Fatty acid composition as a function of latitude in barnacle cyprid larvae

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Abstract

Broadly distributed species need to perform well in a range of environmental conditions, but knowledge of how wide-ranging marine larvae perform along latitudinal gradients remains limited. The fatty acid composition of larvae is important for their physiological responses to changing conditions. Here, we investigated the fatty acid composition of the last, non-feeding stage of barnacle larvae (cyprids) using an integrative (larvae–environment) and comparative (latitudinal) approach. We measured fatty acids in the pelagic particulate matter and cyprids from *Chthamalus bisinuatus*, *Chthamalus proteus*, and *Semibalanus balanoides* from tropical to polar (Arctic) latitudes to identify potential food sources during the feeding larval stages (nauplius) that precede the cyprids and to ascertain larval capacity to integrate neutral (energetic) and polar (structural) fatty acids. We demonstrate that particulate matter in tropical waters mainly consisted of low-quality saturated fatty acids derived from detrital pathways, while particulate matter from polar waters was rich in polyunsaturated fatty acids originating from living microalgae. Across the studied regions, neutral fatty acids were assimilated from various food sources including diatoms, dinoflagellates, detritus, and microeukaryotes. Cyprids consistently retained higher essential fatty acid levels than the relative share in the particulate matter. Particularly, the essential docosahexaenoic acid (22:6ω3), which was scarce in the particulate matter, was highly retained across all species but highest for the tropical cyprids. We argue that this latitudinal pattern in fatty acid retention is related to periods of reduced nutrient intake, increased energetic and/or synthetic requirements, and responses to physical large-scale differences in environmental conditions.

For species with a larval stage, the physiological condition of larvae plays an important role in habitat selection (Miron et al. 1999; Miron et al. 2000), metamorphosis and recruitment success (Minchinton and Schheibling 1991; Jarrett and

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Pechenik 1997; Jarrett 2003), and ultimately population dynamics (Pechenik et al. 1993). Barnacles exhibit a complex life cycle involving a benthic adult phase and a planktonic larval phase where the last larval stage (cyprid) is non-feeding (Høeg et al. 2015; Chan et al. 2021). The cyprids' physiological condition is largely determined by the duration of the pelagic life (Lucas et al. 1979; Pechenik et al. 1993; Miron et al. 2000), the maternal provisioning (Freuchet et al. 2015; Kasten et al. 2019), and the quantity and quality of food ingested by the previous larval (nauplius) stages (Thiyagarajan et al. 2003; Leal et al. 2021). The dietary fatty acids ingested by the nauplius larvae are stored as droplets and are eventually utilized by the cyprid to survive and maintain membrane functionality and structural integrity (Holland and Walker 1975; Hazel 1995; Tremblay et al. 2007). The physiological condition of cyprids varies considerably among daily cohorts and the stored energy is gradually consumed until the larvae settle on a suitable substrate (Miron et al. 2000; Jarrett 2003; Leal et al. 2020). Initial

low-energy reserves impair the substrate exploration behavior of the cyprid (Miron et al. 2000; Tremblay et al. 2007) and reduce the early growth and survival of recently metamorphosed benthic recruits (Jarrett and Pechenik 1997; Leal et al. 2020).

Lipids are an ideal storage material for marine invertebrate larvae because they release twice as much energy as an equal mass of carbohydrate or protein (Lee et al. 2006), but they are one of the least studied compounds (Parrish 2013; Mejri et al. 2021). Fatty acids can be stored either as intracellular neutral lipids (triacylglycerols, wax esters) or as polar lipids in the cell membrane and organelles (e.g., phospholipids, cholesterol) (Copeman and Parrish 2003). Neutral lipids are conservatively assimilated, providing an indication of the ingested food (preceding days to weeks) and, once stored, are available for catabolism and the released energy can be captured in high-energy compounds such as adenosine triphosphate (O'Connor and Gilbert 1968). Polar lipids are essential structural components of biological membranes and are composed of a wide variety of phospholipids different by the nature of their polar head and/or by their fatty acids. In marine invertebrates, many mechanisms governing biological functions and membrane integrity involve changes in the qualitative composition of polar lipids, as their acid composition, their polar head, or the nature of the connection of the fatty acid chain to the glycerol skeleton determine their cellular integrity (Hazel and Williams 1990; Kraffe et al. 2008; Mathieu-Resuge et al. 2020). Some long-chained polyunsaturated fatty acids (PUFA) are considered as essential fatty acids for normal development. For example, the essential docosahexaenoic acid (22:6ω3) and eicosapentaenoic acid (20:5ω3) incorporated in membrane phospholipids are involved in maintaining the functional and structural integrity of biological membranes (Stillwell and Wassall 2003). Eicosapentaenoic acid and arachidonic acid (20:4ω6) can also be precursors of eicosanoids, a group of highly biologically active hormones such as prostaglandins and leukotrienes (Smith and Murphy 2016). It is generally accepted that the production of ω-3 PUFA occurs almost exclusively in marine phytoplankton, since it has been demonstrated that several marine invertebrates cannot biosynthesize ω-3 PUFA de novo and, from them, long-chain PUFA (Kanazawa et al. 1979; Nelson et al. 2006). However, recent research suggests that marine invertebrates could biosynthesize, at least partially, long-chained essential PUFA or some specific PUFA with potential similar function to maintain membrane integrity, like non-methylene-interrupted fatty acids (Barnathan 2009; da Costa et al. 2015; Monroig and Kabeya 2018). Nevertheless, this biosynthesis seems partial or requires a well-grounded evaluation, as many studies showed insufficient biosynthetic capacity in several marine invertebrate species to produce long-chain PUFA to meet nutritional requirements, which rely on essential fatty acids assimilated from dietary food sources (*see* review by Glencross 2009).

The fatty acid composition in available food sources varies across latitudes. In high-latitude coastal waters, phytoplankton is abundant during the spring bloom, which is often dominated by diatoms and dinoflagellates containing high levels of PUFA (Parrish et al. 1995). At low latitudes, coastal primary production rates are low throughout the year and filter-feeders rely on other food items in addition to phytoplankton (e.g., macroalgal detritus and bacteria) that are characterized by low levels of PUFA (Dalsgaard et al. 2003; Kelly and Scheibling 2012). The low availability of food sources rich in essential PUFA may result in nutritional deficiency and reduced growth and development (Webb and Chu 1983). While food is plenty in spring blooms at higher latitudes, the cold water increases cell membrane rigidity, resulting in the loss of membrane integrity and functioning without biochemical regulation (Hazel and Prosser 1974; Hazel 1995). To avoid a dysfunctional membrane, organisms living in cold waters integrate specific PUFA in the cell membrane to increase its unsaturation levels. This selective accumulation of specific fatty acids is called selective fatty acid retention (Copeman et al. 2002; Pernet et al. 2005; Tremblay et al. 2007). Previous work has shown that a higher proportion of fatty acids retained in the polar fraction in comparison to the neutral fraction (Thériault and Pernet 2007) indicates selective incorporation of fatty acids into membranes at the expense of readily available storage lipids.

Barnacles are cosmopolitan filter-feeders and can be important foundation species on rocky shores and recognized as bioindicators of environmental changes (Belt et al. 2009; Leal et al. 2020). They are dominant primary-space occupiers in the intertidal zone, providing food and habitat for a range of associated fauna. Barnacles have a broad latitudinal distribution, therefore experiencing a wide range of environmental conditions that require different levels and composition of neutral and polar fatty acids. However, the capacity of the larvae to integrate fatty acids in different environments remains poorly studied. The present study investigates how the composition of fatty acids in barnacle cyprids changes across a latitudinal gradient from 30.15°S to 64.17°N. Specifically, we hypothesized that cyprid larvae have a different fatty acid composition across tropical, subtropical, cold-temperate, and polar (Arctic) regions, reflecting latitudinal differences in the available food sources and environmental conditions. We measured the fatty acid levels in the nearshore pelagic particulate matter (the food source) and in cyprid larvae using an integrative (larvae–environment) and comparative approach to identify latitudinal changes in the available food sources and the capacity of larvae to store essential PUFA.

Materials and methods

Study areas and species

We sampled acorn barnacles during low tides over a 3-week period at two sites from four regions spanning a latitudinal gradient from 30.15°S to 64.17°N: *Chthamalus proteus* from Caribbean Panama (tropical region), *Chthamalus bisinuatus* from southeastern Brazil (subtropical region), and *Semibalanus balanoides* from both southeastern Canada (cold-temperate region) and western Greenland in the Arctic (polar region)

(Fig. 1). These species occupy the mid-to-upper intertidal zone and play a similar ecological role in their communities (e.g., food source for benthic predators and habitat engineering maintaining species diversity). Barnacles were identified to species level according to morphological features published in identification keys (Dando and Southward 1980; Southward et al. 1998; Southward 2008).

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) (Fig. 2), located on the exposed coastline of Colon Island in the Bocas del Toro Archipelago, were sampled during March 2017. Adult *C. proteus* barnacles with developing eggs are present at all times of the year, indicating a year-round larval production (Zabin et al. 2007). Around the Colon Island, the average sea surface temperature of the coldest month of the year, February, with 27.9°C, is not far from that of the warmest month of the year, October, with 29.6°C (Kaufmann and Thompson 2005).


Fig. 1. Photographs of the cyprid larvae (left panel, in lateral view) of each barnacle species in this study (adult form and recruits in the right panel): **(a)** *Semibalanus balanoides*, Nova Scotia, Canada, **(b)** *Chthamalus bisinuatus*, São Sebastião, Brazil, **(c)** *Chthamalus proteus*, Bocas del Toro, Panama. Larvae display conspicuous lipid droplets in the anterior part of the body, their prime lipid storage. Scale bar corresponds to 200 μm. Photos by Inês Leal.

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) (Fig. 2) were sampled during October 2016. Facing the São Sebastião Channel, these shores are characterized by a steep rocky habitat with a clear 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). In the study area, average sea surface temperature exhibits the lowest values in July, 21.9°C, and the highest in February,

28.6°C (Valentim et al. 2013). The São Sebastião Channel is a meso-oligotrophic system year-round (Leal et al. 2018).

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) (Fig. 2) were sampled during June 2018. Located on the southern coast of the Gulf of St. Lawrence, the sampled sites exhibit barnacle recruitment every spring (Scrosati and Ellrich 2016). *S. balanoides*, the only intertidal barnacle species on this coast, shares its niche with other sessile species such as fucoid algae (*Fucus* spp. and *Ascophyllum nodosum*) and blue mussels, *Mytilus edulis* (Ellrich et al. 2015; Scrosati and
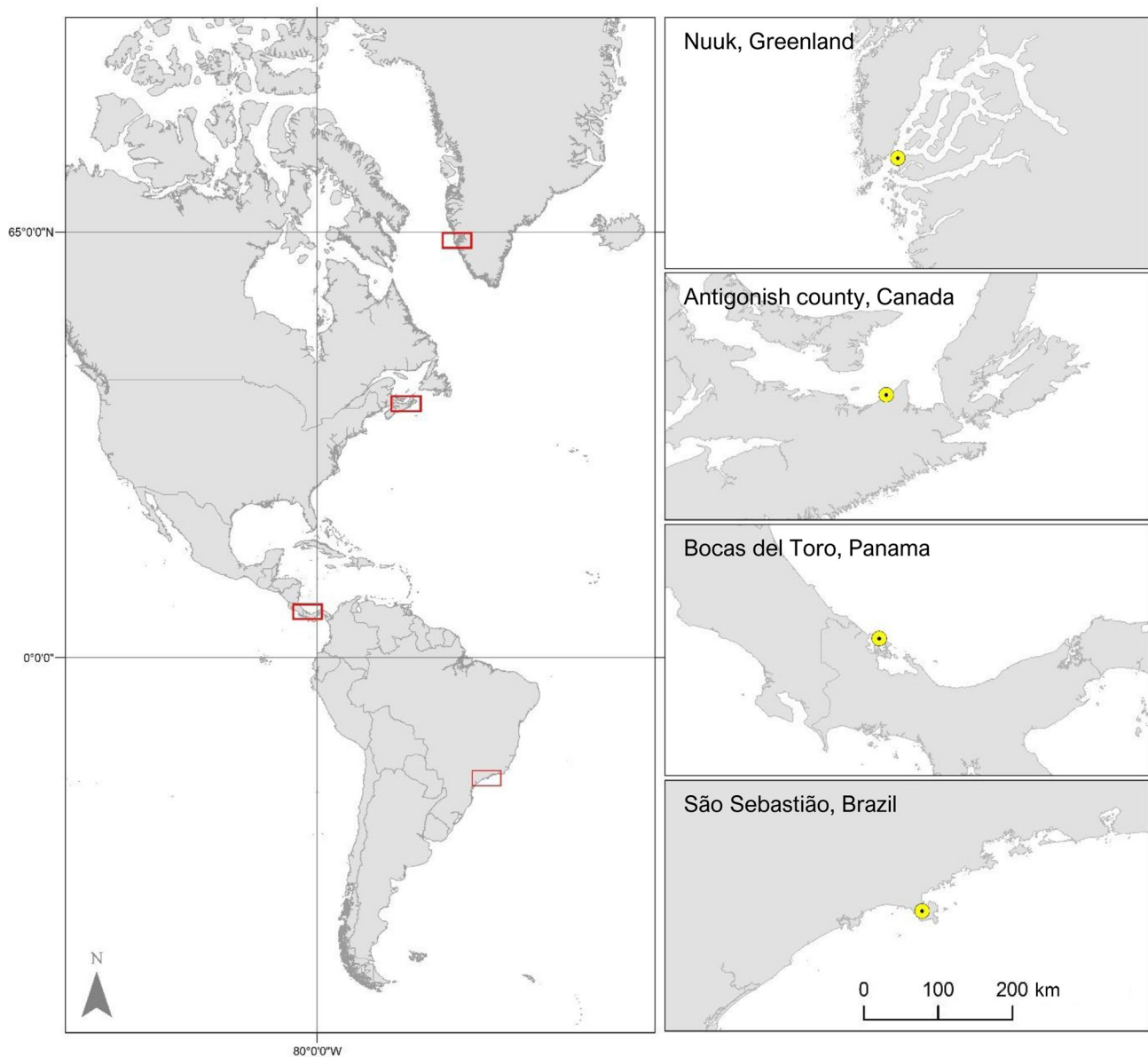
Fig. 2. Map of study regions, Nuuk, western Greenland (Arctic, polar region), Antigonish county, southeastern Canada (cold-temperate), Bocas del Toro, northwestern Panama (tropical), and São Sebastião, southeastern Brazil (subtropical). Yellow circle marks the area of the sampled shores within each region, that is, two shores < 3 km apart.

Ellrich 2016). After the sea ice melts in late winter or early spring, barnacle settlers appear on the intertidal substrate during a limited recruitment season between early May and late June (Ellrich et al. 2015). Low sea surface temperatures are observed on this coast in early spring (below 3°C in April; Scrosati and Ellrich 2016).

In Greenland, the rocky shores of Nuuk (site 1, 64°11′48.71″N, 51°41′59.76″W; site 2, 64°11′50.19″N, 51°42′24.02″W) (Fig. 2), facing the Nuup Kangerlua (Godthåbsfjord) fjord system, were sampled during June 2016. The brown alga *A. nodosum* completely covered the sampled rocky shores, with adult *Mytilus* mussels and *S. balanoides* frequently found attached to the rock underneath (Leal I, *pers. obs.*). *S. balanoides* is a common species with peak settlement during mid-summer months, particularly July (Sejr et al. 2021; Thyrring et al. 2021). Mean sea surface temperatures typically range from −1.5°C during winter to ∼ 13°C in the fjord during summer (Sejr et al. 2014; Clark et al. 2021).

Field sampling

Daily measurements of key biotic and abiotic variables were conducted for the same periods of time at each of the study sites. Intertidal air and water temperatures experienced by intertidal organisms were measured using waterproof data loggers (HOBO Pendant® Temperature/Light 64 K) at 10-min intervals. Four measurements of sea surface salinity were obtained from coastal waters using a probe (EcoScan SALT 6 Plus Salinity Meter with an electrode). Nearshore trophic resources available for filter-feeders were measured by collecting six replicate seawater samples (2 L each) per day. Samples were first stored in opaque bottles, then filtered through a 100-μm mesh sieve, and then through Whatman® glass microfiber filters (GF/F 25 mm, pore size 0.7 μm, preburnt, and weighted). Upon filtration, three glass microfiber filters were rinsed with isotonic 0.3-M ammonium formate to dissolve residual salts (Aminot and Chaussepied 1983), subsequently heated at 70°C for 24 h, and weighted to measure total particulate matter (PM, mg L^{-1}), a proxy of dietary sources. Three other glass microfiber filters were stored at −80°C in dichloromethane-methanol vials (2:1, v/v) after filtration to measure the mass of total fatty acids and their composition, a proxy of the quality and composition of pelagic food (*see* below).

A 150-μm plankton net (30 cm of mouth diameter) was manually towed just below the water surface, daily, along a 100-m transect following the shoreline at each site. The collected plankton was sorted under a stereomicroscope to identify and collect cyprids. Identified cyprids were photographed alive in lateral view to measure their size (mm; $n = 90$ per study area, Fig. 1) using the software Fiji (Schindelin et al. 2012), of which 20 individuals were pooled and preserved in dichloromethane–methanol (2:1, v/v) at −80°C to have enough biological material to analyze their fatty acid content and composition (*see* below).

Settlement plates (10 cm × 10 cm; $n = 10$) covered with 3 M Safety-Walk™ tape (620 series) to provide surface roughness (a normal feature of rocky intertidal habitats known to facilitate settlement; Menge et al. 2010) were haphazardly attached to the mid-intertidal substrate over hundreds of meters to provide suitable settlement substrate for cyprid larvae. The settlement plates were taken daily to the laboratory to assess settlement rates, and new plates were reinstalled in the field. Newly settled barnacles were identified using a stereomicroscope.

Fatty acid analysis

Fatty acids from the pelagic particulate matter were recovered through direct transesterification without prior extraction (Lepage and Roy 1984) and analyzed by gas chromatography–mass spectrometry (GC–MS) to determine the mass of total fatty acids (μg mg^{-1} pelagic particulate matter) and its composition (% fatty acids). 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 Programmable Temperature Vaporizer 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 component FAME Mix (Supelco). Fatty acids were further discriminated into saturated (fatty acids with no double bond), monounsaturated (fatty acids with one double bond), polyunsaturated (fatty acids with ≥ 2 double bonds), and essential (ω3 and ω6) fatty acids. A detailed description of the number of samples processed for fatty acid analyses is available in Supplementary Table S1.

Three pools of 20 individual cyprids were sampled each week at each site for fatty acid analyses (data are therefore expressed as μg larva^{-1}). Lipids were extracted by a modified Folch procedure (Folch et al. 1957) and the neutral fraction (including wax esters, sterols, fatty alcohols, and triglycerides) was separated from the polar fraction (including mainly phospholipids) by silica gel, hydrated with 6% water (Marty et al. 1992; Couturier et al. 2020). 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 analyzed with the GC–MS, as above, to determine the total energetic content in individual larvae (sum of all fatty acids identified; μg larva^{-1}) and its composition (% fatty acid). A detailed description of the number of larvae processed for fatty acid analyses is available in Supplementary Table S2.

The ratio between the fatty acids in cyprids and those in the pelagic particulate matter at each site (larval-to-food ratio) was used to evaluate the relative role of larval selective fatty acid retention (Mejri et al. 2021). We focused on three essential PUFA: eicosapentaenoic acid (20:5ω3), docosahexaenoic acid (22:6ω3), and arachidonic acid (20:4ω6). If this ratio is ≤ 1, then an absence of selective retention is assumed, which

indicates good physiological condition of the larvae (Gendron et al. 2013). A ratio > 1 indicates that the larvae have incorporated a particular fatty acid from the food.

Fatty acid trophic markers

Latitudinal patterns in consumed food sources were investigated using fatty acid trophic markers, a method widely applied to study the origin of ingested food (Dalsgaard et al. 2003; Kelly and Scheibling 2012). The method relies on the observation that fatty acids are incorporated almost unaltered into the storage (neutral) lipids such as wax esters, fatty alcohols, and triglycerides reflecting the fatty acid composition of the consumed food and reliably indicating the food ingested in the preceding weeks. Different classes of primary producers have distinguishable fatty acid compositions, thus allowing detection of the food sources (Dalsgaard et al. 2003; Parrish 2013). For instance, pelagic diatoms are rich in palmitoleic (16:1ω7) and eicosapentanoic (20:5ω3) acids, dinoflagellates are characterized by high levels of stearidonic (18:4ω3) and docosahexaenoic (22:6ω3) acids (Kelly and Scheibling 2012), and bacteria contain high levels of methyltetradecanoic (i15:0) and heptadecenoic (17:1) acids (Parrish 2013). In this study, food sources were classified into six trophic groups: bacteria, detrital matter, macroalgae, microeukaryotes, dinoflagellates, and diatoms (Table 1).

Statistical analysis

Permutational multivariate ANOVAs (PERMANOVAs) using PRIMER (v. 7.0.13) were used to make latitudinal comparisons in fatty acid composition of the pelagic particulate matter, the mass of total fatty acids, the fatty acid trophic markers, and the selective retention of eicosapentaenoic (20:5ω3), docosahexaenoic (22:6ω3), and arachidonic (20:4ω6) acids among

study regions (fixed factor with four levels: tropical, subtropical, cold-temperate, and polar), with sites nested within region (random factor with two levels—the two sites). Analyses were conducted with fatty acids expressed as percentages (arcsin square-root transformed) using a PERMANOVA based on Bray–Curtis similarities and 9999 permutations. Estimates of p values for pairwise comparisons among regions were obtained using Monte Carlo tests (p_{MC}) in PRIMER. To uncover the nature of the latitudinal differences detected by each PERMANOVA, we carried out principal component analysis (PCA) (Gabriel, 1971). PCA biplots, displaying information both on samples (points) and variables (vectors), were used to visualize the variation in neutral and polar composition of fatty acids among the study regions. 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) for visual presentation.

Results

Nearshore conditions

Intertidal temperature variation ($T_{max} - T_{min}$) was considerable among all sampled shores, being consistently greater than 20°C ($\Delta T_{Tropical} = 24.1°C$; $\Delta T_{Subtropical} = 26.5°C$; $\Delta T_{Cold-temperate} = 21.3°C$; $\Delta T_{Polar} = 27.1°C$), but mean intertidal temperature (measured by the installed temperature loggers) decreased with increasing latitude (Fig. 3a). The tropical region experienced the highest average temperature (29.8°C ± 1.57 SD), followed by the subtropical (24.1°C ± 1.17 SD), cold-temperate (10.6°C ± 1.84 SD), and polar (7.0°C ± 1.57 SD) regions. The same latitudinal trend was found for salinity, with the tropical (36.9 ± 0.52 ppt) and subtropical regions

Table 1. List of fatty acid trophic markers used to describe the origin of nearshore particulate matter available as dietary source for filter-feeders.

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	Nutritionally rich matter
	20:5ω3 (EPA)	Diatoms	
	22:6ω3 (DHA)	Dinoflagellates	

EFA, essential fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
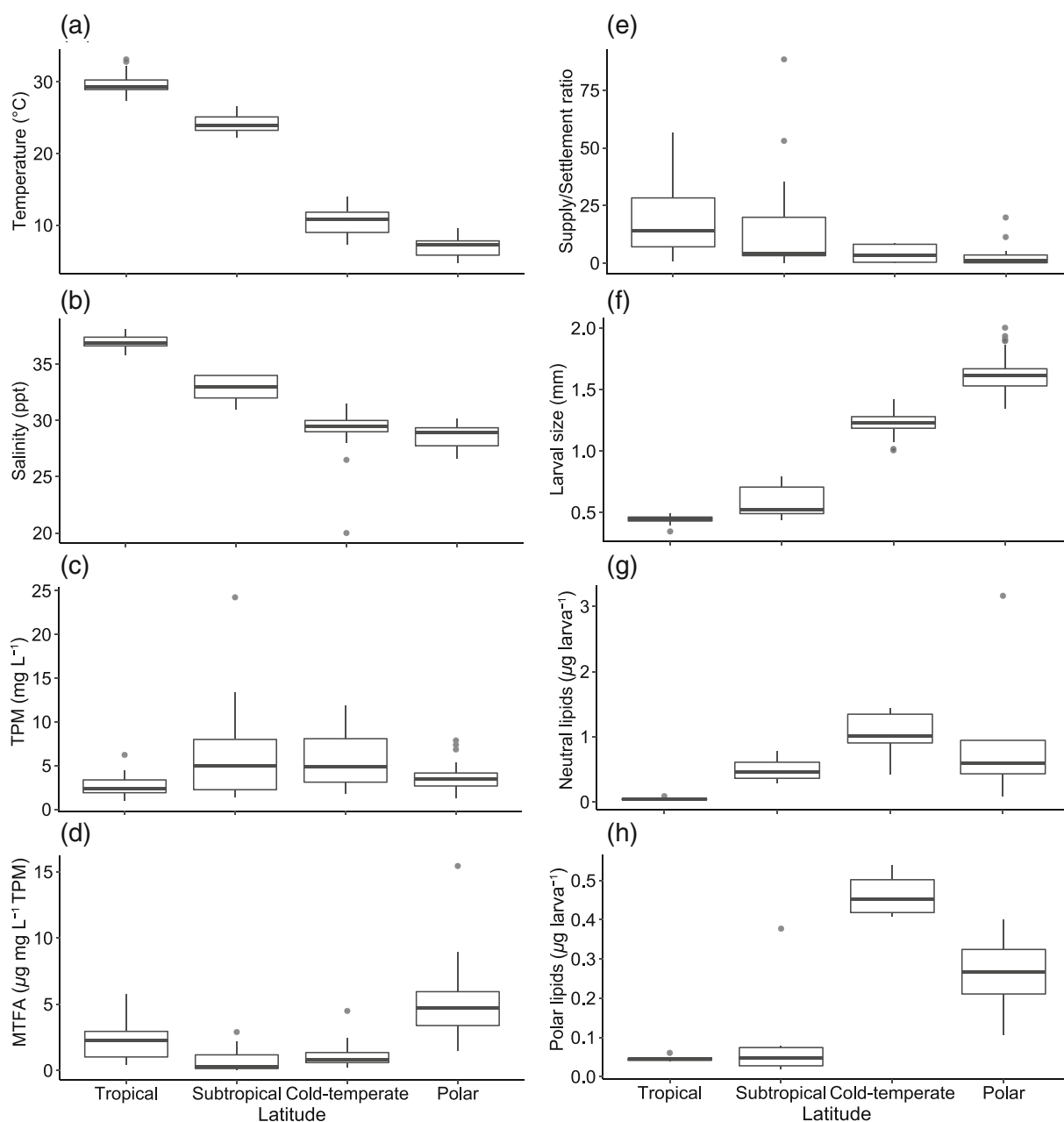
Fig. 3. Nearshore variation in (**a**) temperature (intertidal logger), (**b**) salinity, (**c**) total particulate matter (TPM), (**d**) mass of total fatty acids (MTFA) in the PM, (**e**) ratio between number of cyprids in the water column and newly settled barnacles, (**f**) larval size, (**g**) larval neutral lipid content, and (**h**) larval polar lipid content at the four study regions. The horizontal line in each boxplot is the median, the boxes define the hinges (25–75% quartile) and the whisker is 1.5 times the hinges. Black dots represent data outside this range.

$(33.0 \pm 0.28 \, \text{ppt})$ having higher salinities compared to the cold-temperate $(29.1 \pm 1.91 \, \text{ppt})$ and polar regions $(28.6 \pm 1.05 \, \text{ppt})$ (Fig. 3b). In contrast, no latitudinal pattern emerged for the concentration or mass of fatty acids in the pelagic particulate matter. The highest concentration was measured in subtropical waters $(6.08 \pm 1.01 \, \text{mg L}^{-1})$, while tropical waters had the lowest $(2.6 \pm 0.20 \, \text{mg L}^{-1})$ (Monte Carlo pairwise test; $p_{MC} = 0.04$; Fig. 3c). The mass of total fatty acids was highest at the polar region $(5.3 \pm 0.56 \, \mu\text{g mg}^{-1}$ of

particulate matter) and lowest at the subtropical region $(0.8 \pm 0.2 \, \mu\text{g mg}$ of particulate matter) (Monte Carlo pairwise test; $p_{MC} = 0.01$; Fig. 3d).

The larval supply-to-settlement ratio (ratio between the density of cyprids in the water column and the density of newly settled individuals) declined with latitude, revealing a higher settlement at higher latitudes relative to the available larval pool (Fig. 3e). The sizes of *C. proteus* and *C. bisinuatus* averaged $0.45 \pm 0.02 \, \text{mm}$ and $0.60 \pm 0.11 \, \text{mm}$ respectively,

while *S. balanoides* cyprids averaged 1.22 ± 0.08 and 1.61 ± 0.14 mm at the cold-temperate and polar regions, respectively (Fig. 3f). The average content of neutral fatty acids was lowest in the tropical (0.05 μg per *C. proteus* larva) and subtropical (0.5 μg per *C. bisinuatus* larva) regions, while the content in the cold-temperate and polar *S. balanoides* was ~ 1.0 μg larva^{-1} (Fig. 3g). Similarly, the average content of polar fatty acids was lowest for tropical *C. proteus* (0.05 μg larva^{-1}) and subtropical *C. bisinuatus* (0.1 μg larva^{-1}), compared to *S. balanoides* from the cold-temperate (0.5 μg larva^{-1}) and polar (0.3 μg larva^{-1}) regions (Fig. 3h).

Fatty acid composition of pelagic particulate matter and cyprids

The amount of saturated fatty acids present in the pelagic particulate matter declined with latitude, constituting 57.3% in the tropics, followed by the subtropics (55.3%), cold-temperate (45.4%), and polar (38.9%) regions (Fig. 4a).

Polyunsaturated fatty acids (PUFA) were more abundant at high latitudes than at low latitudes and constituted 40.1% of the pelagic particulate matter collected at the polar regions, but only 14.3% in the tropics (Fig. 4a). Eicosapentaenoic acid (20:5ω3) were the most abundant essential fatty acid in pelagic particulate matter across latitudes from the tropical (4.3%), subtropical (6.5%), cold-temperate (5.7%), and polar (22.5%) regions (Supplementary Table S1).

No obvious latitudinal trend was observed for the neutral fatty acid composition of cyprids. Energetic neutral lipid reserves of cyprids in the tropical, subtropical, and polar regions consisted mainly of saturated fatty acids, 51.9% for *C. proteus*, 58.2% for *C. bisinuatus*, and 54.4% *S. balanoides*, respectively, while cold-temperate *S. balanoides* consisted mainly of mono-unsaturated fatty acids (43.2%) (Fig. 4b, Supplementary Table S2). Similar levels of PUFA were found in the neutral lipid fraction of cyprids across all latitudes, comprising 14% of total lipid content for tropical, 17.8% for subtropical, 17.7% for
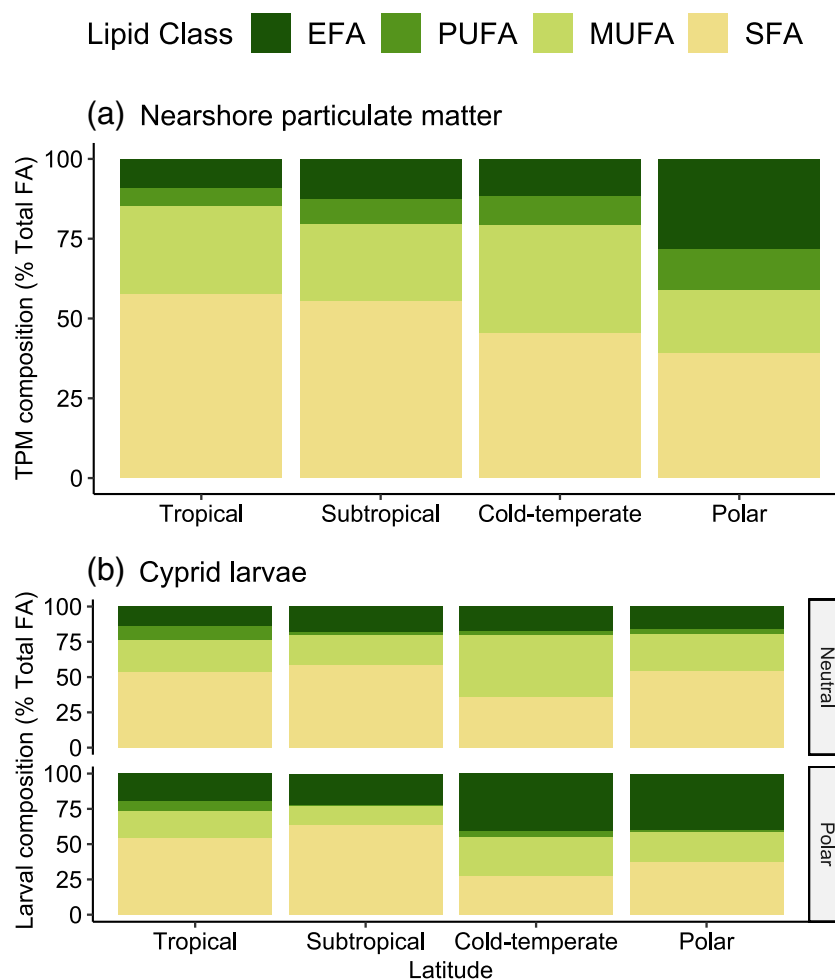
Fig. 4. Relative composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and essential fatty acids (EFA) in the (**a**) nearshore pelagic particulate matter, (**b**) neutral and polar fractions of fatty acids of cyprid larvae at the four latitudinal regions studied. Color gradient depicting a decreasing gradient in fatty acid nutritional quality (highest for EFA, lowest for SFA).

Fig. 5. Principal component analysis biplots of fatty acid trophic markers attributed to the six trophic sources, (i) bacteria, (ii) detrital matter, (iii) macroalgae, (iv) microeukaryotes, (v) dinoflagellates, and (vi) diatoms, present in (**a**) the particulate matter collected from nearshore waters, showing the origin of the food available for filter-feeders; (**b**) the neutral fraction of cyprids, showing the short-term source of the dietary fatty acids assimilated as energy fuel; (**c**) the polar 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. Ellipses extend to the 95% confidence interval for each of the four study regions.

suggesting that they play similar functional roles and likely have similar ancestral adaptively evolved physiologies. Furthermore, no small-scale variation among sites within regions was detected in larval physiological condition, and we argue that

the demonstrated latitudinal patterns of fatty acid retention are related to periods of reduced nutrient intake, increased energetic and/or synthetic requirements, and responses to physical large-scale differences in environmental conditions.
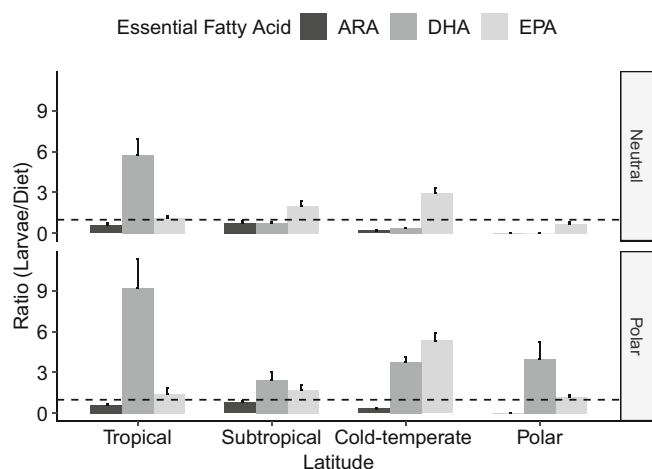
Fig. 6. Ratio of essential fatty acids in cyprid larvae to dietary essential fatty acids (i.e., from nearshore particulate matter). The dashed line indicates equal amounts of fatty acids in larvae and their food. A ratio ≥ 1 indicates that larvae are selectively incorporating fatty acids into energetic reserves (neutral fraction) and/or cellular structure (polar fraction). Essential fatty acids: arachidonic acid (20:4ω6, ARA); docosahexaenoic acid (22:6ω3, DHA); eicosapentaenoic acid (20:5ω3, EPA).

The fatty acid composition of nearshore particulate matter showed a latitudinal trend. Nutritionally rich PUFA food sources peaked in the polar region and the percentage of PUFA decreased from the polar to the tropical latitudes. The analysis of fatty acid trophic markers showed that neutral fatty acids were assimilated from various food sources including phytodetritus (14:0, 16:0, 18:0, and 18:1ω9), diatoms (16:1ω7 and 20:5ω3), and dinoflagellates (18:4ω3 and 22:6ω3) across latitudes. The identified phytodetritus markers could be associated to other sources as they can be biosynthesized by several autotrophic and heterotrophic marine organisms (Kelly and Scheibling 2012). However, high levels of these fatty acids have been observed at sites dominated by detrital organic matter (Freites et al. 2002; Cabrol et al. 2015; Leal et al. 2018). Thus, while barnacle larvae at high latitudes consume planktonic primary producers during the Arctic summer, they also incorporate significant amounts of phytodetritus and resuspended macroalgal material. The same pattern has been observed in adult individuals of other benthic invertebrates (Renaud et al. 2015; Gaillard et al. 2017; Thyrring et al. 2017). At low latitudes, the more readily available heterotrophic food (detrital and bacterial particles) may result from allochthonous inputs from land transported to the shore through run-off (Leal et al. 2022). For example, heavy rainfalls at the onset of the rainy season produce low-salinity plumes that flow over the typically warm Caribbean waters in Panama (Kaufmann and Thompson 2005), providing an input of nutrients and detrital particles, to nearshore benthic communities (Herbeck et al. 2011; Gorman et al. 2019). Fatty acids that constitute trophic markers for macroalgae, including green and brown macroalgal detritus as well as terrestrial detritus (18:1ω9,

18:2ω6, 18:3ω3 and 18:3ω6), contributed to larval nutrition at subtropical and cold-temperate latitudes. The fatty acids 18:2ω6 and 18:3ω3 are found in elevated amounts in most littoral vegetation (Budge and Parrish 1998; Parrish et al. 2000), terrestrial runoff material (Copeman and Parrish 2003; Colombo et al. 2017), mangroves (Meziane et al. 1997), seagrass (Kelly and Scheibling 2012), and macroalgae (Cui et al. 2015). Furthermore, 18:1ω9 is also found to be more abundant in brown algae (Kelly and Scheibling 2012). In recent research, macroalgal-derived particulate matter has been suggested to be a non-negligible food source in marine invertebrate nutrition (De Cesare et al. 2017; Gaillard et al. 2017; Leal et al. 2022). Some of the biomass of dominant primary producers (from mangrove leaves to bacteria) enters the food web via detrital pathways rather than being directly grazed (Meziane et al. 1997; Alfaro et al. 2006), and we suggest that macroalgal-derived particulate matter (identified by the fatty acid 18:1ω9 [7–20% of total particulate matter] across all latitudes) could be a substantial food source in benthic food webs. It should be noted that the composition of the fatty acids from the pelagic particulate matter described here is probably not wholly representative of benthic food supply. Temporal variability in nearshore conditions, for example, could not be captured and might be significant in individual study regions. Shifts over fine temporal scales (days) have been reported at both tropical (e.g., shifts in particulate matter composition following rainfall; Leal et al. 2019) and subtropical shores where scattered oceanographic processes (1–2 d) can lead to localized vertical mixing and surplus production in the water (Kasten and Flores 2013). For high-latitude regions, important seasonal shifts have been reported (Gaillard et al. 2017; Thyrring et al. 2017; Bridier et al. 2019) and future research should, therefore, aim to capture such temporal variability for a better understanding of dietary sources entering benthic food webs.

The amount of saturated fatty acids entering the benthic food web comprised a substantial fraction of cyprid energy reserves across latitudes, exceeding 50% of neutral fatty acids in tropical, subtropical, and polar cyprids. The accumulation of saturated fatty acids have been shown to be important for invertebrate species under high energetic demands given the efficient oxidation and high energy yield of this class of fatty acids (Cabrol et al. 2015; Leal et al. 2019). This result aligns with previous research showing that high levels of the saturated fatty acids 16:0 and 18:0 fatty acids are found in rocky-shore mussels inhabiting environments abundant in detritic matter (Freites et al. 2002). Furthermore, for all sampled species, cyprids contained higher levels of PUFA than the relative share in the water. The low level of 18-carbon precursor fatty acids in food sources suggests selective retention rather than biosynthesis, as de novo synthesis of PUFA by crustaceans is probably minimal (Kanazawa et al. 1979). Recent research suggests potential PUFA biosynthesis by marine invertebrates like crustaceans (Monroig and Kabeya 2018), but this biosynthesis

seems partial and requires a well-grounded evaluation (Glencross 2009). 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 were selectively incorporated into the cell membranes at the expense of neutral fatty acids. The highest docosahexaenoic acid (22:6ω3) retention in neutral lipids was observed in the tropical *C. proteus*. Since the docosahexaenoic acid is preferentially retained during food deprivation (Martinez-Silva et al. 2018), cyprid larvae in tropical regions may retain docosahexaenoic acid during naupliar development in response to limited availability in pelagic food sources. Preferential retention of important fatty acids in unfavorable conditions is of great importance for maintaining normal cellular functioning (Werbrouck et al. 2016).

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 water temperature usually induces an increase in the proportion of unsaturated fatty acids in the cellular membrane (i.e., an increase in membrane fluidity) to avoid lethal membrane damage (Hazel 1995). To compensate for the effect of temperature, organisms remodel the membrane composition of fatty acids, a process termed homeoviscous adaptation (Hazel 1995), rendering the membrane more or less fluid. Thus, while the neutral fatty acid composition provides reliable information on food web structure, the polar fatty acid composition can reveal how ectotherms physiologically adjust to their environment. To maintain membrane fluidity in cold waters, cyprids likely increase the content of PUFA (i.e., unsaturation state) in their membrane. Indeed, *S. balanoides* cyprids from cold-temperate and polar waters had a far greater proportion of PUFA in their polar lipids compared to tropical and subtropical *Chthamalus* spp. (≥ 40% compared to 25.7% and 22.9%, respectively). The essential docosahexaenoic acid, which allows for rapid conformational changes in biological membranes (Feller et al. 2002), was selectively incorporated in membranes across all studied regions. The retention of polar PUFA in cyprids inhabiting low-latitude environments could allow for an increased metabolic activity of membrane proteins driven by high environmental temperatures, while the retention of PUFA at high latitudes serve to maintain optimal membrane fluidity. Thus, the selective incorporation of polar PUFA may vary as a function of latitude, reflecting local adaptation to environmental temperature. Membrane remodeling may also be critical for cyprid larvae to quickly adjust to the variable temperatures faced upon settlement (> 20°C daily oscillations found at all sampled intertidal regions). Although in some intertidal species there seems to be no correlation between membrane unsaturation state and freeze tolerance (Thyrring et al. 2020, 2023), persistent exposure to water temperatures, either above or below those required to maintain optimal fluidity, initiates acclimatory (within the lifetime of an individual) or

adaptational (over generations) membrane changes in fatty acid composition that may offset the direct effects of temperature on membrane lipid fluidity during the pelago-benthic transition (Hazel 1995).

Finally, *C. proteus* settlers have been shown to face increasing levels of thermal stress in the tropics (Leal et al. 2020) and it is likely that higher larval mortality rates would occur during the pelago-benthic transition. This increased mortality may be reflected in the elevated larval supply-to-settlement ratio at the tropical and subtropical regions, but this notion needs to be tested (so we hope to stimulate further research into the settlement dynamics of benthic species). Although *S. balanoides* supply-side ecology (influence of new recruits onto population dynamics) has been extensively studied on northern Atlantic coasts (e.g., Knight-Jones 1953; Lucas et al. 1979; Jarrett 2003; Scrosati and Ellrich 2018), we provide the first latitudinal description of the fatty acid composition of barnacle cyprid larvae from tropical to polar regions. In particular, our results demonstrate that fatty acid composition in barnacle cyprid larvae varies as a function of latitude, seemingly reflecting local food and environmental conditions. In tropical waters, available fatty acids are derived mainly from detrital particles, and PUFA appear to be selectively retained to tackle food deprivation. In productive polar waters, fatty acids were mainly obtained from living microalgae, and PUFA likely used to increase membrane unsaturation to cope with low water temperatures. Thus, our investigation provides useful and novel information concerning fatty acid signatures of late-stage barnacle larvae and pelagic food sources entering benthic food webs across multiple latitudes.

Data availability statement

The data that support the findings of this study are openly available in Zenodo.org at http://doi.org/10.5281/zenodo.11142679 (Leal et al 2024).

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Conflict of Interest

The authors have no conflict of interests related to this publication.