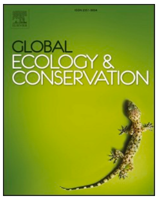
Global Ecology and Conservation 67 (2026) e04154

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: www.elsevier.com/locate/gecco



Original research article

Effects of dietary fatty acid composition and salinity on copepod reproduction in a eutrophic estuary

Mianrun Chen [a], Qi Chen [d], Yueyue Si [e], Zhiwei Liu [c], Zhuo Xu [b], Dingyu Luo [b,*], Xian Sun [b,*]

[a] *South China Sea Development Research Institute, Ministry of Natural Resources (Remote Sensing Technology Application Center of South China Sea, NMR), Guangzhou 510300, China/Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519082, China*
[b] *School of Marine Sciences, Zhuhai Key Laboratory of Marine Bioresources and Environment, Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering, Pearl River Estuary Marine Ecosystem Research Station, Ministry of Education, Research Center of Ocean Climate, Sun Yat-Sen University, Zhuhai 519082, China*
[c] *School of Ecology, Sun Yat-sen University, Guangzhou 510275, China*
[d] *Guangzhou pearl river resources protection technology development Co., Ltd, Guangzhou 510611, China*
[e] *School of Ocean and Earth Science, University of Southampton, Southampton SO14 3ZH, UK*

ARTICLE INFO

Keywords:
Copepod reproduction
Polyunsaturated fatty acids
Pearl River Estuary
Salinity effect

ABSTRACT

Estuarine ecosystems are critical for fisheries and biodiversity, with copepods serving as essential trophic links. Accurate prediction of copepod reproductive success, measured via egg production rate (EPR), is vital for ecosystem management. Traditional monitoring relies on biomass indicators like chlorophyll-a, but emerging evidence underscores the importance of dietary biochemical quality, particularly polyunsaturated fatty acids (PUFAs) as indicators of dietary biochemical quality. This study, conducted during seasonal cruises in the Pearl River Estuary in May (spring), August (summer), and November (autumn) 2015, and January (winter) 2016, tested a hierarchical framework where PUFA availability is the primary regulator of EPR, modulated by environmental factors like salinity. Generalized Additive Models revealed that PUFA variability were the strongest predictor of EPR, exhibiting nonlinear responses that reflect complex nutritional trade-offs. Salinity influenced reproduction indirectly by shaping phytoplankton community composition and subsequent PUFA quality, rather than through direct physiological stress. In contrast, chlorophyll-a showed limited explanatory power. These findings advocate for a paradigm shift from biomass-based to mechanism-driven assessments, with the PUFA-salinity relationship serving as a sensitive diagnostic tool for early detection of ecosystem degradation. This framework enables proactive management before population declines manifest and has implications for sustainable fisheries. Future research should validate causal links through controlled experiments and develop cost-effective PUFA monitoring methods.

1. Introduction

Estuarine ecosystems are the world's most productive and biologically diverse habitats, sustaining rich fisheries and serving as vital nursery and foraging grounds for numerous fish and bird species of commercial importance (Anahi et al., 2014). Within these systems,

---

* Corresponding authors.
E-mail addresses: luody27@mail.sysu.edu.cn (D. Luo), sunx27@mail.sysu.edu.cn (X. Sun).

https://doi.org/10.1016/j.gecco.2026.e04154
Received 14 October 2025; Received in revised form 25 January 2026; Accepted 5 March 2026

zooplankton, particularly copepods (Copepoda), serve as a critical trophic link, channeling energy from primary producers to higher trophic levels (Sterner, 2009). Their reproductive success and population dynamics directly influence fish recruitment and broader ecosystem functioning. A defining feature of estuarine habitats is the pronounced spatiotemporal variability in physicochemical conditions, such as salinity, temperature, and nutrient concentrations, driven largely by seasonal cycles. These fluctuations induce significant shifts in zooplankton community structure and functionality (Hansen et al., 2016; Mungenge et al., 2024). Consequently, accurately predicting copepod reproductive performance is essential for the conservation, management, and sustainable use of estuarine ecosystems (Yi et al., 2022).

Zooplankton growth, egg production, and maturation are strongly influenced by the availability and nutritional quality of their diet (Dodds et al., 2009; Hantzsche and Boersma, 2010). Therefore, identifying the factors that regulate copepod recruitment potential, commonly measured as the weight-specific egg production rate (EPR, in eggs female^{-1} day^{-1}), is crucial for assessing population dynamics. Phytoplankton abundance, typically proxied by chlorophyll-a (Chl a) concentration, has traditionally been regarded as the principal driver of zooplankton reproduction, implying a direct quantity-dependent relationship. However, growing evidence

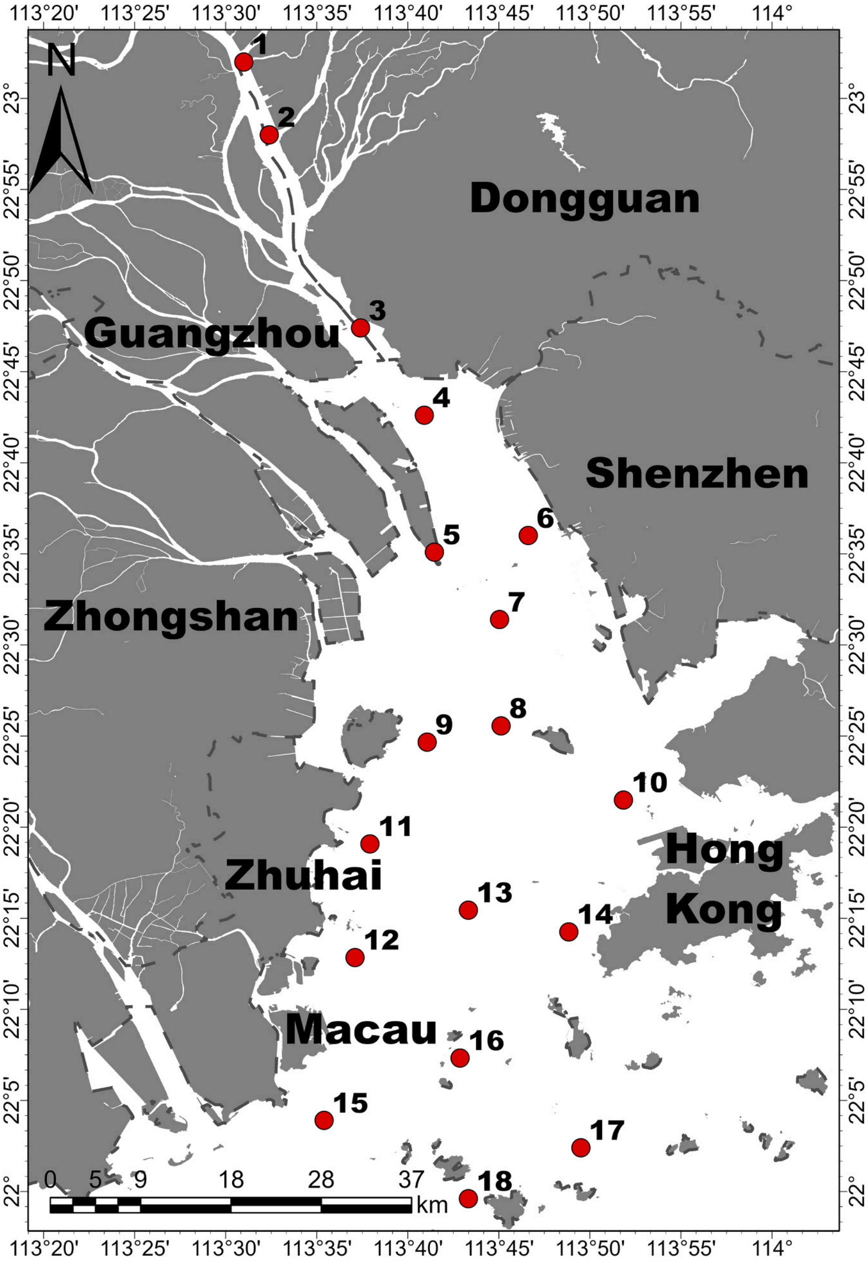


Fig. 1. Sampling site in Pearl River Estuary from the South China Sea.

challenges this paradigm, demonstrating that algal prey quality, particularly its biochemical composition, exerts a more profound influence on copepod life-history traits, including egg production and viability (Anahi et al., 2014; Milione and Zeng, 2007). This conceptual shift underscores the limitations of biomass-based metrics, which often fail to capture early warning signals of reproductive impairment, and highlights the need for more direct indicators of nutritional value. Biochemical traits such as essential fatty acid profiles, for instance, polyunsaturated fatty acids (PUFAs) like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are increasingly recognized as functional indicators that define food quality and its transfer efficiency across trophic levels (Dickman et al., 2008; Matsui et al., 2021).

Although the effects of various environmental drivers on copepod reproduction are well-documented, a significant knowledge gap remains in the systematic comparison, ranking, and validation of multiple candidate indicators within a hierarchical framework that prioritizes mechanism-driven factors over traditional metrics. While numerous studies have isolated the influences of temperature (Chinnery and Williams, 2003; Choi et al., 2021), salinity (Mabidi et al., 2018; Nguyen et al., 2020), and food quality, specifically PUFA availability (Anahi et al., 2014; Milione and Zeng, 2007), their relative importance, interactions, and pathways, including the possibility that salinity gradients alter trophic conditions and thereby modulate the relevance of PUFA availability for reproduction, remain poorly quantified under field conditions. Importantly, PUFA–salinity relationships have rarely been evaluated in a unified manner because PUFA indicators require dedicated biochemical analyses and are therefore seldom sampled at spatial and temporal resolutions comparable to estuarine salinity variability. Furthermore, strong co-variation among salinity, temperature, nutrients, and phytoplankton structure complicates the disentanglement of joint effects using traditional single-driver or purely biomass-based approaches. The absence of a validated, mechanism-driven indicator hierarchy limits the ability to identify reliable predictors of EPR and to evaluate reproductive impairment as an early-warning signal of ecosystem change.

We hypothesize that a hierarchical indicator system, where biochemical quality (e.g., PUFA availability) serves as the primary regulator, modulated by environmental stress factors (e.g., salinity), and supersedes traditional biomass metrics, will provide a more comprehensive and mechanistic explanation of EPR. To address this gap, we quantitatively assessed and compared the relative explanatory power of physical, biomass-based, and biochemical parameters on the EPR of copepods in the Pearl River Estuary, employing advanced modeling techniques to elucidate nonlinear responses and causal pathways. Our goal was to establish a robust framework for identifying the most reliable indicators of copepod reproductive performance, thereby enhancing the evaluation of trophic transfer efficiency and supporting the development of proactive management strategies in changing estuarine environments.

2. Materials and methods

2.1. Study site and sample collection

We conducted four seasonal cruises in the Pearl River Estuary, China, in May (spring), August (summer), and November (autumn) 2015, and January (winter) 2016. On each cruise, we measured salinity, temperature, particulate organic carbon (POC), ciliate abundance, mesozooplankton biomass, phytoplankton community structure, and food quality (defined by food item composition and fatty acid profiles) at all sites (Fig. 1). Fatty acids were measured only during the summer and winter cruises, at four stations per season ($n = 4$ in summer; $n = 4$ in winter). For biochemical analyses, we collected triplicate samples at each station.

2.2. Measurement

The measurement procedures for environmental parameters followed those described by Chen et al. (2021). Briefly, we measured in situ temperature and salinity with a hand-held multi-parameter water quality analyzer (YSI 556MPS, YSI Incorporated, Yellow Springs, OH). For POC determination, surface water (0.25 –3 l) was filtered through pre-combusted (450°C, 4 h) Whatman GF/F filters (diameter: 25 mm; pore size: 0.7 μm) and analyzed with an elemental analyzer (Model 240-XA, Control Equipment Corporation). We then calculated POC concentrations using Hewlett Packard (HP-150) analysis software. For Chl a, surface water (0.25 –1 l) was filtered through a cascade filtration system fitted with a 20 μm polycarbonate membrane, a 2 μm polycarbonate membrane, and a Whatman GF/F filter sequentially under low vacuum pressure (<100 mm Hg). Filter concentrations were determined using a Turner Designs fluorometer (Model 7200, Turner Trilogy) (Parsons et al., 1984).

We conducted vertical net tows using a plankton net equipped with a digital flow meter. An aliquot of the collected mesozooplankton sample was filtered onto a pre-weighed 10-μm-aperture polycarbonate (PC) membrane. The membrane was placed in a covered Petri dish, and the volume of the filtered aliquot was recorded. In the laboratory, the membrane was dried at 60 °C for 4 h and re-weighed. Mesozooplankton dry weight (DW, mg) was calculated as the difference between post- and pre-drying weights. The DW biomass concentration (mg m^{-3}) was calculated, calculated as:

$$B_{Dw} = \frac{DW}{V}$$

where DW is the mesozooplankton dry weight (mg) retained on the membrane and V is the corresponding seawater volume (m^3) filtered.

2.3. Community structure

We analyzed phytoplankton community composition using pigment biomarkers (Howell et al., 2004). Seawater (0.25 –0.5 l) was filtered through GF/F filters, and filters were extracted with N,N-dimethylformamide for 1 h at −20°C. Extractions were analyzed within 24 h using an UltiMate 3000 HPLC system (Thermo Fisher Scientific). The relative contributions of each phytoplankton group to the total chl a level were then calculated using the CHEMTAX program (Mackey et al., 1996; Latasa, 2007).

2.4. Biochemical quality indicator: seston fatty acids

For fatty acid analysis, 1.5–3 l of seawater was filtered onto GF/C filters (diameter: 47 mm; pore size: 0.45μm) during the summer and winter cruises. We determined the composition and concentrations of fatty acids using a gas chromatograph (Agilent7890A, equipped with a 30 m×0.32 mm×0.25 μm capillary column). Before analysis, the filters were freeze-dried, and 19:0 fatty acid methyl ester was added as an internal standard. The filters were then placed in a mixture of chloroform and methanol (2:1, V/V) with BHT (butylated hydroxytoluene, 0.005%) added as an antioxidant to extract lipids. The filters were extracted 2–3 times and the resulting extract was washed with 0.88% KCl solution and centrifuged (1500 rpm, 5 min) to isolate the organic phase. The separated organic phases were dried under high-purity nitrogen gas. We added 1 mL of 1 mol/L KOH-methanol solution to the dried samples for saponification (80 °C, 2 h) under nitrogen. After cooling, we added 1 mL of 14% BF3 methanol solution for methylation (80 °C, 1 h) under nitrogen. The fatty acid methyl ester was extracted with n-hexane and diluted to 0.4 mL for analysis. All filters mentioned above were immediately wrapped in aluminum foil and frozen in liquid nitrogen.

2.5. Measurement of response variable: in situ egg production (EPR)

Egg production experiments were carried six locations across the estuary: upper (sites 7, 8, 9), middle (site 13), and lower (sites 16, 18). Mesozooplankton were collected by towing a 505 μm plankton net equipped with a digital flow meter (HYDRO-BIOS, Kiel, Germany). The cod-end contents were diluted to 500 mL with 0.2 μm-filtered seawater.

We used a nested beaker design for the incubation, where the bottom of the upper beaker was replaced with a 200 μm mesh. After gently mixing, we added 50 mL of the sample to the upper beakers. This design separates the copepods and their eggs during the incubation, as copepod might feed on their own eggs and nauplius (Boersma et al., 2014), which would complicate the quantification of egg production. Simultaneously, a second 50 mL aliquot was oven-dried to determine the biomass of the incubated assemblage (mg dry weight), which was then used to calculate in situ mesozooplankton biomass.

Incubation at each site was performed in triplicate. The beakers were kept in the dark at ambient temperature for 24 h. After incubation, we recovered and identified copepods to species. As we used natural assemblages, EPR estimates represent community-level reproductive output standardized by dry weight. Eggs were filtered onto 20 μm polycarbonate membranes, eluted with 0.2 μm-filtered seawater into 15 mL centrifuge tubes and fixed with 5% Lugol's acid. In the laboratory, we counted eggs and nauplii were counted under an inverted microscope (Leica Microsystems, DM IL LED, Wetzlar, Germany). Weight-specific egg production rate (wEPR; egg dw mg^{-1} d^{-1}) was calculated as follows:

$$\mathrm{wEPR} = \frac{\mathrm{Ne}}{\mathrm{dw} \times \mathrm{T}}$$

where Ne is the total number of eggs, dw is the dry weight of mesozooplankton (mg) and T is the incubation duration (d).

2.6. Spatial and nonlinear analyses of environmental effects

To visualize the spatial distribution of copepod fatty acid profiles, we applied inverse distance weighting (IDW) interpolation using ArcGIS Pro (v10.8; Esri, Redlands, CA, USA). PUFA concentrations served as the input Z variable, with geographic coordinates projected prior to interpolation. The resulting continuous surface was used to analyze spatial patterns relative to salinity gradients.

To evaluate the effects of environmental variables on copepod reproductive performance, we employed generalized additive models (GAMs) to capture potential nonlinear relationships. We natural log-transformed in situ EPR prior to modeling to correct for right-skewed distributions. GAMs were fitted using the *mgcv* package in R (R Foundation for Statistical Computing, 2016; Wood, 2017), with restricted maximum likelihood (REML) estimation, cubic regression splines, and a basis dimension of $k = 4$. We assessed predictor contributions via single-term deletion (ΔDeviance/χ^2 test) and permutation importance.

2.7. Structural equation modeling of food quality pathways

To disentangle the direct and indirect pathways linking environmental forcing, food quality, and copepod reproduction, we developed a series of structural equation models (SEMs) using the *lavaan* package (v0.6–17) in R (Rosseel, 2012; Gana and Broc, 2019). We quantified phytoplankton community structure as the relative biomass of micro-, nano-, and pico-phytoplankton, transformed into orthogonal isometric log-ratio (ILR) balances. Specifically, we derived two interpretable balances, *bal_micro_vs_others*, contrasting microphytoplankton against the combined nano + pico fractions, and *bal_nano_vs_pico*, contrasting nano- against pico-phytoplankton.

Fatty acid profiles were summarized into three biochemical classes, saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA), and the first principal component (FA_PC1) derived from the normalized SAFA–MUFA–PUFA matrix was used as an integrative indicator of dietary fatty acid quality. We expressed reproductive output as the log-transformed in situ EPR (*Hatch_ln*, eggs m^{-3} d^{-1}).

Given the strong hydrographic variability of the estuary, salinity was incorporated as an exogenous driver hypothesized to influence both phytoplankton structure and reproductive performance, either directly or via changes in fatty acid quality. We tested four model structures: a fully saturated model (C2), a mediation-only model (FA_PC1 as the sole mediator), and two partially mediated models (H1, H2).

Models were fitted using maximum likelihood estimation with 5000 bootstrap replicates to obtain robust standard errors and bias-corrected accelerated (BCa) confidence intervals. Model adequacy was evaluated using χ^2, RMSEA, SRMR, CFI, TLI, and information criteria (AIC, BIC), with the most parsimonious structure (H2) retained for interpretation.

3. Results

3.1. Spatiotemporal patterns of key variables

We observed pronounced seasonal shifts in PUFA concentrations and salinity across the Pearl River Estuary (PRE) (Fig. 2). During summer (Fig. 2A), intensive freshwater discharge generated a strong salinity gradient, with low-salinity waters (<5) extending southward. Elevated PUFA levels (10–15 μg L^{-1}) were concentrated in these low-salinity zones, suggesting enhanced nutritional quality under high riverine influence. In contrast, winter conditions (Fig. 2B) showed reduced freshwater input and a weakened salinity gradient, with surface salinity exceeding 25 in most offshore areas. Correspondingly, PUFA concentrations declined substantially (0–4 μg L^{-1}), showing a more homogeneous pattern. The spatial coupling between PUFA enrichment and freshwater-driven salinity gradients highlights the dominant control of seasonal hydrodynamics on estuarine trophic quality and the potential cascading effects on higher trophic levels.

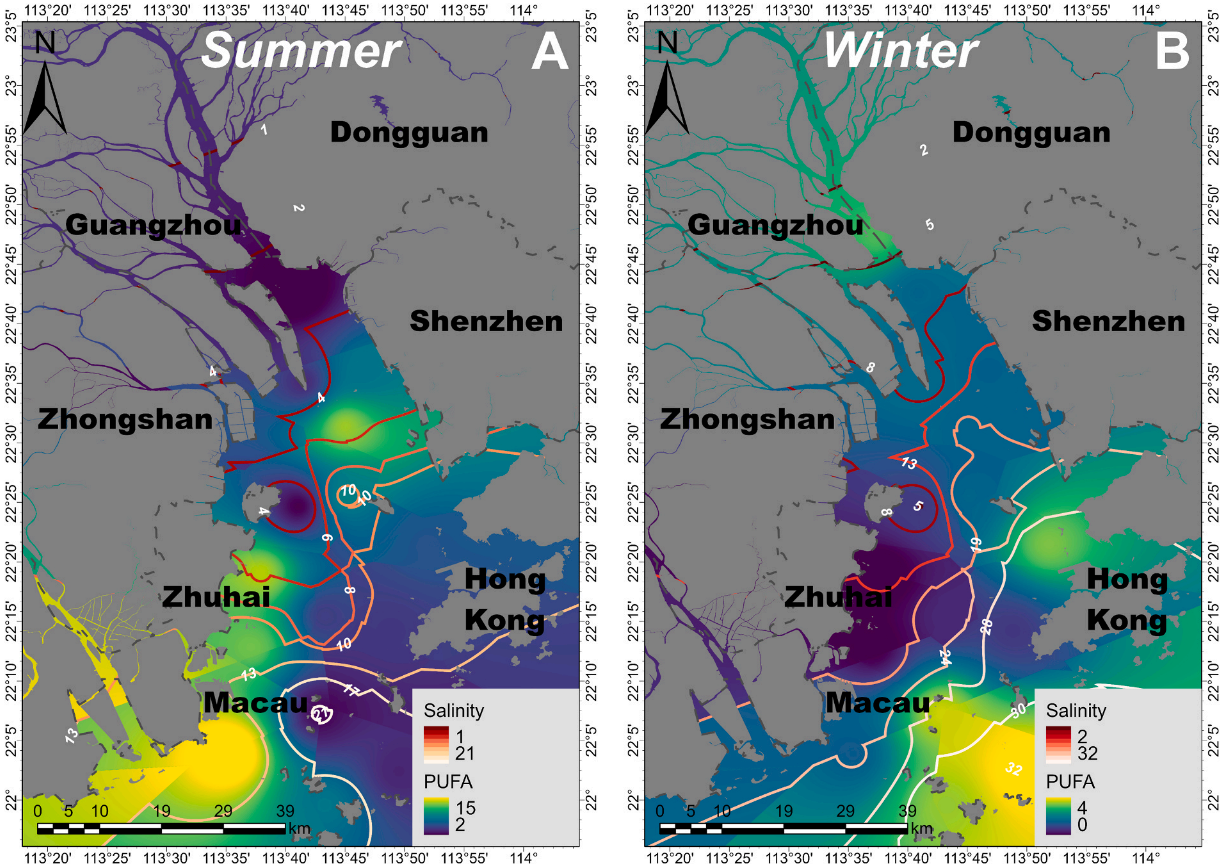


Fig. 2. Spatial distribution of polyunsaturated fatty acids (PUFA; μg L^{-1}, color gradient) and salinity (isohalines) in the Pearl River Estuary during (A) summer and (B) winter. Interpolated PUFA concentrations were derived from inverse distance weighting (IDW), illustrating seasonal contrasts in copepod dietary fatty acid composition in relation to freshwater discharge and estuarine salinity gradients. The PUFA color scale is season-specific to preserve within-season spatial contrast (summer: 2–15 μg L^{-1}; winter: 0–4 μg L^{-1}); therefore, colors should not be compared quantitatively between panels.

3.2. Biochemical and environmental drivers

GAMs revealed that biochemical and environmental variables contributed differentially to copepod reproductive responses (Fig. 3). Among the predictors tested, SAFA, PUFA, and Chl a emerged as the dominant drivers of reproductive output. The strong covariation between PUFA and total fatty acids (Total FA) resulted in a diminished marginal effect of PUFA in the ΔDeviance test, yet its importance remained evident in the permutation analysis. MUFA exerted a moderate influence, whereas other variables, including Total FA, temperature, TIN, salinity, and POC, contributed weak or redundant information. Integrating both ΔDeviance and permutation approaches provided a consistent and robust identification of key predictors, underscoring that copepod reproductive performance in eutrophic estuarine systems is primarily governed by lipid composition and phytoplankton availability rather than by direct physicochemical gradients.

3.3. Nonlinear responses of key predictors

The GAMs further characterized the nonlinear functional responses of copepod reproduction to the major biochemical drivers (Fig. 4). PUFA exhibited a distinct U-shaped relationship, where reproductive probability declined at intermediate concentrations but increased at both low and high levels (Fig. 4A). In contrast, SAFA showed a unimodal response, peaking at moderate concentrations before declining at higher values (Fig. 4B). Chl a showed a positive relationship up to intermediate concentrations, followed by a gradual decrease (Fig. 4C). MUFA, meanwhile, displayed a weak and nearly linear negative effect (Fig. 4D). Collectively, these nonlinear patterns highlight that copepod reproductive success depends on a balanced lipid composition and moderate phytoplankton availability. Either nutrient limitation or excessive lipid saturation may constrain reproductive output, reflecting the physiological trade-offs and metabolic constraints of copepods in eutrophic estuarine environments.

3.4. Mechanistic pathways via fatty acids

SEMs elucidated how salinity, phytoplankton community structure, and dietary fatty acid quality jointly regulate copepod reproduction (Fig. 5). The ecologically constrained SEM (H1) identified a trophic pathway in which phytoplankton size structure modulates dietary fatty-acid quality, which in turn regulates copepod egg production (Fig. 5). The nano vs. pico contrast was a strong positive driver of dietary fatty acids (FA_PC1; $\beta = 0.68, p < 0.001$), whereas the micro vs. others balance had a weaker, non-significant negative association ($\beta = -0.36$). FA quality then exerted a significant positive effect on reproduction ($\beta = 0.63, p < 0.05$). Salinity acted as a distal physical control: it increased both phytoplankton balances ($\beta = 0.38$–$0.46, p < 0.05$), but reduced FA quality (Salinity to FA_PC1: $\beta = -0.47, p < 0.01$). Salinity's influence was primarily indirect, yielding a net negative total effect (≈ -0.25). In parallel, microphytoplankton dominance directly suppressed egg production ($\beta = -0.51, p < 0.05$). Together, these pathways indicate a hierarchical control in which salinity sets the phytoplankton size template, size structure sets dietary FA quality, and FA quality is the proximal regulator of reproductive output.

4. Discussion

This study establishes a hierarchical framework for assessing estuarine copepod reproductive success, prioritizing biochemical

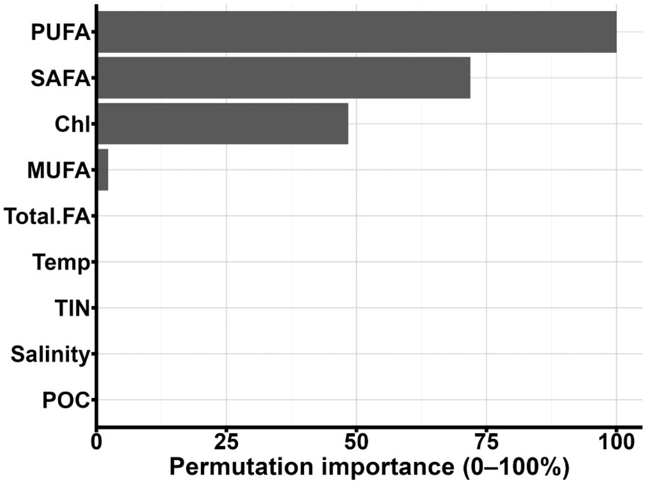


Fig. 3. Relative importance of environmental and biochemical predictors derived from permutation analysis in generalized additive models (GAMs). Saturated fatty acids (SAFA), polyunsaturated fatty acids (PUFA), and chlorophyll *a* emerged as the primary drivers of copepod reproductive responses, whereas monounsaturated fatty acids (MUFA) had secondary influence and total fatty acids (Total FA), temperature, total inorganic nitrogen (TIN), salinity, and particulate organic carbon (POC) showed weaker or redundant effects.

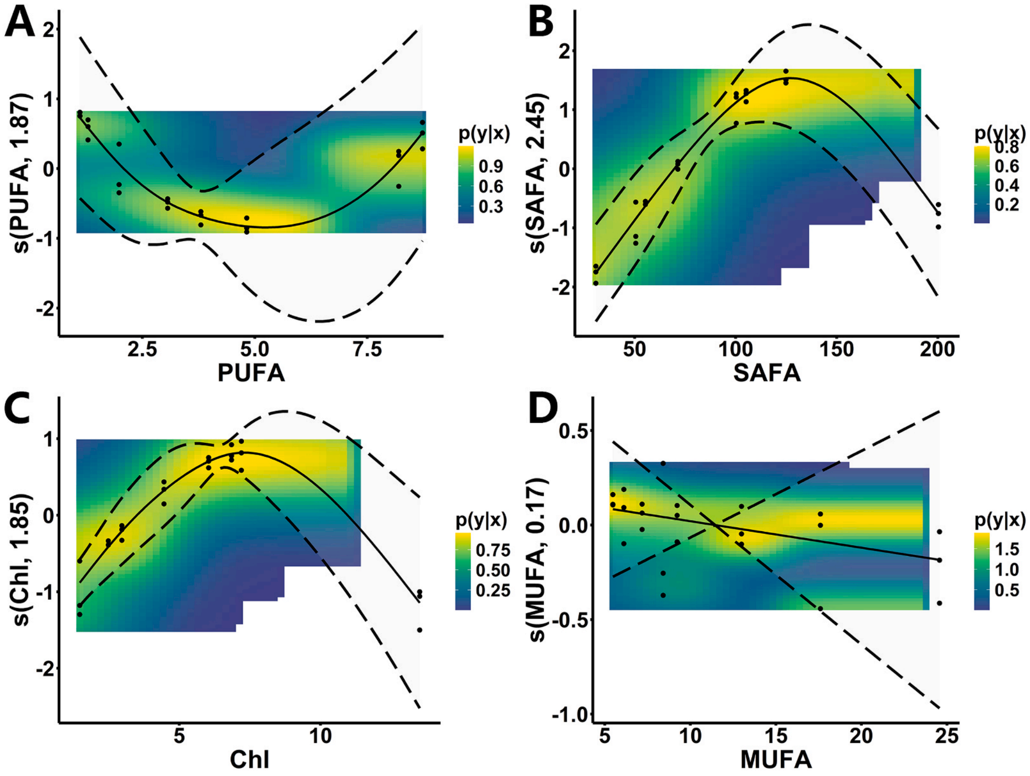


Fig. 4. Partial effects of key predictors on copepod reproductive responses derived from generalized additive models (GAMs). Panels show smoothed relationships with 95% confidence intervals (dashed lines) for (A) polyunsaturated fatty acids (PUFA), (B) saturated fatty acids (SAFA), (C) chlorophyll *a*, and (D) monounsaturated fatty acids (MUFA), with background color scales indicating the conditional probability of reproductive success.

quality (notably PUFAs) over environmental stressors and traditional biomass metrics. Our findings underscore a paradigm shift from abundance-based monitoring to mechanism-driven approaches grounded in nutritional ecology.

4.1. Core physio-ecological mechanisms of PUFAs and limitations of traditional biomass indicators

Our results demonstrate that PUFA bioavailability is the most decisive positive correlate of copepod reproductive performance. This association stems from the multifaceted physiological roles of PUFAs. PUFAs are essential for maintaining cellular structural integrity (Valentine and Valentine, 2004). DHA ensures optimal fluidity of phospholipid bilayers (Tiselius et al., 2012), a critical requirement for rapid cell division during early development. PUFA deficiency has been linked to membrane defects, disrupted cell signaling, and impaired development, leading to arrested embryogenesis, reduced hatching success, and elevated larval deformity (Knuckey et al., 2005; Wang et al., 2022). In bioenergetics and resource allocation, PUFAs serve as high-energy metabolic substrates and essential nutrients. Copepods lack key desaturase enzymes for synthesizing long-chain PUFAs and must acquire them directly from their diet (Anahi et al., 2014). They prioritize the allocation of ingested PUFAs to reproductive tissues as core components of vitellogenin, thereby supplying vital energy and structural modules for offspring development (Matsui et al., 2021; Tiselius et al., 2012). Consequently, parental dietary PUFA profiles determine egg viability and recruitment potential (Frisch et al., 2007). At the ecosystem level, PUFAs act as an integrative nutritional indicator, reflecting both the quality and quantity of the food base (Chen and Liu, 2020). PUFA concentrations and composition in seston mirror phytoplankton community structure (Canavate, 2019). Empirical evidence from the Pearl River Estuary robustly supports this mechanism. GAMs identified PUFA as the strongest predictor of reproductive output, exhibiting a nonlinear U-shaped relationship (Fig. 4A). This pattern suggests physiological trade-offs, where intermediate PUFA levels may coincide with suboptimal phytoplankton assemblages or competing metabolic demands, whereas both low and high concentrations enhance reproduction, potentially by optimizing membrane properties and energy allocation. SEMs further confirmed the direct positive effect of dietary fatty acid quality (largely PUFA-driven) on egg production ($\beta = 0.63$, $p < 0.05$; Fig. 5).

In contrast Chl a exhibited systematic predictive failure. Its fundamental limitation is “functional blindness”: an inability to distinguish nutritional properties among phytoplankton functional groups (Carstensen et al., 2011). In eutrophic estuaries, high Chl a often coincides with diatom blooms which may lack essential long-chain PUFAs (Milione and Zeng, 2007). Moreover, some diatoms produce secondary metabolites like PUAs, which can disrupt copepod cell cycles and severely reduce egg hatching success, creating a “high biomass–low reproductive output” paradox (Ask et al., 2006; Uriarte et al., 2005). Thus, in complex estuarine environments,

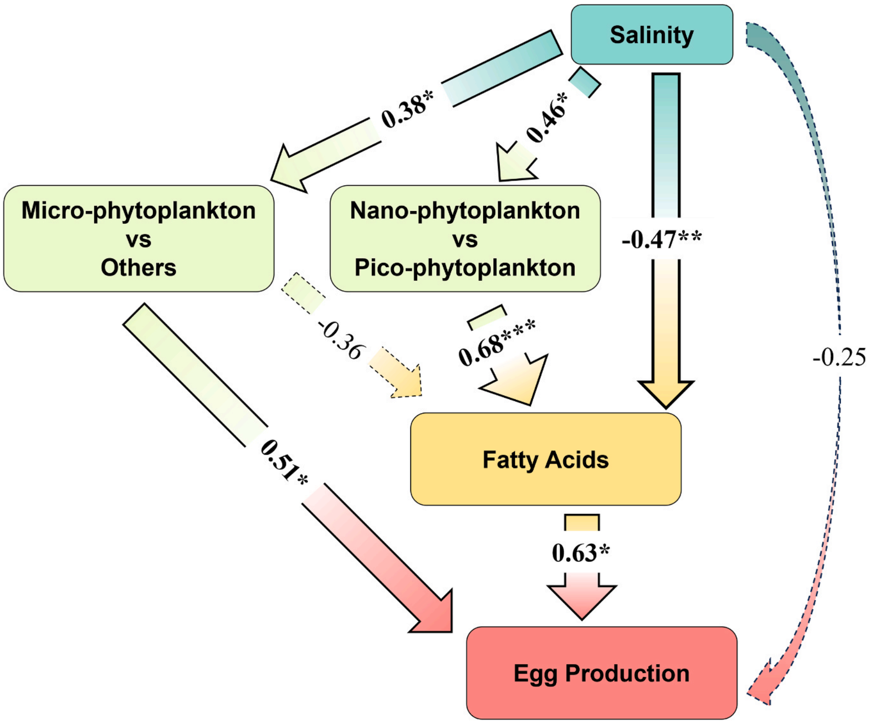


Fig. 5. Structural equation model (H1) depicting direct and indirect pathways linking salinity, phytoplankton size structure (micro vs. others; nano vs. pico), dietary fatty-acid quality (FA_PC1), and copepod egg production in the Pearl River Estuary. Salinity increased both size-structure balances ($\beta = 0.38$ and 0.46; $p < 0.05$) but reduced FA quality ($\beta = -0.47$; $p < 0.01$). The nano vs. pico balance enhanced FA quality ($\beta = 0.68$; $p < 0.001$), whereas micro vs. others showed a weak, non-significant negative link to FA ($\beta = -0.36$). FA quality promoted egg production ($\beta = 0.63$; $p < 0.05$), while microphytoplankton dominance directly suppressed reproduction ($\beta = -0.51$; $p < 0.05$). The total effect of salinity on reproduction was negative but modest (≈ -0.25), reflecting primarily indirect transmission through FA quality. Arrows denote standardized path coefficients; solid lines indicate significant paths ($p < 0.05$), dashed lines indicate non-significant paths. Asterisks indicate significance levels of path coefficients (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

elevated Chl a may signal a nutritionally inadequate or even inhibitory prey environment, explaining the failure of biomass-based indicators to accurately predict reproductive output. The GAM results (Fig. 3) corroborate this, showing the inadequacy of linear biomass metrics in capturing complex ecological dynamics.

4.2. Integrated effects of environmental stress: salinity as a key regulatory factor

Salinity emerged as the second most influential factor, regulating copepod reproductive dynamics. The negative correlation between salinity and reproductive performance arises from physiological and environmental mechanisms. Physiologically, osmoregulation in high-salinity settings imposes significant energetic demands. As osmoconformers, copepods must continuously expend ATP to maintain osmotic balance via ion regulation (Hansen et al., 2012), leading to a trade-off in energy allocation: resources are diverted from reproduction to maintenance, suppressing egg production and hatching rates (Annabi-Trabelsi et al., 2018). Ecologically, salinity acts as an environmental integrator like pollution and hypoxia (Lauer and Bianchini, 2009), and with physical processes like stratification and hypoxia (Choi et al., 2024). Salinity thus embodies a composite stressor, reflecting osmotic pressure, pollution risk, and habitat suitability. Our findings indicate that copepod reproductive success depends on their ability to acquire high-quality food (PUFA) to offset metabolic costs imposed by salinity-mediated stress. Critically, SEM analyses revealed that the effect of salinity is primarily indirect, mediated through trophic interactions rather than direct physiological stress (Fig. 5). Salinity negatively influenced dietary fatty acid quality ($\beta = -0.47$, $p < 0.01$) while positively affecting phytoplankton size structure ($\beta = 0.38$–0.46, $p < 0.05$), ultimately suppressing reproduction via reduced PUFA availability (total indirect effect ≈ -0.25). This mechanistic pathway explains spatiotemporal patterns in the PRE, where summer low-salinity zones correlated with elevated PUFA levels and enhanced reproduction, whereas winter high-salinity conditions coincided with oligotrophy and reproductive decline. Thus, salinity functions as a distal control, shaping the biochemical foundation of the food web rather than acting solely as an osmotic stressor.

4.3. Limitations and prospects

Fatty acid profiles of environmental particulate organic matter (POM) represent community-level averages and may not precisely reflect the composition of prey selectively ingested by copepods. This "trophic upgrading" effect (Bi and Sommer, 2018) could affect causal inferences between diet quality and reproductive performance, particularly given the nonlinear responses observed in GAMs (Fig. 4). For instance, the U-shaped PUFA relationship may be influenced by species-specific feeding preferences or temporal lags. Furthermore, correlative insights from SEMs and GAMs require experimental validation. Future work should incorporate compound-specific profiling, such as DHA:EPA ratios, for greater mechanistic resolution. While mixed-species incubations may introduce bias, our standardized protocol ensures comparability; future studies should employ species-specific incubations.

The "salinity–food quality" framework developed here supports proactive management. We advocate integrating PUFA analysis into estuarine monitoring programs alongside traditional metrics like Chl a. Although this requires greater analytical investment, it provides high-fidelity insight into trophic transfer efficiency and true ecosystem carrying capacity (Tiselius et al., 2012). This framework also serves as a diagnostic tool, enabling rapid attribution of population declines to either environmental stress or nutritional constraints. Future research should prioritize controlled feeding trials to establish causal links, development of cost-effective proxies for PUFA content, and extension of the nutritional framework to higher trophic levels to assess dose-dependent relationships between copepod PUFA and fish recruitment, thereby bridging biochemical quality to fisheries management.

5. Conclusion

This study establishes that copepod reproductive success in estuarine systems follows a hierarchical framework where biochemical quality, specifically PUFA availability, serves as the primary regulator, modulated by environmental stressors like salinity, while traditional biomass indicators show limited utility. Salinity exerts its influence indirectly by altering phytoplankton size structure and dietary fatty acid quality. These findings necessitate a paradigm shift from biomass-based monitoring to mechanism-driven assessments focusing on biochemical quality. Future research should establish causal links through controlled experiments, develop cost-effective PUFA monitoring methods, and extend this nutritional framework to higher trophic levels.

CRediT authorship contribution statement

Zhuo Xu: Formal analysis. **Zhiwei Liu:** Writing – review & editing. **Xian Sun:** Writing – review & editing, Validation, Resources, Project administration. **Dingyu Luo:** Visualization, Software, Formal analysis. **Qi Chen:** Software, Formal analysis. **Mianrun Chen:** Writing – original draft, Project administration, Funding acquisition. **Yueyue Si:** Software, Methodology, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Project of Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai) (SML2021SP308 and 311024004), the National Natural Science Foundation of China (NSFC:42376129), and the Science and Technology Development foundation of South China Sea Bureau, Ministry of Natural Resources (230203), which are led by MC.

Data availability

Data will be made available on request.

References

Anahi, B.A., Lopez-Abbate, M.C., Biancalana, F., Hoffmeyer, M.S., 2014. Influence of Experimental Thermal Shifts and Overcrowding on Fecundity in Wild Females of Acartia tonsa of the Bahía Blanca Estuary. Braz. J. Oceano 62, 201–207. https://doi.org/10.1590/S1679-87592014064706203.

Annabi-Trabelsi, N., Rebai, R.K., Ali, M., Subrahmanyam, M.N.V., Belmonte, G., Ayadi, H., 2018. Egg production and hatching success of ParAcartia grani (Copepoda, Calanoida, Acartiidae) in two hypersaline ponds of a Tunisian Solar Saltern. J. Sea Res. 134, 1–9. https://doi.org/10.1016/j.seares.2017.12.002.

Ask, J., Reinikainen, M., Båmstedt, U., 2006. Variation in hatching success and egg production of Eurytemora affinis (Calanoida, Copepoda) from the Gulf of Bothnia, Baltic Sea, in relation to abundance and clonal differences of diatoms. J. Plankton Res. 28, 683–694. https://doi.org/10.1093/plankt/fbl005.

Bi, R., Sommer, U., 2018. Food Quantity and Quality Interactions at Phytoplankton–Zooplankton Interface: Chemical and Reproductive Responses in a Calanoid Copepod. Front. Mar. Sci. 7, 68–74. https://doi.org/10.3389/fmars.2020.00274.

Canavate, J.P., 2019. Advancing assessment of marine phytoplankton community structure and nutritional value from fatty acid profiles of cultured microalgae. Rev. Aquac. 11 (3), 527–549. https://doi.org/10.1111/raq.12244.

Carstensen, J., Sánchez-Camacho, M., Duarte, C.M., Krause-Jensen, D., Marbà, N., 2011. Connecting the dots: responses of coastal ecosystems to changing nutrient concentrations. Environ. Sci. Technol. 45 (21), 9122–9132. https://doi.org/10.1021/es202351y.

Chen, J., Liu, H., 2020. Nutritional indices for assessing fatty acids: A mini-review. Int. J. Mol. Sci. 21 (16), 5695. https://doi.org/10.3390/ijms21165695.

Chinnery, F.E., Williams, J.A., 2003. The influence of temperature and salinity on Acartia (Copepoda: Calanoida) nauplii survival, 1 Mar. Biol. (1), 1. https://doi.org/10.1007/s00227-004-1354-2.

Choi, S.Y., Lee, E.H., Soh, H.Y., Jang, M.-C., 2021. Effects of Temperature and Salinity on Egg Production, Hatching, and Mortality Rates in Acartia ohtsukai (Copepoda, Calanoida). Front. Mar. Sci. 8, 704479. https://doi.org/10.3389/fmars.2021.704479.

Choi, S.Y., Belmonte, G., Hyun, B., Jang, P.-G., Lee, W.-J., Soh, H.Y., Seo, M.H., Seo, J.-Y., Shin, K., Jang, M.-C., 2024. Effects of hypoxia on copepod egg hatching success: An in situ study. J. Mar. Syst. 245, 103979. https://doi.org/10.1016/j.jmarsys.2024.103979.

Dickman, E.M., Newell, J.M., González, M.J., Vanni, M.J., 2008. Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. Proc. Natl. Acad. Sci. 105 (47), 18408–18412. https://doi.org/10.1073/pnas.0805566105.

Dodds, L., Black, K., Orr, H., Roberts, J., 2009. Lipid biomarkers reveal geographical differences in food supply to the cold-water coral Lophelia pertusa (Scleractinia). Mar. Ecol. Prog. Ser. 397, 113–124. https://doi.org/10.3354/meps08143.

Frisch, D., Green, A.J., Figuerola, J., 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. Aquat. Sci. 69, 568–574. https://doi.org/10.1007/s00027-007-0915-0.

Gana, K., Broc, G., 2019. Structural equation modeling with lavaan. John Wiley & Sons.

Hansen, B.W., Drillet, G., Pedersen, M.F., Sjøgreen, K.P., Vismann, B., 2012. Do Acartia tonsa (Dana) eggs regulate their volume and osmolality as salinity changes? J. Comp. Physiol. B. 182, 613–623. https://doi.org/10.1007/s00360-012-0646-y.

Hansen, B.W., Buttino, I., Cunha, M.E., Drillet, G., 2016. Embryonic cold storage capability from seven strains of Acartia spp. isolated in different geographical areas. Aquaculture 457, 131–139. https://doi.org/10.1016/j.aquaculture.2016.02.024.

Hantzsche, F.M., Boersma, M., 2010. Dietary-induced responses in the phagotrophic flagellate Oxyrrhis marina. Mar. Biol. 157, 1641–1651. https://doi.org/10.1007/s00227-010-1437-1.

Knuckey, R.M., Semmens, G.L., Mayer, R.J., Rimmer, M.A., 2005. Development of an optimal microalgal diet for the culture of the calanoid copepod Acartia sinjiensis: Effect of algal species and feed concentration on copepod development. Aquaculture 249, 339–351. https://doi.org/10.1016/j.aquaculture.2005.02.053.

Lauer, M.M., Bianchini, A., 2009. Chronic copper toxicity in the estuarine copepod *Acartia tonsa*at different salinities. Mar. Ecol. Prog. Ser. 29, 2297–2303. https://doi.org/10.1002/etc.285.

Mabidi, A., Bird, M.S., Perissinotto, R., 2018. Increasing salinity drastically reduces hatching success of crustaceans from depression wetlands of the semi-arid Eastern Cape Karoo region, South Africa. Sci. Rep. 8, 5983. https://doi.org/10.1038/s41598-018-24137-0.

Matsui, H., Sasaki, T., Kobari, T., Waqalevu, V., Kikuchi, K., Ishikawa, M., Kotani, T., 2021. DHA Accumulation in the Polar Lipids of the Euryhaline Copepod Pseudodiaptomus inopinus and Its Transfer to Red Sea Bream Pagrus major Larvae. Front. Mar. Sci. 8, 632876. https://doi.org/10.3389/fmars.2021.632876.

Milione, M., Zeng, C., 2007. The effects of algal diets on population growth and egg hatching success of the tropical calanoid copepod, Acartia sinjiensis. Aquaculture 273, 656–664. https://doi.org/10.1016/j.aquaculture.2007.07.014.

Mungenge, C.P., Wasserman, R.J., Cuthbert, R.N., Dondofema, F., Dalu, T., 2024. Salinisation of arid temporary pools alters crustacean hatching success but not phenology dynamics. Hydrobiologia 851, 181–193. https://doi.org/10.1007/s10750-023-05325-0.

Nguyen, T.T., Le, M., Doan, N.X., Nguyen, S.T., Truong, T.S.H., Vu, M.T.T., Dinh, K.V., 2020. Salinity and temperature effects on productivity of a tropical calanoid copepod *Pseudodiaptomus incisus*. Aquac. Res. 51, 3768–3779. https://doi.org/10.1111/are.14727.

Rosseel, Y., 2012. lavaan: An R package for structural equation modeling. J. Stat. Softw. 48 (1), 1–36. https://doi.org/10.18637/jss.v048.i02.

Sterner, R.W., 2009. Role of zooplankton in aquatic ecosystems.

Tiselius, P., Hansen, B., Calliari, D., 2012. Fatty acid transformation in zooplankton: from seston to benthos. Mar. Ecol. Prog. Ser. 446, 131–144. https://doi.org/10.3354/meps09479.

Uriarte, I., Cotano, U., Villate, F., 2005. Effects of estuarine conditions and organic enrichment on the fecundity and hatching success of Acartia clausi in contrasting systems. Exp. Mar. Biol. Ecol. 320, 105–122. https://doi.org/10.1016/j.jembe.2004.12.021.

Valentine, R.C., Valentine, D.L., 2004. Omega-3 fatty acids in cellular membranes: a unified concept. Prog. Lipid Res. 43 (5), 383–402. https://doi.org/10.1016/j.plipres.2004.05.004.

Wang, S., Wang, L., Wang, Y., Chen, Y., Chen, J., Chen, N., 2022. Embryo Development and Effects of Temperature, Salinity, and Light Intensity on Egg Hatching of Calanoid Copepod Bestiolina amoyensis (Calanoida: Paracalanidae). Front. Mar. Sci. 9, 940303. https://doi.org/10.3389/fmars.2022.940303.

Wood, S.N., 2017. Generalized additive models: an introduction with R. chapman and hall/CRC.

Yi, J., Ma, F., Li, J., Liu, W., Cao, Y., Wan, Y., Wang, C., Chen, M., Yu, D., Xu, H., 2022. China Biodiversity Observation Network for better global sustainability developments. EcoEnviron. & Health 1 (4), 201–203. https://doi.org/10.1016/j.eehl.2022.11.001.