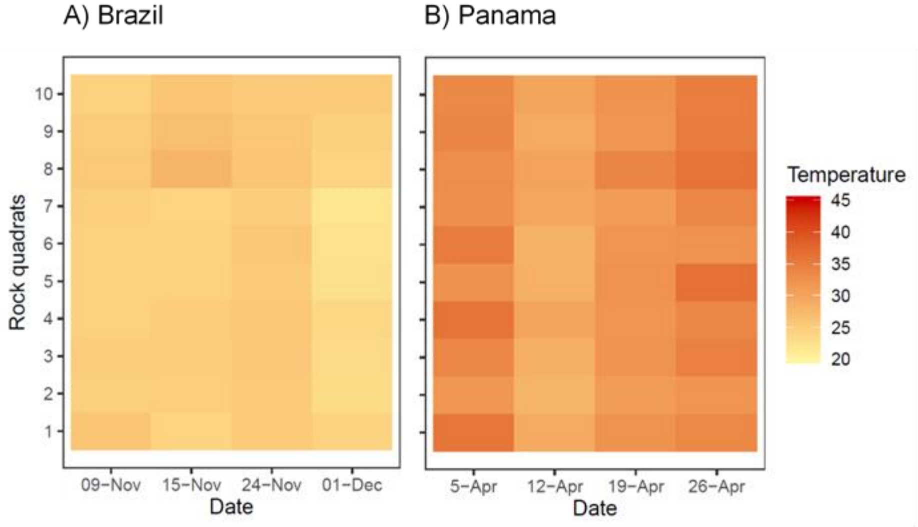


Figure 38. Air temperature and tidal regime, including daytime and nighttime periods, present at the time of samplings.

Natural rock temperature (IR measurements during low tide; Figs. 39,40) averaged 25 °C in Brazil, and 32 °C in Panama, reflecting the local air and sea-surface temperatures. The thermal maps produced (Fig. 39) visually highlight the spatial variability in temperature among quadrats of the same 100-m stretch of rocky shore. In Brazil, rock temperatures varied up to 4 °C. In Panama, there was up to 5 °C of variation within each day; and, higher variability among dates. When comparing the IR temperature of natural *versus* artificial plates (Fig. 40, Table 18), we observe that for both study areas 1) the temperature of coloured, artificial plates was significantly different, i.e., the thermal manipulation of substrate temperature was successful, and 2) the temperature of natural rock was similar to that of white plates, but significantly different from black plates, i.e., in the present experiment, white plates mimicked natural rock temperature. However, the degree of increase in temperature by artificial substrates differed between locations. In Brazil, temperatures were on average 3 °C higher on black plates, and in Panama 8 °C (Fig. 40). This likely reflects the local air temperature and solar radiation conditions experienced during low tide, which also differed between locations. A summary of the remaining key abiotic variables examined is provided in Fig. 41.



C) Example of one rock quadrat and IR image in Brazil

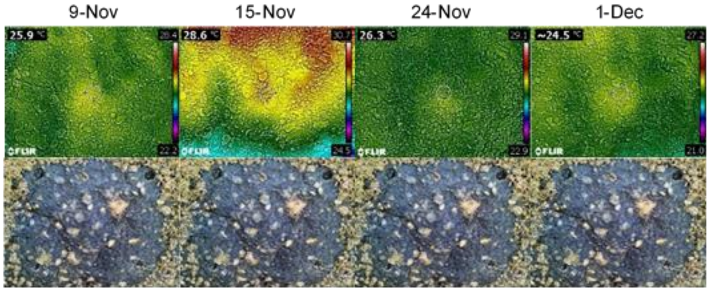
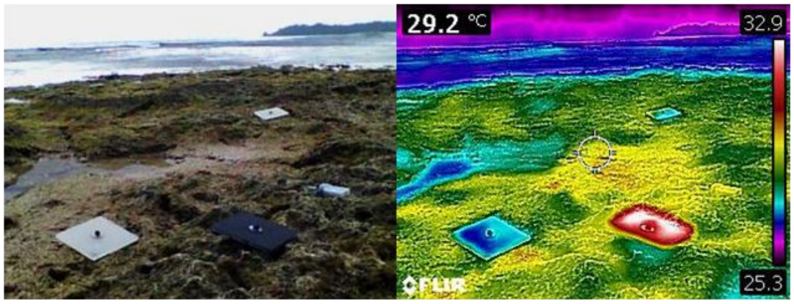


Figure 39. Color maps illustrating the infra-red (IR) temperature of rock quadrats (10 ×10 cm, *n* = 10) on 4 sampling dates, in A) Brazil and B) Panama, together with C) an example of a measurements made for the same quadrat for the 4 different dates in Brazil. Temperature scale in degrees Celsius.

A) *In situ* thermal manipulation



B) Temperature of natural *versus* artificial substrates

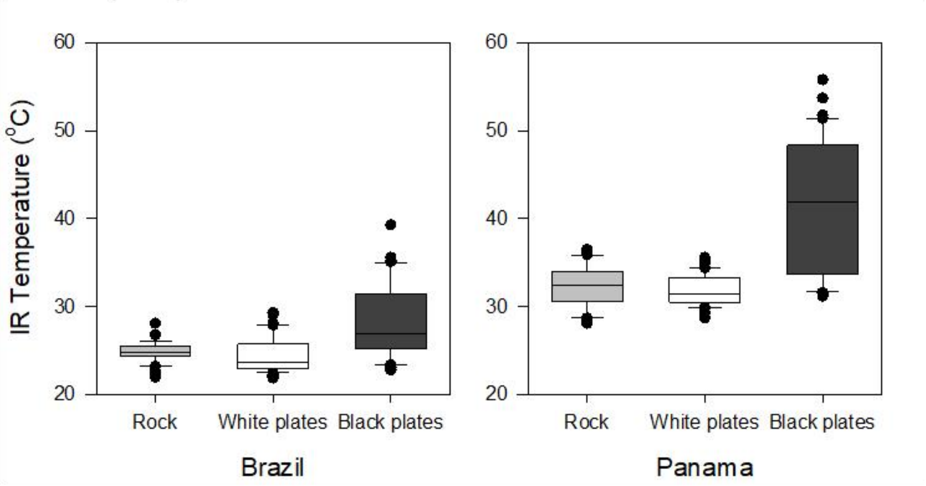


Figure 40. Characterization of the *in situ* experiment during low tide. Upper panel shows the set-up of the plates and respective IR image in visible light in Paunch beach, Panama (as a scale reference, plates are 10 × 10 cm). Lower panel comprises a series of boxplots describing the infra-red (IR) temperature of rock quadrats (grey), white plates (white) and black pates (black) measured during low tide at each sampling location.

Regarding biological variables likely to influence early juvenile performance, we looked further into larval physiological condition and post-metamorphic size, i.e., size at settlement (Fig. 42). The lipid droplet cover of *C. bisinuatus* cyprids overlapped with that of their tropical congeners *C. proteus* (frequency distributions, Fig. 42A). Yet, lipid droplet covers for *C. proteus* never exceeded 35 %, whereas *C. bisinuatus* cyprids reached lipid covers as high as 52 %, likely indicating the presence of two different-quality larval cohorts. Size at settlement also overlapped in both chthamalids (Fig. 42B), averaging 0.458 mm for *C. bisinuatus*, and 0.476 mm for *C. proteus*. Note that trait variability (% CV) differed, being higher for larval condition (36 % for *C. bisinuatus*, and 29 % for *C. proteus*) compared to size at settlement (11 % for *C. bisinuatus*, and 12 % for *C. proteus*).

Table 18. Result table of the one-way ANOVA on Ranks regarding the differences in infrared (IR) temperature according to type of substrate (rock *vs*. black plates *vs*. white plates).

Source of variation	***df***	***Median***	***H***	***p***
Brazil				
Type of substrate	2		24.745	<0.001
Rock		24.85		
White plates		23.70		
Black plates		26.90		
Panama				
Type of substrate	2		45.635	<0.001
Rock		32.40		
White plates		31.50		
Black plates		41.95		

6.5.2 Post-settlement response: *C. bisinuatus* and *C. proteus*

GLS results revealed that the biological response of the two *Chthamalus* species differed from the pattern described for the manipulated *in situ* temperatures. Differences in post-settlement mortality between black and white plates were not significant for *C. bisinuatus* (*t*-value = 0.590; *p*-value = 0.560). For *C. proteus*, although differences were not significant, an almost significant effect of plate was found (*t*-value = -1.904; *p*-value = 0.065), and average mortality was over six times higher on the warmer, black plates. Both chthamalids reached ≈ 0.5 mm by the third day of benthic life, growing at a rate of 2 % (*C. bisinuatus*) and 1 % (*C. proteus*) increment per day. Local biotic and abiotic variables influenced the growth of the two species differently (Tables 19,20). The same trends were apparent from early settlement (1-day old to 3-days old juveniles), although the level of predictors' significance varied. For the subtropical *C. bisinuatus*, no effect of type of plate (white, black) on juveniles' growth was found, but there was a positive combined effect of larval condition (cyprids energetic content) and size at settlement on growth increment. The growth of 3-days old *C. bisinuatus* juveniles also showed an apparent positive effect of temperature, although not quite significant (ΔT °C, *p* = 0.078; Table 19). For the tropical *C. proteus*, an effect of type of plate was found, and juveniles' growth was affected positively by size at settlement and negatively by temperature. No effect of larval condition was found on the growth of *C. proteus* settlers, yet food availability (TPM, *p* = 0.01; Table 20) played a positive role by the third day of benthic life, which coincides with the onset of feeding.

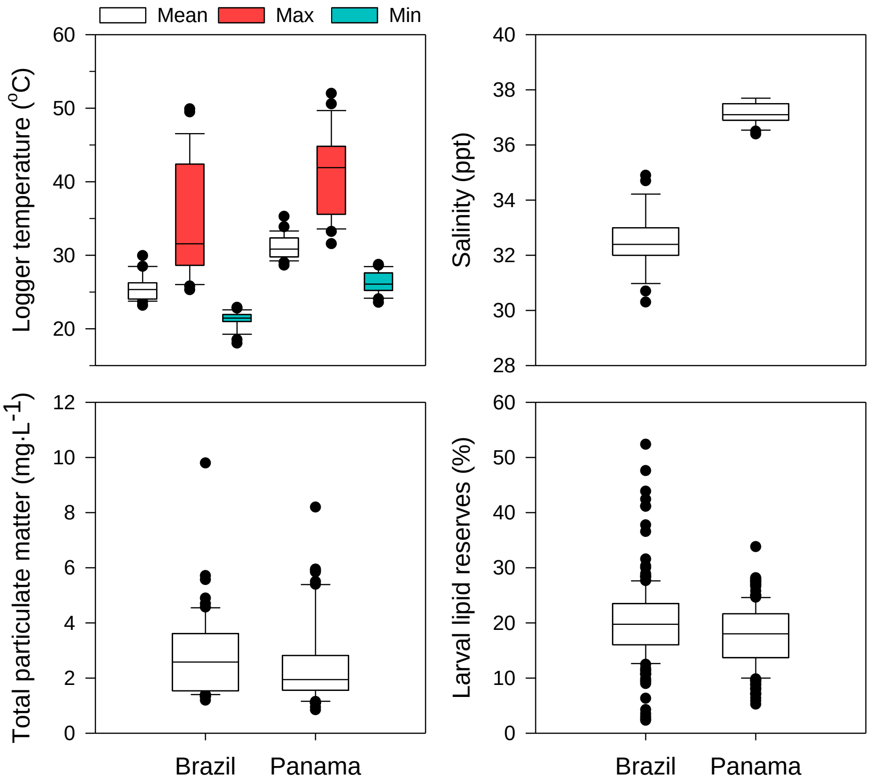


Figure 41. Series of box-plots describing the environmental conditions found at both sampling locations: A) Temperature recorded by *in situ* loggers in the intertidal, B) Salinity, C) Total particulate matter, and D) Larval condition (lipid droplet cover). Plots include the 25th percentile, the median (line within the box) and the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

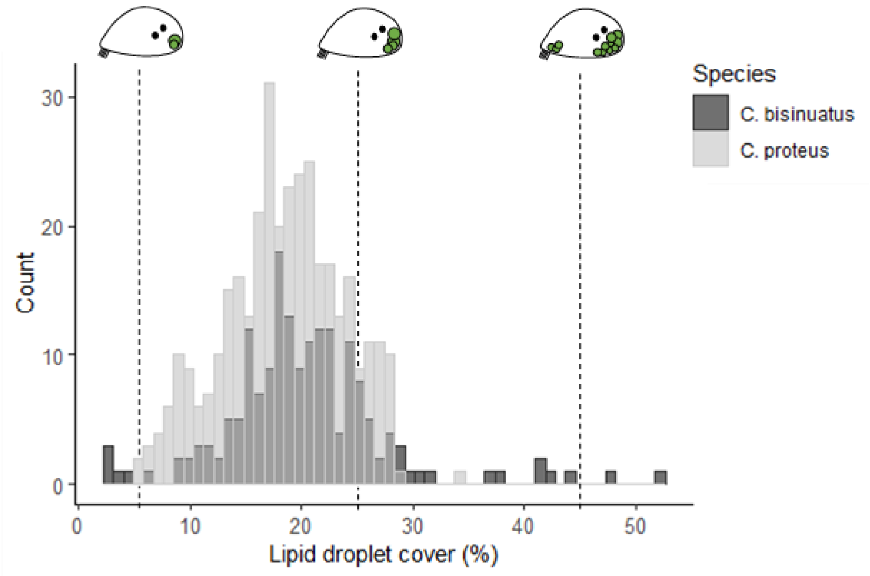
Table 19. GLS coefficients for the post-settlement growth of the subtropical chthamalid *C. bisinuatus*. Significance denoted as ‘***’ 0.001; ‘**’ 0.01; ‘*’ 0.05; ‘.’ 0.1.

Days since settlement	***Estimate***	***Std. Error***	***t-value***	***Pr(>|t|)***
1-day old juvenile				
(Intercept)	-6.817	3.468	-1.966	0.063 .
Larval condition	0.088	0.048	1.825	0.082 .
Size at settlement	16.201	6.546	2.475	0.022 *
2-days old juvenile				
(Intercept)	0.735	0.662	1.110	0.282
Larval condition	0.085	0.034	2.485	0.023 *
3-days old juvenile				
(Intercept)	-7.267	2.613	-2.781	0.015 *
Larval condition	0.111	0.030	3.643	0.003 **
Size at settlement	14.542	5.226	2.782	0.015 *
ΔT°C	0.039	0.020	1.931	0.078 .

Table 20. GLS coefficients for the post-settlement growth of the tropical chthamalid *C. proteus*. Significance denoted as ‘***’ 0.001; ‘**’ 0.01; ‘*’ 0.05; ‘.’ 0.1.

Days since settlement	***Estimate***	***Std. Error***	***t value***	***Pr(>|t|)***
1-day old juvenile				
(Intercept)	-3.089	1.510	-2.046	0.049 *
Tape colour	0.478	0.213	2.227	0.033 *
Size at settlement	10.486	2.980	3.518	0.001 ***
ΔT°C	-0.040	0.017	-2.326	0.028 *
2-days old juvenile				
(Intercept)	-5.136	1.505	-3.414	0.002 **
Tape colour	0.494	0.171	2.890	0.008 **
Size at settlement	13.794	2.869	4.808	<0.001 ***
ΔT°C	-0.026	0.014	-1.802	0.083 .
3-days old juvenile				
(Intercept)	-3.105	1.196	-2.595	0.017 *
Tape colour	0.371	0.157	2.359	0.028 *
Size at settlement	8.962	2.257	3.971	<0.001 ***
Total particulate matter	0.223	0.079	2.867	0.01**
ΔT°C	-0.042	0.012	-3.593	0.002 **

A) Larval physiological condition



B) Size at settlement

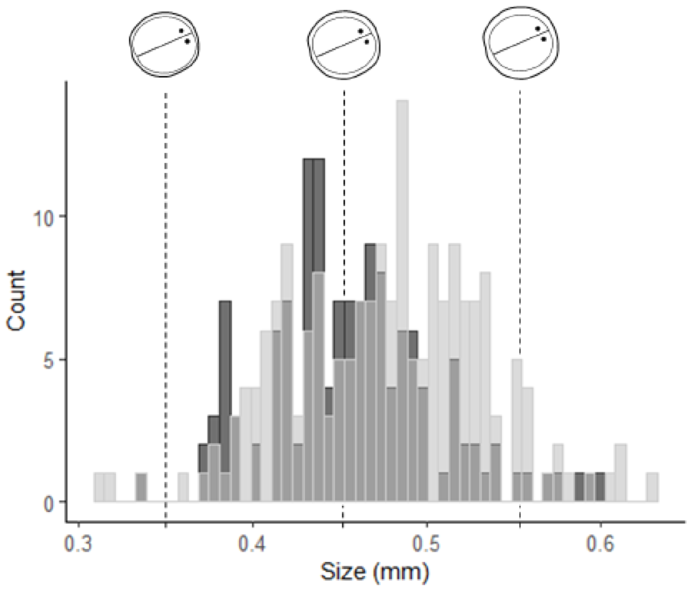


Figure 42. Frequency distribution of A) Larval lipid droplet cover (%) and B) Size at settlement (mm) of *C. bisinuatus* (dark grey) and *C. proteus* (light grey). Hatched lines schematically represent an increasing gradient in physiological quality (A) and size (B).

6.6 DISCUSSION

6.6.1 Effects of increasing substrate temperatures

The present study shows that the early post-settlement performance of low-latitude *Chthamalus* spp. is influenced by increasing substrate temperatures, the effect of which depends on the local thermal regime experienced. Effects of increasing substrate temperatures have been already reported for temperate barnacle species (*Tesseropora rosea*, Lathlean and Minchinton, 2012; *Balanus glandula* and *Chthamalus dalli*; Kordas et al., 2015). Here, we add a comparison of the effects of *in situ* thermal manipulations for subtropical and tropical chthamalid species. The same experimental set-up (different colored plates) in the two latitudes had different effects, reflecting the local physical conditions and likely resulting in the differing biological responses of the two species.

The sub-critical temperatures early *C. bisinuatus* settlers experienced had no apparent effect on their post-settlement performance. There was a positive interaction between larval energetic condition and size at settlement on the early growth of these subtropical chthamalids, with higher quality cyprids and bigger post-metamorphic settlers resulting in juveniles that grew more. Individual growth rates usually display a unimodal relationship with temperature (Kordas et al., 2011), so that increased growth in response to higher temperatures is possible as long as energy reserves and/or food availability meet the required energetic demands (Thiyagarajan et al., 2003). The apparent beneficial effects of temperature on *C. bisinuatus* growth might be explained by positive effects on metabolic activity and/or feeding efficiency. For the first few days following metamorphosis, juvenile barnacles rely on stored energy (e.g., Rainbow and Walker, 1977). Upon feeding, temperature–growth relationships largely depend on the ample supply of food and other resources (Kordas et al., 2011). During the sampling period in Baleeiro Head, food availability was higher than reported for other periods of the year (see Leal et al., 2018). This variability in trophic resources is likely reflected in 1) the larval lipid budget of *C. bisinuatus*, leading to cohorts with different condition (which might explain the 36 % variability observed in cyprid’s lipid content), and/or 2) the energetic budget of feeding juveniles. Such differences likely result in differential fitness of juveniles (Jarrett and Pechenik, 1997). The response of *C. proteus* settlers to increased temperatures differed from that of *C. bisinuatus*. Elevated temperatures had a deleterious effect in the performance of the young Caribbean chthamalids, leading to higher mortality and slower growth. Yet, similarly to *C. bisinuatus*, size at settlement had a role to play, with bigger settlers growing more. It is likely that manipulated temperatures exceeded the tolerance threshold of *C. proteus*. Whereas the intertidal temperatures recorded in Brazil averaged 25.4 ^{o}C, and were typical of the onset of the subtropical summer (Valentim et al., 2013), in Panama recorded temperatures averaged 31.1 ^{o}C, oscillating from 24 ^{o}C to 52 ^{o}C (Fig. 37-B), significantly exceeding the yearly sea-surface temperatures for the region (28.6 ^{o}C; Collin et al., 2009). The negative effects of temperature on the growth of *C. proteus* means that early settlers take longer to reach a refuge size, being likely more vulnerable to predation and competition (e.g., Connell, 1961). As space is a limiting resource in benthic habitats, rapid growth rates are an advantage for sessile species such as barnacles (e.g., Sutherland and Karlson, 1977). Therefore, differences in growth capacity may ultimately hamper recruitment of *C. proteus* populations (Connell, 1961; Jarrett and Pechenik, 1997); this is further discussed below.

6.6.2 Importance of the local interplay of tides and temperature

The cycling of tides interacts with shoreline topography to control the opportunities for functional recovery (feeding, respiration), particularly for sessile filter feeders (Mislan et al., 2011). In the present study, the thermal regime during the harshest tidal phase, i.e., daytime hot aerial exposure, considerably differed between studied locations. Higher tides observed in Brazil during the sampling period suggest that chthamalids in the upper-midlittoral might have benefited from lower temperatures (27.1 ± 1 ^{o}C) and an ameliorating sea-spray effect; contrary to Panama, where temperatures during daytime low tides were considerably higher (34.4 ± 1 ^{o}C; Fig. 38). Finke et al. (2007) investigated the tidal regimes of temperate coasts and their influences on aerial exposure for intertidal organisms and suggested that the potential effects of a warming climate through increased body temperatures during low tides would be substantially greater towards lower latitudes.

6.6.3 Natural substrate thermal variability

A closer look at the IR temperature of natural substrate revealed a complex nature of small-scale thermal variability in the rocky intertidal (Fig. 39). We observed that quadrats within the same mid-littoral zone, only meters apart and with similar orientation, slope, and exposure, displayed mean temperatures that varied by up to 5 ^{o}C (similar results reported by Lathlean et al. 2012). This illustrates the importance of considering spatial temperature variability, as such variability may result in extreme temperature events having a mosaic effect on local populations, limiting our ability to predict effects of large-scale temperature variability in species distributions (Helmuth 2002; Lathlean et al., 2012; Lathlean and Minchinton, 2012). Substratum type has already been shown to affect survival of chthamalid species (*Chthamalus anisopoma*; Raimondi, 1988). Yet, impacts of increasing temperatures might be highly specific to certain individuals based not only on their relative exposure or protection within the mosaic (Miller and Dowd 2019), but also to each individual’s physiological traits, as suggested by the present study.

6.6.4 Potential outcomes

As the vertical distribution of chthamalids on the shore is often limited by their temperature and desiccation resistance (Foster, 1969; Southward et al., 1995; Power et al., 2001; O’Riordan et al., 2010), impacts for these organisms could act primarily to alter their distribution across local environmental gradients (down the rocky shore; Thompson et al., 2002). Chthamalids’ greater resistance enables them to inhabit the refugial high intertidal, a niche that other barnacles (e.g., balanids) cannot exploit (Southward and Crisp, 1954). The services provided by foundation species as barnacles (bentho-pelagic coupling, habitat engineering) are vital for generating and maintaining local species diversity in the intertidal (Bertness and Leonard, 1997; Koh et al., 2005; Kordas et al., 2015). A shift in vertical distribution of chthamalids could lead to habitat loss in the upper intertidal zone, and to biological interactions with low-intertidal species (e.g., competition with *Semibalanus* sp.; Wethey, 1983, 1984), the outcomes of which are difficult to predict. Moreover, increasing temperatures may firstly impact chthamalid barnacles at the equatorial margins of their distribution. The distribution of *C. bisinuatus* spans the Atlantic Coast of South America, up to Uruguay (Dando, 1987). *C. proteus* is native of the Gulf of Mexico, Caribbean and South America, with its distribution extending as far as Parana State, Brazil (Dando and Southward, 1980; Zabin et al., 2007). Therefore, the Caribbean population sampled is closer to the warmest part of its distributional range compared to the Brazilian population, that is at mid-range.

The use of physiological data in forecast models has been suggested to be crucial to better predict the biological responses of organisms to a changing environment (Kish et al., 2016). Our data provides a latitudinal comparison showing that the post-settlement performance of closely-related barnacle species inhabiting the same ecological niche is affected by an interplay of 1) local abiotic parameters and 2) pre-settlement and early settlement physiological traits. The evidence provided here complements the prevalent *ex situ* approaches used to address the impacts of environmental change (e.g., laboratory experiments, Wernberg et al., 2012). One should bear in mind however, that factors other than temperature may be at play in such *in situ* manipulations (larval phototaxis, substrate texture, biofilm composition; Olivier et al., 2000; Finlay et al., 2008; Prendergast et al., 2009; Dobretsov et al., 2013; Matsumura and Qian, 2014). Importantly, effects found at a particular site may apply only at sites with the same type of environmental regime (Connell 1985). Our results add to this conclusion, and suggest that biological responses to climate warming may be site-specific. The significance of this suggestion becomes even more apparent when one considers evidence showing that intertidal temperatures do not necessarily follow a linear latitudinal gradient (Helmuth, 2002; Miller and Dowd, 2019). Current experimental designs are often unable to capture the complexity of environmental conditions (Miller and Dowd, 2019). We hope that our *in situ* experimental approach contributes to the understanding of the factors underpinning the critical post-settlement events that drive patterns of abundance and distribution of barnacles in low-latitude systems.

6.7 CONCLUSION

Subtropical *C. bisinuatus* juveniles seem to be able to cope with small increases in temperature through the important positive interplay between larval quality, post-metamorphic size and temperature on the initial growth of settlers. Conversely, tropical *C. proteus* populations, already living close to their thermal threshold, may be at risk from increasing habitat temperature through declining survival and growth. Higher temperatures can affect the ability of these juveniles to grow and possibly compete for space. These *in situ* findings add to the growing understanding of where species will likely be most affected by increasing temperatures, and to the growing interest in thermal stress, energetics, and extreme thermal events in marine coastal habitats.

6.8 ACKNOWLEDGMENTS

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6.9 SUPPLEMENTARY MATERIAL

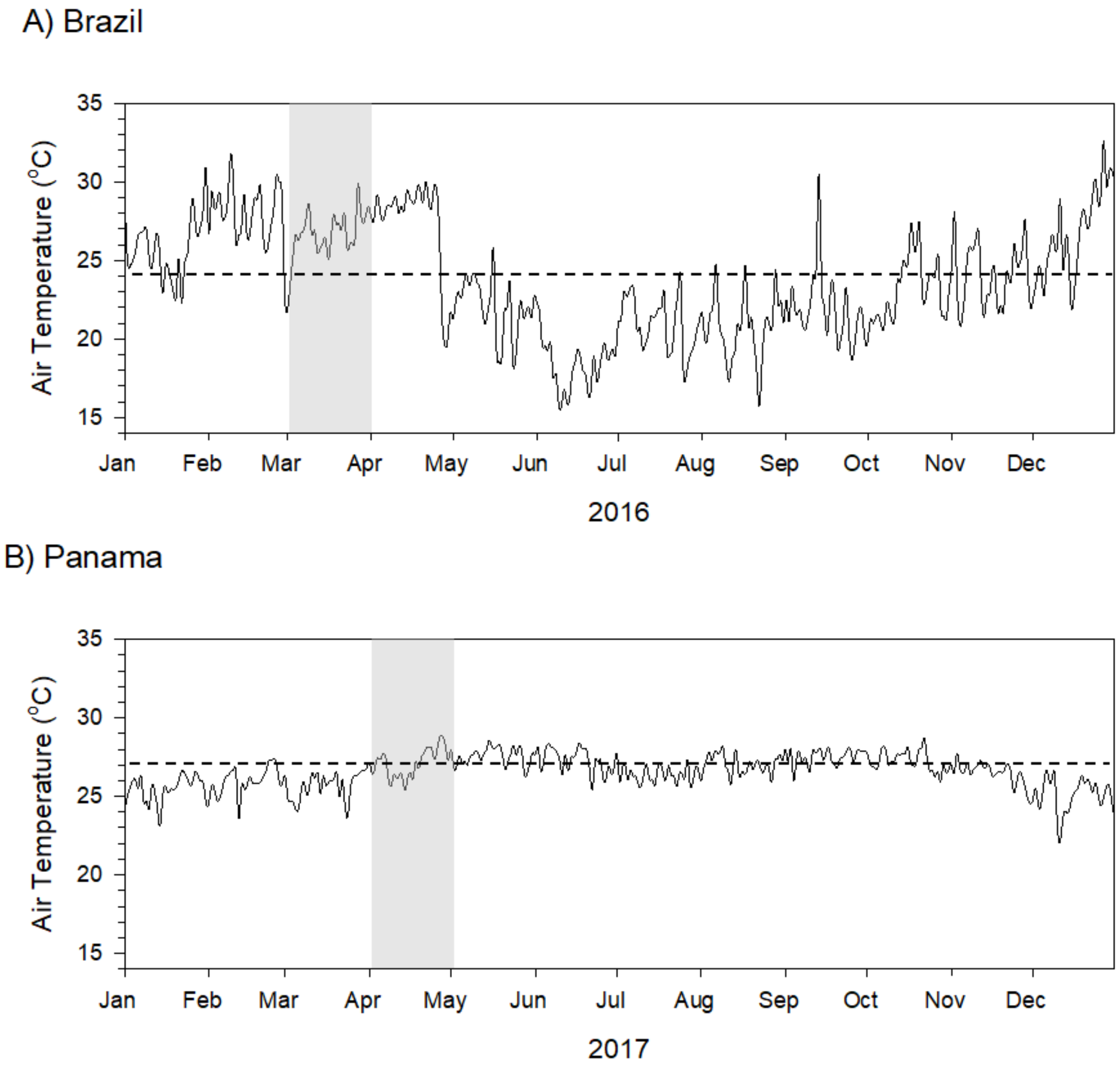

Figure 43. Daily averages of air temperature for the year A) 2016 in Brazil, B) 2017 in Panama, plotted using data sets from the Meteorology Station of CEBIMar/USP and the Physical Monitoring Program of STRI. Dashed lines correspond to the yearly average. Grey shades represent the sampling window of the present work.

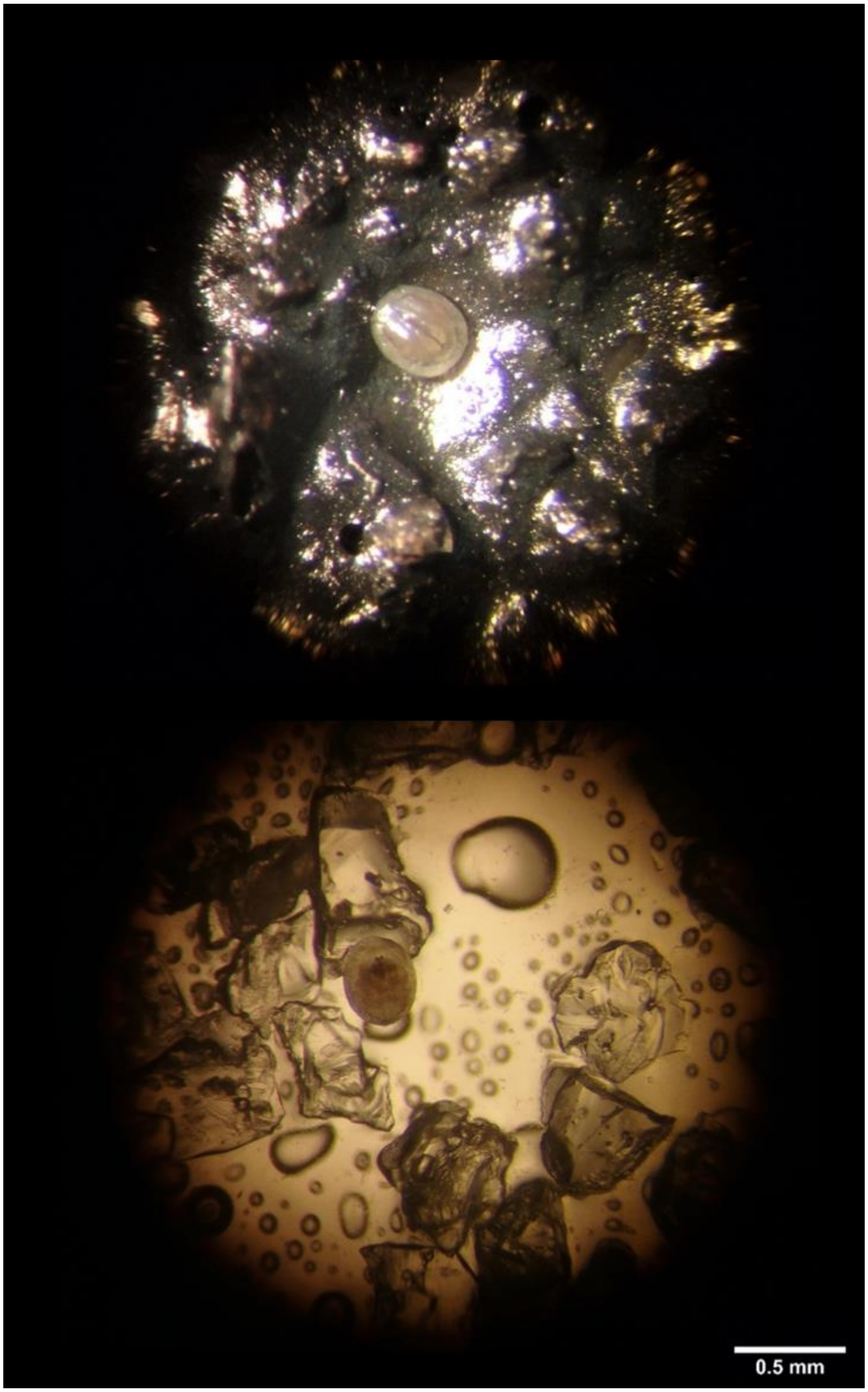

Figure 44. Example of images of juveniles of *Chthamalus bisinuatus* settled on black (top) and white (bottom) tapes used for rostro-carinal diameter measurements on Fiji software.

Figure 45. *Chthamalus proteus* cypris larvae in lateral view to measure lipid droplet cover (%), the proxy used for larval physiological condition. This cyprid, for example, had 23 % of lipid droplet cover. Measurements made using the software Fiji. Lipid droplets are visible at the anterior end of the cyprid.

ARTICLE 7

CYPRID LARVAE OF THE ACORN BARNACLE *SEMIBALANUS BALANOIDES* (LINNAEUS, 1767) (CIRRIPEDIA: SESSILIA: ARCHAEOBALANIDAE) CAN METAMORPHOSE TO JUVENILES WITHOUT BEING PERMANENTLY ATTACHED TO A SUBSTRATE

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7.1 ABSTRACT

It is commonly assumed that the pelagic cyprid larvae of acorn barnacles must permanently attach to a substrate before metamorphosing to the benthic juvenile stage. We show that this is not always the case and demonstrate that some cyprids can metamorphose in the water column, i.e., without first becoming cemented to a surface. We observed early-metamorphosing cyprids to fully developed juveniles in coastal plankton samples during the 2018 recruitment season of *Semibalanus balanoides* (Linnaeus, 1767) in Atlantic Canada. Through a laboratory experiment, we demonstrated that cyprids can be induced to fully metamorphose into pelagic juveniles. These novel findings raise the question of whether this phenomenon may also occur in other barnacle species.

Keywords: Benthic invertebrates, Intertidal, Larval ecology, Rocky shores

7.2 RÉSUMÉ

Il est communément admis que les larves pélagiques compétentes à la fixation de balanes doivent s’attacher en permanence à un substrat avant de se métamorphoser au stade juvénile benthique. Nous démontrons que ce n’est pas toujours le cas et que certains cypris peuvent se métamorphoser dans la colonne d’eau, c’est-à-dire sans être préalablement cimentés à une surface. Nous avons observé des cypris se métamorphoser en juvéniles pleinement développés dans des échantillons de plancton côtier pendant la saison de recrutement 2018 de *Semibalanus balanoides* (Linnaeus, 1767) au Canada atlantique. Grâce à une expérience en laboratoire, nous avons démontré que les cypris peuvent être amenés à se métamorphoser complètement en juvéniles pélagiques sans contact avec une surface. Ces nouvelles découvertes soulèvent la question de savoir si ce phénomène peut également se produire chez d’autres espèces de balanes et le potentiel de survie de ces individus en dérive post-métamorphique.

Mots clés : Invertébrés benthiques, Intertidal, Écologie larvaire, Côtes rocheuses

7.3 ACORN BARNACLE METAMORPHOSIS

Acorn barnacles (Crustacea: Cirripedia: Sessilia) have a complex life cycle that includes benthic juvenile and adult stages as well as pelagic larval stages (Crisp, 1955). The final larval stage, the cyprid, plays a crucial role because it is specialized in the search of a suitable site for settlement, an irreversible step that involves the permanent cementation of the larva to the substrate (Crisp, 1955). Settlement is followed by metamorphosis of the settled cyprid to a sessile juvenile, a dramatic life-history transition that entails changes in habitat, morphology, and physiology (Bishop et al., 2006a). Metamorphosis is regulated by hormones (Heyland and Moroz, 2006) and a variety of environmental cues (Letourneux and Bourget, 1988; Rittschof, 1993). The capacity to undergo metamorphosis (i.e., competence) can last from days to months (Pechenik, 1990), allowing the larvae to explore a number of surfaces until an adequate one on which to settle is found (Crisp, 1955). Ultimately, a subset of the larvae that settle and metamorphose will survive and be recruited to the adult benthic populations (Bertness et al., 1992; Pineda et al., 2006).

We used the boreo-arctic *Semibalanus balanoides* (Linnaeus, 1767) to provide field and laboratory evidence of an alternative path whereby cyprids can successfully metamorphose to juveniles in the water column instead. While sampling plankton by rocky intertidal locations on the Gulf of St. Lawrence coast of Nova Scotia, Canada (45°45'43.34"N, 62°10'20.79"W) in May 2018, we found postmetamorphs of *S. balanoides* spanning a range of developmental phases, from early-metamorphosing cyprids to fully developed juveniles (Fig. 46). Given that postmetamorphs were found on multiple occasions and constituted an appreciable proportion (ca. 4 %) of all the post-naupliar *S. balanoides* found in these samples, 6 postmetamorphs amid 154 unmetamorphosed cyprids, it appears that, while uncommon, these were not isolated events.

In Nova Scotia, cyprids of *S. balanoides* settle and metamorphose in intertidal habitats in May and June (Ellrich et al., 2015). As is typical for cirripedes, the entire larval development of *S. balanoides* occurs in the pelagic environment, comprising six naupliar stages followed by the non-feeding final larval stage, the cyprid. Nauplii of *S. balanoides* feed for approximately 5–6 weeks in coastal waters before reaching the cyprid stage (Drouin et al., 2002). Cyprids engage in two forms of locomotion: (1) swimming in short bursts using their six pairs of thoracopods and (2) exploratory "walking" using their single pair of antennules to explore the intertidal substrate until they find an appropriate place to settle (Lagersson and Høeg, 2002). The antennules allow for a wide range of movements of the cyprid, such as turns of up to 180° along the antennular axis without losing contact with the substrate (Lagersson and Høeg, 2002; Høeg and Møller, 2006). The third segment of each antennule is specialized as an attachment organ, from which the cyprid secretes different adhesive compounds for final cementation (Crisp, 1960; Khandeparker and Anil, 2007). Permanent adhesion to the substrate is considered to be critical for metamorphosis from cyprid to juvenile (Crisp, 1955), and cyprids that fail to permanently attach to substrata are assumed to continue searching for suitable attachment sites (Maki et al., 1988; Essock-Burns et al., 2017). Our observations, however, suggest that this might not always be the case.

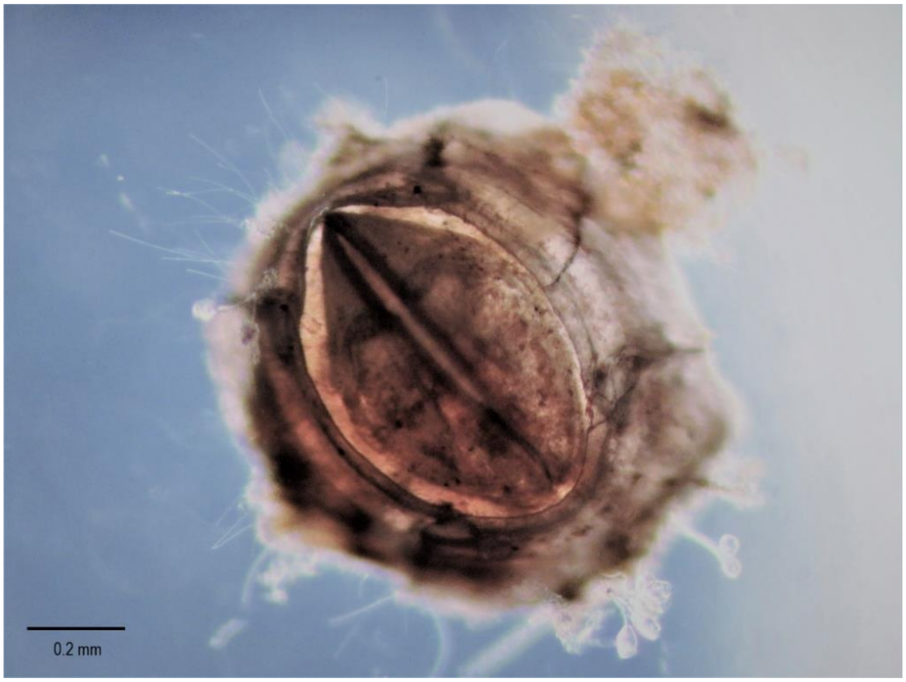

Figure 46. Live juvenile of *Semibalanus balanoides* collected in a coastal pelagic environment in Nova Scotia, Canada.

The ability of cyprid larvae of *S. balanoides* to metamorphose without first permanently attaching to a substrate was tested at the Aquaculture Station of Pointe-au-Père (UQAR/ISMER). Cyprids of *S. balanoides* were collected from the bay of Métis-sur-Mer, Québec (48°40'47.45"N, 68° 1'57.97"W) in June 2018, with a 63 µm mesh plankton net towed manually just below the water surface, and reared individually as described in detail below in 6-well, flat-bottom culture plates (VWR® Multiwell cell culture plates; Mississauga, ON, Canada). Because surface roughness, a normal feature of rocky intertidal habitats, is known to facilitate settlement of *S. balanoides* (Letourneaux and Bourget, 1988), two sets of well-plates were prepared: plates with and without a rugose substrate (3M™ Safety-Walk™ 600-series tape; 3M, London, ON, Canada) covering the bottom of each well, the latter serving as controls. Three replicate plates per treatment, each with 6 wells (7 ml volume), thus containing 36 cyprids in total, were kept in a controlled-temperature room (8 °C) under conditions of constant agitation using an orbital shaker (BT302, Benchmark Scientific Orbi-Shaker™ JR; Sayreville, NJ, USA) at 100 rpm to simulate a turbulent environment, characteristic of most intertidal habitats. The water in each well was exchanged every second day for 7 ml of 0.5-µm filtered, UV-sterilized seawater from a flow-through system. After 10 d of acclimation to laboratory conditions, all cyprids were exposed to a metamorphosis-inducing cue consisting of a tissue extract from conspecific adult barnacles (Rittschof, 1993). This extract was prepared by crushing five wild adult *S. balanoides* (tissue and shell) in 20 ml of filtered seawater, suspending the resulting slurry in a 15 ml centrifuge tube, and vortexing it followed by centrifugation for 2 min at 450 g (Z206-A compact centrifuge; Benchmark Scientific, Sayreville, NJ, USA). The supernatant was removed, and 0.5 ml of it was added to each well. If metamorphosis ensued, juveniles were fed with a microalgal mix of *Pavlova lutheri* and *Tetraselmis suecica* (1:1, total of 60 cell μl^{-1}) after each water change. The experiment was carried out for a total of 50 d, and individuals were observed and photographed (Olympus Stereo Zoom Microscope SZ61; Olympus Canada, Toronto, ON, Canada) on a daily basis in order to estimate the time to metamorphosis and the survival rate.

The major finding of this experiment was that cyprid-to-juvenile metamorphosis of *S. balanoides* could occur without permanent attachment to a surface, as 56 % of the cyprids in wells with no added rugose surface underwent pelagic metamorphosis (10 out of 18 cyprids). These metamorphosing cyprids were found suspended in the water column during the daily photographic monitoring. Whether metamorphosis started after brief contact of the cyprid with the substrate or whether it took place while cyprids remained suspended in the water column cannot be ascertained, given that larval behaviour could not be monitored continuously due to the use of the orbital shaker. The fact that all metamorphic steps were observed in the water column indicates, however, that metamorphosis can take place without permanent attachment to a substrate. When provided with a rugose substrate, 72 % of cyprids settled and then metamorphosed (13 out of 18 cyprids). Regardless of substrate, cyprids took, on average, 5 d to metamorphose upon exposure to the inducing cue. Post-metamorphic mortality was low over the 50 d of the experiment, with only 1 out of the 13 juveniles attached to the substrate dying, and 4 out of the 10 pelagic postmetamorphs.

In agreement with balanid cyprids undergoing metamorphosis on a substrate (Maruzzo et al., 2012), “pelagic metamorphosis” in *S. balanoides* started with the dispersion of the lipid droplets in the anterior part of the carapace, together with body contractions and the separation of the epidermis from the carapace (Fig. 47A). As metamorphosis continued, pelagic specimens shed the carapace, and their antennular cuticle and compound eyes were expelled through the ventral opening of the mantle cavity (Fig. 47B). These pelagic postmetamorphs then completed the metamorphic process, becoming fully developed juveniles with shell plates while still suspended in the water column (Fig. 47C). It appears that, upon reaching competence and being presented with a metamorphosis-inducing cue, an individual cyprid may undergo a complete metamorphosis to a juvenile barnacle without attaching to a substrate. The comparison between our photographic evidence and that of recent studies (Maruzzo et al., 2012) makes it clear that these specimens follow the normal course of metamorphosis, except for them being unattached.

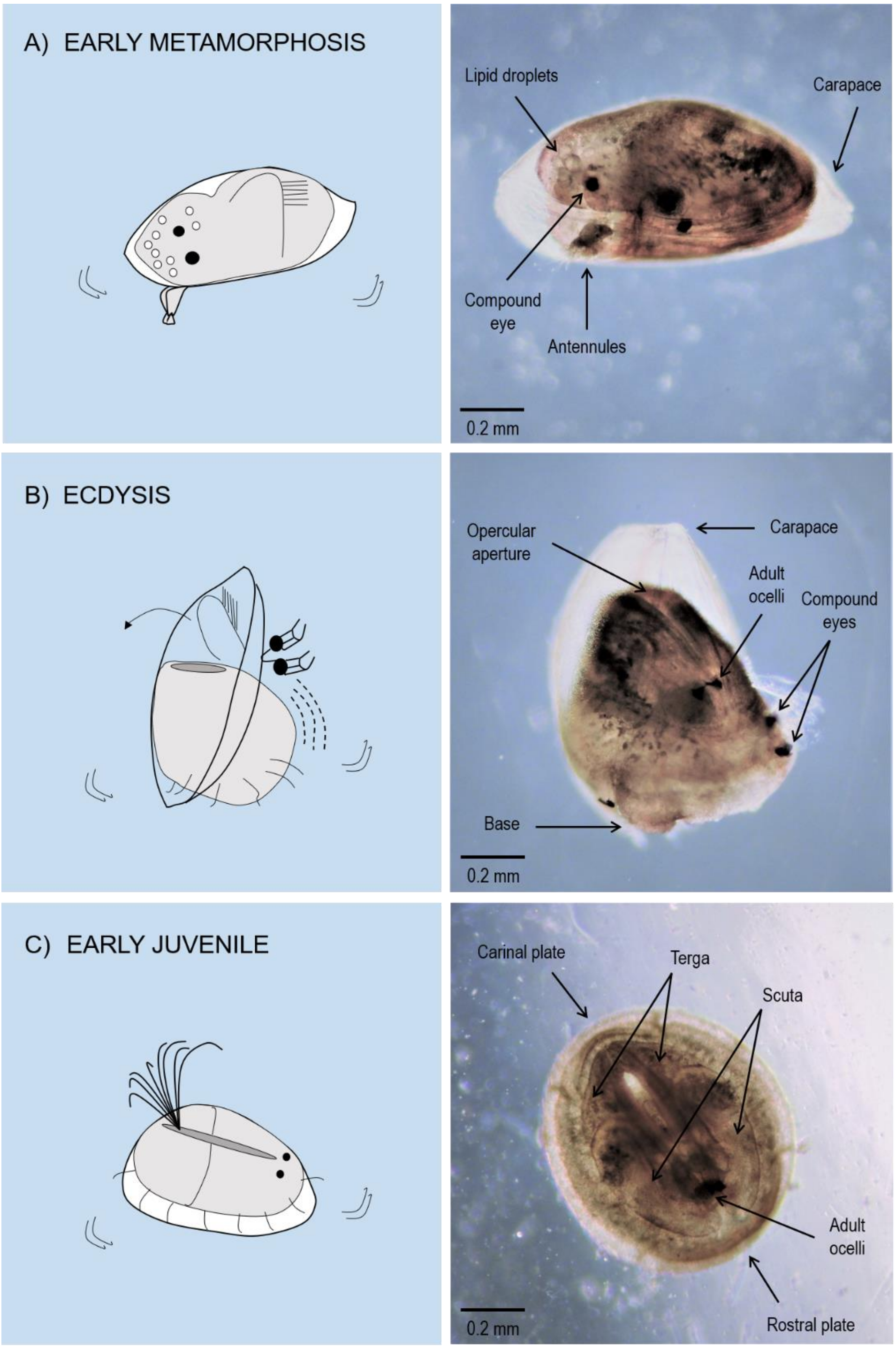

Figure 47. Schematic representation of the metamorphosis of a *Semibalanus balanoides* cyprid larva unattached to a surface, together with microphotographs of live specimens at each respective stage of metamorphosis from the experiment described herein. Early metamorphosis (**A**); ecdysis (carapace loss) (**B**); early juvenile (**C**). Key structures identified by arrows (adapted from Maruzzo et al., 2012).

Settling barnacle cyprids are under considerable time pressure because their preferred intertidal substrate is only immersed for a few of hours during high tide and they must complete metamorphosis before the tide ebbs. Along with predation, bulldozing by mollusks, and removal by wave action and/or algal sweeping, barnacles face numerous risks as they recruit into adult benthic populations (e.g., Beermann et al., 2013; Ellrich et al., 2015). The results described here suggest that, if a cyprid's antennular cement cannot bind strongly enough to a surface (due to substrate unavailability in crowded communities, inhibitory biofilms, and/or depletion of energetic reserves; Thiyagarajan et al., 2002; Tremblay et al., 2007b), the larva might simply undergo metamorphosis in the pelagic environment instead of continuing to search for a suitable substrate. If so, the pelagic *S. balanoides* postmetamorphs we caught were not necessarily dislodged from a substrate (e.g., Larsson et al., 2010), but may instead reflect some form of "larval desperation" (Elkin and Marshall, 2007). Indeed, unattached metamorphosis has been induced artificially in parasitic barnacles with highly selective larval stages (Glenner and Brodin, 1997; Glenner et al., 2008). Cyprids of rhizocephalan barnacles are extremely selective in their choice of substrates without settling unless a specific host or part of a host (e.g., the gills) is provided (Glenner and Brodin, 1997). These studies add to our conclusion that metamorphosis in cirripedes can occur and follow a natural morphological course even without attachment.

As has been supposed for dispersing cyprids that fail to recruit (Pineda et al., 2006), pelagic postmetamorphs may constitute a demographic sink rather than a supply to local populations because it is unlikely that they will be able to attach to a substrate. Instead, they will become denser and sink at random to inappropriate bottoms as their plates calcify and enlarge (but note that detached adult barnacles can reattach to other surfaces; Saroyan et al., 1970; Khandeparker and Anil, 2007). We suggest that by the nature of their metamorphic process, a subset of the competent larval pool might metamorphose and drift away, contributing to the mismatch between the number of cyprids in the water column and those counted as settlers in the benthos (Barbosa et al., 2016). The consequences of our findings are therefore two-fold: 1) ecological, in the sense that "pelagic metamorphosis" entails a loss of larvae that do not settle, and 2) developmental, in the sense that cementation is not strictly necessary for the initiation of cyprid-to-juvenile metamorphosis. These novel findings raise the question of whether this phenomenon may also occur in other barnacle species.

7.4 ACKNOWLEDGEMENTS

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GENERAL DISCUSSION

This thesis presents novel insights into supply-driven population dynamics in the intertidal realm by integrating key aspects of physiology with ecology. The originality of this work emerges from the array of sampled environments and species, the diverse methodologies used to sample and analyze data, and the fruitful collaborations established with researchers from different institutes, countries, and backgrounds. I begin this general discussion by summarizing and integrating the major contributions of the presented research, followed by addressing the broader question of how ecophysiological processes shape settlement dynamics of marine benthic invertebrates, and finish by proposing future research directions.

STUDY CONTRIBUTIONS

NEARSHORE TROPHIC CONDITIONS & LARVAL ENERGETICS

The studies presented in Chapters 1 and 2 provide a detailed assessment of the fatty acid composition of nearshore particulate matter across different coastal waters. I find it particularly interesting the important contribution of saturated fatty acids (SFAs) to the composition of all nearshore pelagic matter sampled (minimum of 39 % collected from arctic areas; Leal et al., *in prep*-c). This likely reflects the land-ocean connectivity typical of intertidal shores, that receive considerable amounts of allochthonous detritus, contributing to strong detrital-based food webs close to shore. Despite the considerable fraction of SFAs, different sources contributed to the bulk of dietary fatty acids available for suspension-feeders over different environments. The analysis of fatty acid trophic markers (FATM) revealed that the composition of nearshore particulate matter at low latitudes had higher percentages of detrital particles compared to living microalgae (Leal et al., 2018, 2019), and that of arctic ones, higher percentages of diatom and dinoflagellate markers that imply higher local primary productivity (Leal et al., *in prep*-c).

The high dependence on heterotrophic food chains based on detrital matter found at low latitudes (SFAs reaching values as high as 87 % at times in southern Brazil; Leal et al., 2018) was reflected in the larval fatty acid composition of both tropical (*Isognomon alatus*; Leal et al., 2019; *Chthamalus proteus*, Leal et al., *in prep*-c) and subtropical species (*Brachidontes solisianus*; Leal et al., *in prep*-a; *Chthamalus bisinuatus*, Leal et al., *in prep*-c), whose share of SFAs surpassed 50 % of both energetic (neutral) and structural (polar) lipid content. Nevertheless, essential fatty acids (EFAs) were found in larval neutral reserves (e.g., lipid droplets) of all competent larvae collected. Similar percentages were found in lecithotrophic barnacle larvae from arctic to tropical areas (14 – 18 %; Leal et al., *in prep*-c), and lower percentages in planktotrophic bivalve larvae from subtropical Brazilian waters (6 – 8 %; Leal et al., *in prep*-a) and the tropical Caribbean (2 %; Leal et al., 2019). This suggests that EFA accumulation in triacylglycerol (TAG; neutral) reserves seems to be physiologically regulated to serve as a buffer for membrane needs, independently of availability in the food, up to a minimum nutritional requirement threshold (see discussion below "Selective Fatty Acid Retention").

The importance of energetic reserves in settling larvae is further supported by the study investigating the larval physiology of closely related species from the genus *Chthamalus* (subtropical *C. bisinuatus* vs. temperate *C. montagui*), where despite inhabiting shores with considerably different levels of food supply, the cyprids equally stored TAG reserves (≥ 50 % of total lipid content; Leal et al., *in prep*-b). I hypothesize that barnacle naupliar stages explore various dietary sources build their energetic (TAG) reserves. 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, so that although both species have similar TAG contents, their composition is different. This is further confirmed by a multi-latitudinal comparison (Leal et al., *in prep*-c), that shows that in order to build up their energetic reserves, barnacle larvae assimilate dietary fatty acids (during naupliar development/maternal allocation) from various food sources, spanning detritus, bacteria, macrophyte residues, and microalgae. Dietary fatty acids associated with diatoms (16:1 and 20:5ω3) and dinoflagellates (22:6ω3) reached 46 % in *S. balanoides* cyprid larvae, 30 % in *C. bisinuatus* and 20 % in *C. proteus*, showing the importance of a microalgal diet across these taxa. At low-latitudes, there was a considerable contribution of terrestrial inputs and macrophyte residues, with markers of these sources (18:1ω9, 18:2ω6 and 18:3ω3; Budge and Parrish, 1998) reaching 9 % in *C. bisinuatus* and 20 % in *C. proteus*. This was similar for low-latitude planktotrophic bivalve larvae, which ingested a significant fraction of terrestrial and macrophyte particles constituting 19 % of the larval lipid content of both *B. solisianus* (Leal et al., *in prep*-a) and *I. alatus* (Leal et al., 2019). The fatty acid signatures of microeukaryotes, detritus and bacteria was evident in the larval structural lipids (polar) of low-latitude species, reflecting the long-term source of the dietary fatty acids assimilated as membrane building blocks.

TROPHIC TRIGGERS MEDIATING SETTLEMENT

The studies presented in Chapter 1 (Leal et al., 2018, 2019, *in prep*-a) add to the growing evidence suggesting that the transition between the planktonic and benthic phase of benthic bivalves is not as abrupt as generally thought (e.g., Martel, 1993; Le Corre et al., 2013; Forêt et al., 2018a,b). The ability of larvae to postpone metamorphosis as well as the high mobility of settlers after the first settlement is demonstrated for the tropical *I. alatus* and subtropical *B. solisianus*, intertidal foundation species at low-latitude shores. In these studies, I reveal that postlarval drifting may be an integral part of the life cycle of these bivalves, so that the spatial distribution of early settlers might not reflect their final distribution.

In the southern coast of Brazil, bottom-up processes appear to contribute to variability in the settlement dynamics of the common bivalve *B. solisianus* (Leal et al., 2018; *in prep*-a). Trophic cues associated with increasing amounts of nearshore organic matter and saturated fatty acids appear to trigger the early response of settlers within a narrow size range (a "pulse" of similar-sized individuals) and limited post-metamorphic drift ("short-term drifters"). The importance of trophic processes in determining settlement in this subtropical meso-oligotrophic system has been already suggested for barnacles (Barbosa et al., 2016), but the origin and importance of pelagic nutrients to competent larvae remained unknown. On a subsequent study, I demonstrate that such trophic cues likely have a terrestrial origin, resulting from pulsed natural disturbances, i.e., torrential rainfalls (Leal et al., *in prep*-a). I suggest that variation in energy sources is likely to be important to the functioning of this coastal ecosystem, and may be mediated by precipitation-driven pulses of organic matter. Higher primary settlement rates coincided with periods of increased rainfall and nearshore terrestrial inputs. Also, a pulse of similarly-sized drifters was found to settle during the same period. Previous research has suggested that the causes and outcomes of dispersal are driven by the interactions of individuals with their environment (Bowler and Benton, 2005; Nanninga and Berumen, 2014). Similarly, I suggest that in this subtropical system, short-term environmental stochasticity in the form of pulsed rainfall events and resulting organic matter plumes, may trigger specific dispersal behaviors in *B. solisianus*, affecting settlement and distribution dynamics in this population.

In the Caribbean waters of Bocas del Toro, no trophic cues capable of deterring postlarval drifters were found (Leal et al., 2019). I hypothesize that in this tropical system, drifters settle as they age, and/or that this species, more adapted to oligotrophy compared to other subtropical (e.g., Leal et al., 2018) and temperate bivalves (e.g., Forêt et al., 2018a), does not respond to trophic triggers. A shift in trophic conditions occurred in coastal waters, however, with organic matter dominated by tracers of microalgae and vascular plant matter shifting to an SFA-dominated composition over a fine temporal scale, suggesting that short-term environmental stochasticity in food availability occurs in this system. It is likely that rainfall events also drive nutritional inputs to the typically oligotrophic waters of Bocas del Toro, however, this remains to be tested. It is also possible that in this region, where the distinction between rainy vs. dry seasons is very marked, differences in trophic conditions occur between seasons. Further studies should aim for longer term samplings to identify possible seasonal (nutritional) effects shaping settlement dynamics of this fascinating isognomid.

LARVAL SELECTIVE FATTY ACID RETENTION

In both Chapters 1 and 2 (Leal et al., *in prep*-a, c), I prove the ability of competent larvae of *B. solisianus*, *C. proteus*, *C. bisinuatus* and *S. balanoides* to selectively retain EFAs in structural lipids. Of the EFAs, the docosahexaenoic acid (DHA, 22:6ω3) was retained the most. I propose that larvae actively regulate the fatty acids incorporated into membrane lipids by metabolizing the readily-available neutral reserves in their lipid droplets. These fatty acids are selectively allocated by the larva according to environmental constraints (nutritional, thermal), i.e., physiological lipid remodeling. I suggest that lipid remodeling may vary as a function of latitude (Figure 48).

The fatty acid composition of nearshore particulate matter showed a latitudinal trend, with low-quality dietary sources (SFAs) reaching the highest values along tropical shores and lowest in arctic ones. For all late-stage barnacle larvae sampled in Leal et al., *in prep*-c, EFA levels in the structural component of larvae were higher than in nearshore organic matter. In subtropical waters, *B. solisianus* pediveliger larvae selectively retained DHA during periods of reduced precipitation and organic inputs. This selective retention coincided as well with longer planktonic larval duration. The pediveliger larvae of the Caribbean flat-tree oyster *I. alatus*, had only 2 % of EFAs in their neutral reserves (Leal et al., 2019). Only the neutral fraction was analyzed, however, which means that the level of EFAs transferred to the membranes (polar lipids), for example, could not be estimated. Given the high level of DHA retention in the co-existing Caribbean *C. proteus*, I hypothesize that the high metabolic demands driven by higher temperatures and low nutritional status of nearshore pelagic matter, may require the transfer of assimilated EFAs to tissue development during metamorphosis.

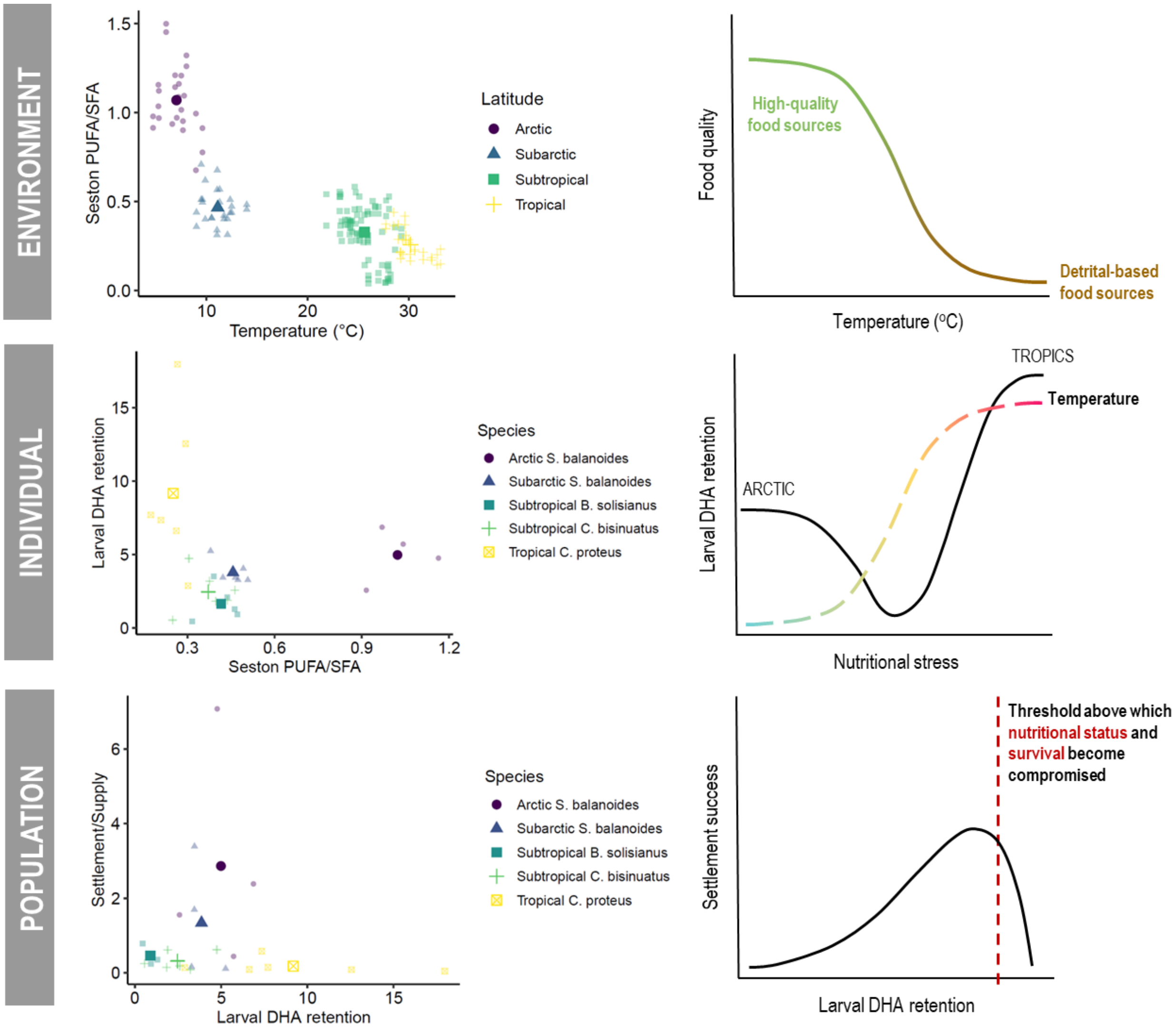

Figure 48. Summary plots of the quality of nearshore organic matter (seston PUFA/SFA; daily averages from all studies presented) collected from arctic, subarctic, subtropical, and tropical waters revealing a measure of nutritional stress. The larval selective DHA retention (weekly larval pools) across species sampled and settlement/supply ratios as a measure of settlement success are also plotted. Untested hypotheses for the observed natural patterns are provided on the right panel.

Persistent exposure to thermal conditions either above or below those required to maintain optimal performance initiates acclimatory (within the lifetime of an individual) or adaptational (over generations) membrane changes in animals' lipid composition, i.e., homeoviscous adaptation (e.g., Hazel, 1995; Pernet et al., 2006; Thyrring et al., 2017). I propose that lipid remodeling as selective DHA retention is, similarly, an acclimatory mechanism. Up to a certain threshold (untested), DHA retention might be a compensatory/coping mechanism that improves performance (Figure 48). In areas where thermal and nutritional stresses act together, DHA retention is so high that it may signal nutritional deficiency, as apparent from the multilatitudinal comparison with late-stage barnacle larvae (Leal et al., *in prep*-c). Further research on nutritional "tipping points" is necessary to validate these hypotheses. Yet, I provide novel knowledge on fatty acid signatures of competent marine benthic invertebrate larvae, and open the door for future research on the importance of larval lipid remodeling in shaping population dynamics of this important group of habitat-forming species.

STUDY LIMITATIONS

Logistics are often problematic in ecological studies, and my work was no exception. The experimental designs of the studies carried out were limited spatially and temporally, clarifying mainly ecological processes at a local scale. The idiosyncrasies associated with each species life-cycle (e.g., spawning behaviour, number of eggs, maternal allocation, duration of pelagic development, settlement window) complicate comparisons and broad extrapolations. Ideally, sampling multiple sites at each latitude, including sites with similar trophic regimes (oligotrophic *vs*. eutrophic), over different time frames (days, months, years) would capture such "environmental factor/life-cycle" interactions. It would have been interesting to sample a single species at different latitudes to explore phenotypic plasticity in larval morpho-physiological traits and its effect on settlement success. Drawing conclusions on ecological processes at broad(er) scales would have benefited from accounting for local wind forcing and small-scale oceanographical features (e.g., wave exposure, tidal amplitude) in conjunction with the *in situ* ecophysiological data collected, given their predominant role in larval supply. It is, however, quite exciting to realize how much there is to explore in a field that has been studied for over 70 years.

The studies here presented raise many interesting questions for the fields of ecology and physiology, and I would like to outline a few:

- **Article 1:** What is the temporal variability in nearshore trophic conditions (e.g., TSM, MTFA, SFAs/PUFAs)? What are the trophic trigger thresholds, and do they change with larval/post-larval age?
- **Article 2:** What is the extent of coastal plumes (meters, hundreds of meters)? Does the proximity to urban sites influences the trophic quality of organic matter?
- **Article 3:** What is the relative importance (spatial extent) of secondary dispersal to population connectivity? What is the impact of rainfall events on local oceanographical features (e.g., water stratification; tidal amplitude)?
- **Article 4:** Is there selectivity in naupliar diet? What are the species-specific clearance rates? Do nauplii switch diet in time (e.g., picophytoplankton *vs*. detritus)?
- **Article 5:** Are there seasonal effects on lipid accumulation/allocation? How do phytoplanktonic blooms at higher latitudes compare to low-latitude blooms with respect to nutritional content? Is there a threshold above which selective fatty acid retention affects individual fitness?
- **Article 6:** At what size do chthamalid juveniles reach a refuge size? Will rising temperatures affect lipid accumulation during larval development?
- **Article 7:** Does “desperate metamorphosis” result from a poor physiological condition (e.g., depleted energy reserves)? Does it occur for other barnacle species? How much does it contribute as a demographic sink?

ECOPHYSIOLOGICAL PROCESSES SHAPING SETTLEMENT DYNAMICS

“Only when settlement is well understood will it be possible to incorporate settlement into specific population models and therefore have a better understanding of temporal and spatial variations. For these reasons, it is essential to (...) integrate aspects of different disciplines (e.g., ecology and physiology).”

– Rodríguez et al., 1993

I begin this thesis by introducing the question: “*How do plastic larval traits driven by contrasting trophic conditions shape natural populations?*”. Solving this question is a herculean task, and I am far from being able to provide an answer that accounts for all the variability inherent of natural systems. However, I do make the contribution of showing that in natural settings, trophic conditions (e.g., amount of organic matter, levels of EFAs, dietary sources) shape the ecophysiology of a few marine benthic invertebrate species by mediating (1) larval and postlarval nutrition (e.g., detritus as main short- and long-term food sources in low-latitude waters; Leal et al., 2018), (2) acclimatory responses in competent larvae (i.e., selective fatty acid retention under nutritional stress; Leal et al., *in prep*-c), (3) size at settlement (e.g., trophic cues promote settlement of subtropical mytilids; Leal et al., *in prep*-a), and (4) post-settlement performance (e.g., organic matter improves growth of juvenile Caribbean chthamalids; Leal et al., 2020).

The trophic ecology of invertebrate larvae is a key theme investigated by benthic ecologists around the globe. Its importance is easily understood given the role of trophic conditions in determining individual’s capacity to grow, develop, metamorphose, settle, compete, and reproduce. The interaction of larvae with a range of physical, chemical, and biological factors determines where settlement occurs and at what intensity, ultimately setting the scene for the distribution, abundance, and dynamics of the adult populations that follow. A summary of these factors can be found in Table 21. There is, therefore, a strong selective pressure for settlement and metamorphosis to be dictated by adequate cues, i.e., physical (water movement), chemical (conspecifics, biofilm), and biological (food supply, quality) cues that act on larval behaviour and response, as is evident from Table 21.

Several researchers have called attention for the need to collect more field data (e.g., Pineda et al., 2009; Wernberg et al., 2012). Understanding the intra-specific variability in organismal traits in natural settings is important and often neglected in most *ex-situ*, i.e., laboratory approaches. My work results largely from sampling *in-situ*, which I find helped to create a baseline of the natural conditions found at the sampled rocky shores. Further, the frequency of the sampling was daily, in line with Connell (1985) who noted that measurement of settlement should be frequent, and that the "closest to the ideal" would be daily. Connell (1985) argued that frequent settlement measurements are needed "to avoid missing any larva that attaches and then become detached within a short period" that add nuisance if sampling events are done far apart. In fact, the protocol I have followed to assess daily settlement rates in the field was identical to that done by Connell three quarters of a century ago.

> *"At every other low tide the stones were brought into the laboratory and examined with a dissecting microscope. The stones were always returned to the shore before the tide rose again."*
>
> – Joseph H. Connell, 1961

It is fascinating that the protocols used by pioneer ecologists remain the same today, simply evolved to the use of more sophisticated gear. J.H. Connell used rock boulders to measure daily settlement rates of *S. balanoides* in Scotland, bringing them daily to the laboratory during low tide. I would like to highlight a finding of Connell (1961) that is pertinent to the study Leal et al., 2020 published in the Journal of Crustacean Biology. Already in the 1950s, Connell points that: "There are several possible sources of error in this method: (…) cyprids, having cemented themselves down, might be detached and then reattach themselves elsewhere. (…) A later study of this species at Woods Hole, Massachusetts, showed that if attached cyprids were removed and placed in dishes of seawater, *they could metamorphose and become reattached*." As Connell (1961) points out, the possibility of reattachment occurring in the turbulent waters of the intertidal area would, however, be small.

Table 21. Examples of factors influencing the settlement response of bivalve and barnacle larvae.

Process	Factor	Example	Reference
PHYSICAL	Temperature	Lower seawater temperatures lead to an increase in *Mytilus edulis* larval duration, i.e., larger PII size.	Pechenik et al., 1990
	Flow velocity	*Semibalanus balanoides* cyprids attach under conditions of water flow, and have the tendency not to attach under stagnant conditions.	Crisp, 1955
	Substrate availability	Where the density of *S. balanoides* settlers was high, it was positively related to the availability of free space.	Minchinton and Scheibling, 1993
	Micro-topography	Preferential settlement of *S. balanoides* in cracks and pits, i.e., rugophilic behaviour.	Wethey, 1986
	Sound	Increased settlement in larval cultures of *Crassostrea virginica* exposed to oyster reefs' sound.	Lillis et al., 2013
CHEMICAL	Conspecifics	Conspecific cues trigger gregarious settlement in *S. balanoides*.	Knight-Jones,1953
	Biofilm	Settlement of *B. amphitrite* larvae is negatively correlated to the density of bacteria attached to the substratum.	Olivier et al., 2000
	Predators	Settling *Balanus glandula* larvae avoid substrata previously occupied by a mobile predator, the whelk *Nucella lamellosa*.	Johnson and Strathmann, 1989
BIOLOGICAL	Food supply	Chl-*a* during naupliar phases linked to *Chthamalus bisinuatus* larval settlement success.	Barbosa et al., 2016
	Food quality	Phytoplanktonic pulses rich in essential fatty acids trigger settlement of *M. edulis*.	Toupoint et al., 2012a
	Dietary sources	Terrestrial cues driven by rainfall pulses trigger primary settlement of *Brachidontes solisianus*.	Leal et al., *in prep*-a
	Morphology	Greater settlement success rates related to *M. edulis* small size at metamorphosis.	Martel et al., 2014
	Behaviour	Depleted TAG reserves in *B. amphitrite* impair habitat selectivity: larvae become "desperate" to settle.	Tremblay et al., 2007b

Further, Pineda (1994) has shown that *Chthamalus* spp. settlement in California was composed of unmetamorphosed settlers (attached cyprids) plus metamorphosed juveniles. The proportion of unmetamorphosed settlers relative to total settlement appeared to be related to periodic short immersion times that did not allow the attached cyprids to metamorphose. The author highlighted the need to document patterns in the proportion of metamorphosed vs. unmetamorphosed cyprids in the field, as the population effects of high proportions of unmetamorphosed settlers is not known. Contrary to the tropics, where warm waters may speed up metamorphosis, the cold waters of high-latitudes pose a constraint to cyprids, which need to metamorphose "as fast as possible". In the particular case of *S. balanoides,* Connell (1961) found an average of 1.5 days between attachment and metamorphosis. There is hence a considerable amount of time that cyprids are vulnerable to physical disturbance, and may not be able to bind strongly to a substrate. Yet, once metamorphosis is triggered, it will continue, even if it occurs in the water column (Leal et al., 2020). This opens the door for researchers to investigate how much this contributes to population sinks in nature, that might be particularly relevant for cold-habitat species.

This brings me to the final point that I would like to address in this topic: generalization. Community ecology, which aims to explain the distribution and abundance of organisms, does not stem from a common narrative as does evolutionary biology (Roughgarden, 2009). The properties within a community such as its species composition and dynamic stability depend on the local situation. As mentioned by J. Roughgarden, "Fusing community ecology with earth science combines the idiosyncrasy of species identities with the idiosyncrasy of geological place. A general model of the rocky intertidal zone would await a general model of coastal circulation, which is not on the horizon".

"So, is there a general theory of community ecology today? No. Can there be? Yes. Should there be? I don't know."

– Joan Roughgarden, 2009

By making a characterization of Environment–Individual–Population in each study here presented, I was able to better define the processes shaping local populations, and I strongly advocate for an integrated ecological approach. This approach made me realize how difficult generalizations can be. The close interplay of local, sometimes micro-spatial and -temporal, conditions affect the habitat or rocky shore in a unique manner (e.g., Leal et al., 2020). However, complexity tends to be greatest at small (i.e., local) scales, where variation is usually explained by particular and unique events. Large-scale patterns can emerge from the apparent stochasticity at small scales, and variation at local scales need not impede tests for similar patterns at broader scales (Fowler-Walker et al., 2005). It is debatable the need of one theory to explain all observed patterns, as it may overshadow local diversity patterns. A balance should be sought between testing for general and specific phenomena. Proper hierarchical sampling designs (e.g., Underwood, 1997) allow researchers to be in a better position to understand the spatial extent and nature of generalities. As mentioned by Underwood et al. (2000) "observations of patterns or lack of patterns are the fundamental starting-blocks for ecological study".

PERSPECTIVES

A CHANGING CLIMATE

Marine invertebrate larvae are a key element in the energetic transfer from lower to upper trophic levels. Similar to other researchers (e.g., Colombo et al., 2017), I found that the contents of PUFAs were greater in larval stages from arctic and subarctic waters compared to those from the tropics. Colombo et al. (2017) demonstrate that high-latitude marine organisms provide a large global share of these essential nutrients to consumers, and called attention for the implications of climate change and other anthropogenic stressors in impacting the global distribution and abundance of ω3 PUFAs within marine ecosystems and on the terrestrial consumers that depend on these subsidies. Changes at the base of marine food webs (i.e., phyto- and zooplankton) can cascade through food webs. Recent studies demonstrate that exposure to heatwaves can increase the degree of saturation of fatty acids in marine organisms (e.g., seaweed *Phyllospora comosa*; Britton et al., 2020). This adjustment counteracts the negative effects of elevated temperatures by decreasing membrane fluidity, which increases at higher temperatures (i.e., homeoviscous adaptation). Thus, although populations at the equatorial margins of their distribution may be the most vulnerable to warming (e.g., Leal et al., 2020), the acclimatory response of species at high-latitudes may have devastating cascading effects, i.e., decreased proportions of essential fatty acids (EPA and DHA) in a variety of organisms as a result of cellular adaptation. It is important, therefore, to identify and conserve geographic areas that have exceptionally high ω3 PUFAs production and subsequent retention in higher trophic levels (e.g., fish), not only for our health but also for the balance of the ecosystems in our planet.

COASTAL DEGRADATION AND HABITAT COUPLING

Growing efforts are being made to integrate landscape and marine ecology. Discharges of major rivers to coastal areas are the most striking example of material transfers in the context of land-ocean coupling (Schlacher et al., 2009; Gorman et al., 2009, 2019). Inputs of river-born nitrates, raw untreated human effluents, and other urban and industrial inputs impact local ecosystems through the classic “paradox of enrichment”, where rich subsidies result in poorer diversity or other declines in abundance (Palumbi, 2003). Nutrient-driven shifts of habitats from perennial (i.e., canopy-forming algae) to opportunistic species (i.e., turf-forming algae) have been observed in the south coast of Australia (Gorman et al., 2009) and in the warm temperate coastline of Brazil (Gorman et al., 2020). Projected increases in storm intensity, duration, and possibly frequency, in combination with enhanced nutrient and sediment inputs related to human activities will expose estuarine and coastal habitats more often, and for longer time periods, to eutrophication and hyposalinity stress (Herbeck et al., 2011). Effects on populations that respond to such nutritional pulses (e.g., subtropical mytilids; Leal et al., *in prep*-a) may include the disruption of settlement patterns, and deserve further attention. Land management of local areas (e.g., agricultural and forestry practices, fragmentation, desertification) affects not only other terrestrial habitats, but the productivity, food webs, and community structure of rivers, estuaries, and oceans (Polis et al., 1997). Subsidies from one ecosystem to another, together with fisheries, population connectivity, and conservation, comprehend some of the most pressing problems in marine ecology.

> *"The message is clear: Ecosystems are closely bound to one another, be they stream and lake, pelagic and intertidal zones, farms and the sea, forest and river, or ocean and desert."*
>
> – Polis et al., 1997

SUSTAINABLE RESOURCE MANAGEMENT

The understanding of the dispersion of early life stages and therefore ecological connectivity is paramount in the design of marine protected areas to ensure genetic and demographic exchange among reserves and replenishment of adjacent areas (Marshall et al., 2012; Ospina-Alvarez et al., 2020). For instance, Ospina-Alvarez et al. (2020) show that in the central Chilean coast it is unnecessary to relocate existing management areas, as increasing no-take marine areas by 10 % could maximize ecosystem service provision, while improving the spatial representativeness of protected areas and minimizing social conflicts. Studies of this kind are encouraging and necessary to make well-grounded decisions in the management of our coastlines. In the first chapter of this thesis, I show evidence for postlarval drifting at low-latitudes, which together with research with temperate species (e.g., Olivier and Retière, 2006; Forêt et al., 2018a) points to secondary dispersal as key factor shaping population connectivity, as such migrations can significantly shift initial settlement sites, even with potential commercial setbacks. For example, catching and relaying wild seed green-lipped mussels *Perna canaliculus* onto grow-out farms in New Zealand has been shown to be extremely inefficient, i.e., seed mussels being lost after the transfer due to secondary settlement behaviour (Carton et al., 2007). Indeed, bedload transport can relocate bivalve juveniles by metres to hundreds of metres in episodes lasting no more than one tidal cycle (Petuha et al., 2006). Understanding dispersal dynamics of coastal foundation species is therefore key in the context of sustainable use of natural resources, especially when the use of early stages, i.e., propagules, offers the possibility to expand the spatial extent of efforts to restore coastal systems (Vanderklift et al., 2020). Once more, an ecophysiological approach can yield great insights in determining the quality of propagules, comprehending traits such as size, energy reserves available for initial growth and establishment, genetic diversity, and origin, which will ultimately define their viability.

CONCLUSION

This thesis provides new insights into the processes shaping settlement dynamics of rocky intertidal foundation species by integrating key ecological drivers (e.g., trophic resources, water temperature, precipitation) with physiological factors (e.g., nutritional status, fatty acid composition, growth) acting on early life stages. I show that the ecophysiology of six keystone species, the bivalves *Isonogmon alatus* and *Brachidontes solisianus*, and the barnacles *Chthamalus proteus*, *Chthamalus bisinuatus*, *Chthamalus montagui*, and *Semibalanus balanoides,* is strongly shaped by the trophic environment. Latitudinal variations in nearshore food sources are pronounced, and shape larval nutritional composition and physiological response (e.g., selective fatty acid retention). This has implications for comparative studies of intertidal species, as the trophic context affects animal physiology. The use of lipidomics brings immense opportunities to clarify the physiological processes mediating the pelago-benthic transition. It is my contention that uncovering the processes shaping the early life-history of marine benthic invertebrates not only advances community ecology, but also has serious implications in the management of natural resources by informing vulnerability hotspots, proper boundaries in marine protected areas, and seed viability in coastal rehabilitation.

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