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Variations in phenotypic plasticity in a cosmopolitan copepod species across latitudinal hydrographic gradients

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Studies assessing latitudinal variations in habitat conditions and phenotypic plasticity among populations yield evidence of the mechanisms governing differentiation in the potential to adapt to current/future habitat changes. The cosmopolitan copepod species *Acartia tonsa* thrives across ocean clines delimiting Seasonal (30–40° S) and Permanent (10–30° S) Upwelling coastal provinces established during the middle–late Pliocene (3.6–1.8 Ma) alongshore the South East Pacific (SEP), nowadays exhibiting contrasting variability features related to several ocean drivers (temperature, salinity, pH, and food availability). Latitudinal variation across the range of environmental conditions of the coastal provinces can contribute toward shaping divergent *A. tonsa's* phenotypes, for example, through specific patterns of phenotypic plasticity in morphological and physiological traits and tolerance to environmental drivers. With the aim of contributing to the understanding of these adaptive processes in a relatively little studied oceanic region, here we compared the expression of parental (i.e., adult size, egg production, and ingestion rate) and offspring (i.e., egg size) traits in relation to variation in environmental habitat conditions across different cohorts of two distant (> 15° latitude) *A. tonsa* populations inhabiting estuarine and upwelling habitats located in the Seasonal and Permanent Upwelling province, respectively. Mean conditions and ranges of variability in the habitat conditions and phenotypic plasticity of parental and offspring traits within and among cohorts of *A. tonsa* populations varied significantly across the different examined regions (i.e., Seasonal vs. Permanent). We also found significant differences in the coupling of habitat variability and trait expression, suggesting that the differences in trait expressions might be related to habitat variability. The phenotypic divergence was translated to cohort-related patterns of trait trade-offs regulating reproduction and tolerance of egg production efficiency that can jointly determine the level of plasticity, genetic structure, or local adaptation. The current findings provide novel evidence of how divergent phenotypes might sustain *A. tonsa* populations across variable coastal provinces of the SEP.

KEYWORDS

coastal ocean, environmental variability, latitudinal gradient, phenotypic plasticity, copepod

Introduction

The variability in morphological and physiological traits that underlie the phenotypic plasticity of individuals is a fundamental component toward understanding the dynamics and persistence of the wild populations against the fluctuating nature of the habitat (Pianka, 1976; Stearns, 1992). Trait-related phenotypic plasticity (i.e., the genotype to have a broader tolerance) in the variable habitats supports the maintenance of individual fitness despite the shifts in the mean habitat conditions and inherent variability (e.g., Schlichting and Pigliucci, 1998; Pigliucci, 2001, 2005). Phenotypic plasticity is manifested during different ontogenetic stages (Senner et al., 2015) and across multiple life history traits (Langerhans, 2018). The expression of traits can vary with that of other traits through trade-offs (Zera and Harshman, 2001; Ortega-Mayagoitia et al., 2018), which can reveal adaptive costs of plasticity (Stillman, 2003; Sasaki and Dam, 2021). In adults, phenotypic plasticity is also related to the phenotype and fitness of their offspring, through parental anticipatory effects or adaptive plasticity (Morrongiello et al., 2012 and references therein). This is mediated by genetic components contributing to the local adaptation (Sunday et al., 2011), such that genetic polymorphism may promote rapid phenotypic responses as a response to changes in the environment (Bitter et al., 2019; Brennan et al., 2019; Sasaki and Dam, 2020). Studies of the drivers and extent of intraspecific variations in phenotypic plasticity within single marine species can yield essential information in determining the mechanisms of populations resilience under current as well as the potential adaptation to future habitat changes (Porlier et al., 2012; Barley et al., 2021; Sasaki and Dam, 2021).

Climate and geographic milestones consolidated during the middle–late Pliocene (3.6–1.8 Ma) (Lindberg, 1991; Cardenas et al., 2009 and references therein) alongshore the South East Pacific (SEP) nowadays promote different climatic (Tapia et al., 2014) and ecogeographic coastal provinces (Camus, 2001; Escribano et al., 2003; **Supplementary Figure 1**). Each province exhibits contrasting mean conditions and variability ranges in several ocean drivers (temperature, salinity, pH, and food) (Vargas et al., 2017), likely conducting to spatial patterns of local adaptation in contemporary populations (Gaitán-Espitia et al., 2017; Vargas et al., 2022). The Seasonal Upwelling province extends from 30° S to 40° S, corresponding to > 1,000 km of coast widely interrupted by river discharges. The river influence on the coastal ocean is maximal during the rainy austral fall-winter, while an intense upwelling activity becomes more important on local hydrography during spring-summer seasons (Vargas et al., 2016). The physical–chemical gradients occurring along and across-shore in these habitats due to river and upwelling influence also experience high-frequency shifts associated with tidal cycles, yielding a temporarily predictable and spatially variable temperature, salinity, and pH mosaic over relatively small coastal areas (Aguilera et al., 2013; Vargas et al., 2016; Garcés-Vargas et al., 2020; Osma et al., 2020). More than 15° latitude toward the north, the Permanent Upwelling province (18–30° S) is located off the Atacama Desert, where nearshore habitat conditions vary in a less predictable way over the synoptic-temporal scale (García-Reyes et al., 2014; Aguilera et al., 2020). Year-round upwelling of cold (< 14°C) and salty and low pH (< 7.7) subsurface water (Torres et al., 1999; Aguilera et al., 2020) leads to synoptic changes in surface ocean conditions, though individual upwelling events are highly variable depending on wind stress and involved water masses (Song et al., 2011; Oerder et al., 2015). Additionally, El Niño (EN) Southern Oscillation can dramatically disrupt the upwelling variability pattern given its proximity to the Equatorial Pacific (Ulloa et al., 2001; Escribano et al., 2004; Aguilera et al., 2019). Empirical evidence scattered over these provinces accumulated over three decades has established strong signatures of higher variability and likely lower predictability in the upwelling habitats (Tapia et al., 2009; Aguirre et al., 2021). These *in situ* studies are fundamental to the ongoing debates in ecology and evolution, specifically, the extensive theoretical and emerging empirical research investigating how organisms have evolved to cope with high heterogeneous habitats (Vargas et al., 2017, 2022).

The cosmopolitan copepod species *Acartia tonsa* (Chaalali et al., 2013) is contemporarily distributed alongshore the SEP from 54° S in the sub-Antarctic region (Aguirre et al., 2012), to the tropical upwelling (20° S) systems (Aguilera et al., 2011; Ruz et al., 2015). In fact, *A. tonsa* is common and abundant enough to be considered as a foundation species in estuarine and upwelling habitats embedded in the contrasting coastal provinces of the SEP (Peterson et al., 1988; Hidalgo and Escribano, 2007; Vargas et al., 2007; Escribano et al., 2009;

Aguilera et al., 2013). Latitudinal gradients occurring in the average and ranges of environmental drivers can influence the expression of traits in copepods, leading to divergent patterns of phenotypic plasticity within populations on a long-term scale (Dunson and Travis, 1991; Pörtner et al., 2004; Lercari and Defeo, 2006). Investigations of variation in physiological traits across space and time can yield evidence for latitudinal differences in phenotypic plasticity among *A. tonsa* populations (Sasaki and Dam, 2019), including acclimation (Stillman, 2003) and developmental phenotypic plasticity (Pereira et al., 2017), and ultimately shape populations' tolerance to variation in environmental conditions (Dam, 2013). Phenotypic divergence can be assessed by analyzing variation in the expression of parental and offspring traits within and across cohorts of the *A. tonsa* populations inhabiting latitudinally distant and climate different coastal provinces. Body size (BS), egg production (EPR), egg size (ES), and ingestion rate (IR) are fundamental traits shaping phenotypic plasticity and acclimation responses outlining the fitness under variable conditions in a given habitat (Kingsolver and Huey, 1998). Recent studies investigated the factors that determine variations in the expression of the traits in heterogeneous habitats (Moran, 1992; Reed et al., 2010; Bernhardt et al., 2020; Bitter et al., 2021) and showed that more heterogeneous habitats, i.e., characterized by a greater difference in the variation, might favor alternative phenotypes, expanding the phenotypic plasticity. Phenotypic plasticity is often modeled in wild populations as the slope of the reaction norm (Stearns, 1992), which can reveal differences among populations in the tolerance to environmental changes (Sasaki and Dam, 2019). On the other hand, dominant drivers, such as temperature, might also act toward reducing phenotypic plasticity (Barley et al., 2021), determining the impact of multiple factors on the development of the population-level phenotypic plasticity. In order to understand the mechanisms that govern the variation in copepod phenotypic plasticity across latitudinal gradients, we studied variation in the expression of morphometric and physiological traits within and across three different cohorts of the two *A. tonsa* populations sourced from an estuarine and upwelling habitat located at the Seasonal (39.8° S) and Permanent (23.3° S) Upwelling provinces of the SEP, respectively. Our study characterized specific habitat variability and phenotypic plasticity at the inter-daily scale of a comprehensive suite of parental (BS, EPR, and IR), and offspring (ES) reproduction-related traits, established for each cohort. Our fundamental question was to investigate the linkage between environmental conditions and variation in the expression of traits within and among cohorts potentially shaping divergent patterns of phenotypic plasticity in *A. tonsa* populations. We found significant differences in the phenotypic plasticity of parental and offspring traits among cohorts of and between *A. tonsa* populations across two examined regions (i.e., Seasonal vs. Permanent). Environmental–biological coupling and cohort-related patterns of trait trade-offs regulating reproduction also diverged between populations, potentially determining the tolerance to environmental drivers. Our study provides novel evidence of how divergent phenotypes might sustain *A. tonsa* populations across latitudinal coastal gradients in the SEP.

Materials and methods

Two divergent study areas

The estuarine and upwelling habitat are located in two distant (>3,000 km, >15° latitude distant) and contrasting climatic-ecogeographic provinces alongshore the SEP (Camus, 2001; Escribano et al., 2003; Tapia et al., 2014; **Supplementary Figure 1**). The first habitat is an estuarine system in Southern Chile (Valdivia River Estuary, 39.8° S), strongly seasonal and affected year-round by river runoff discharges (average 484 ± 300 m^3 s^{-1}; Pérez et al., 2015). Nearshore, river discharges are partially mixed by coastal circulation (Pino et al., 1994), and temporarily modulated by tidal cycles. Such interaction promotes as a whole a marked alongshore gradient in the average and range of temperature (15 ± 2°C), salinity (33 ± 1 psu), and pH_T (7.93 ± 0.3) (Aguilera et al., 2013; Garcés-Vargas et al., 2020; Osma et al., 2020). The second habitat is the Antofagasta upwelling center (Antofagasta, 23.3° S), situated off the most arid desert in the world, the Atacama Desert, which lacks seasonality (Tapia et al., 2014) and freshwater discharges (Hartley et al., 2005). Equatorward wind prevails year-round at this latitude (Strub et al., 1998), favoring permanent upwelling events at the coast. Depending on the intensity and extent of wind stress, the vertical advection of deep-cold (< 14°C) and low pH_T (< 7.7) water masses synoptically affects the nearshore surface habitat (Torres et al., 1999; Aguilera et al., 2020), with upwelling events highly different from each other. Mean profiles of temperature and salinity illustrate vertical differences between habitats and vertical variations as well (**Supplementary Figure 2**). Importantly, ocean clines delimiting the aforementioned coastal provinces can act as effective dispersal barriers for populations of benthic species (Cardenas et al., 2009 and references therein).

Cohorts' assessment and temporal habitat characterization

The copepod *A. tonsa* is a neritic species distributed over the upper 30 m of the water column (Paffenhöfer and Stearns, 1988). Within this range, the peak of chlorophyll-*a* (i.e., copepods food index) is often placed within the upper 10 m in nearshore habitats alongshore the SEP (Escribano et al., 1997, 2009;

TABLE 1 Sampling information within each coastal habitat and population.

Habitat	Mean sampling freq. (d)	Column depth (m)	Sampling depth (m)	Cohort	Year	Surveys
Estuarine	4	20	7*	I	2010–2011	12
			7–12**	II	2011	9
				III	2012	9
Upwelling	6	40	10*	I	2015	9
			20–10**	II	2015	9
				III	2015	10

Habitat conditions and trait expression of three different cohorts were assessed for each population, whose reproductive cycles are either seasonally (estuarine) or year-round (upwelling). Oceanographic (*) and plankton (**) sampling.

Osma et al., 2020). Depending on habitat depth, variability in environmental conditions (temperature, salinity, pH, and food) was in general characterized at 7 m in the estuarine and 10 m in the upwelling habitat (**Table 1**). Because of specific depth measurements, we could not consider vertical variability in the conditions, however, the large bathymetric gradient occurs at the surface (1 m) and near the bottom due to the influence of less dense and low pH freshwater lens (Osma et al., 2020) and turbulent and sediment enriched tidal currents (Pino et al., 1994; Vargas et al., 2003), respectively (see **Supplementary Figure 2**). In the upwelling habitat, *A. tonsa* displays restricted vertical migrations constrained to the upper 30 m likely due to a shallow oxygen minimum zone (Escribano et al., 2009). Habitat conditions were measured interdaily (i.e., each 4–6 days) through 30 estuarine and 28 upwelling nearshore surveys (**Table 1**). The occurrence and reproduction of the estuarine *A. tonsa* population are restricted to the austral spring–summer season (Aguilera et al., 2013). Thus, sampling distributed from 2010 to 2012 was carried out during the austral spring–summer seasons (**Table 1**), corresponding to the seasonal occurrence of the individuals, with no copepods found during fall–winter periods (Aguilera et al., 2013). Variations in habitat drivers (and thus related copepod traits) at the estuarine habitat were measured under the impact of the flood tides, since during ebb tides, the water column is shallower and the abundance of *A. tonsa* individuals was lower. Temperature (°C) and salinity (psu) were recorded at the sampling depth (± 1 m) by a CTD (Ocean Seven 305 Plus[1]). Discrete water samples were collected at 7 m depth with a Niskin bottle for determination of food availability and pH_T. Food concentration was determined in triplicate as counts of nanoflagellates and phytoplankton cells in carbon units ($\mu g\ C\ L^{-1}$) with their bio-volumes assessed with an inverted microscope OLYMPUS IX-51. Bio-volumes were converted to carbon units by using carbon:volume conversion factors available in the literature (e.g., Vargas and González, 2004). Within 2 h of water collection, triplicate pH_T measurements were assessed in a closed 25-ml cell thermostated at 25.0°C using a Metrohm 713 pH meter, and a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0219.100) calibrated with Tris buffer at 25°C. By using temperature, salinity, $pH_{25^\circ C}$, and total alkalinity (not shown) data, pH was calculated at total scale (pH_T) through the CO2CYS software v.3.0 (Pierrot et al., 2021).

Reproductive copepods of *A. tonsa* are found throughout the year in the upwelling habitat with several season-independent recruitment episodes with generation times of two weeks to two months (Hidalgo and Escribano, 2007). Discrete samplings in this habitat were carried out throughout 2015 (**Table 1**). Temperature (°C) and salinity (psu) were recorded at the sampling depth (± 1 m) with a SeaBird SBE19 Plus CTD. Discrete water samples were attained at 10 m depth through oceanographic sample collections, for determinations of chlorophyll-*a* concentration (copepod food) and pH_T. For determinations of chlorophyll-*a* (Chl) concentration, seawater was filtered on 200 μm mesh to remove large-sized grazers and debris while maintaining natural food assemblages. Triplicate samples (200 ml) were filtered onto a GF/F filter (nominal pore size = 0.7 μM). Chl was extracted for 24h in 90% acetone v/v and measured in a TD Turner fluorometer (Strickland and Parsons, 1972). Chl concentration ($\mu g\ Chl\ L^{-1}$) was converted to carbon units by using a Chl:C ratio = 120 (Vargas and González, 2004). Within 2 h of collection, seawater pH_T was measured in triplicate as described above in a closed 25-ml cell thermostated (25°C).

Copepod collection

Parental and offspring traits were assessed in three different cohorts of the estuarine *A. tonsa* population over the period of 2010–2012 (**Table 1**), while in the northern-upwelling habitat, these traits were assessed throughout 2015. Based on intraseasonal changes in temperature and body size observed at the upwelling habitat during sampling, three different cohorts were identified within the upwelling population (see "Results" section). Plankton samples were obtained by gently oblique hauling of a 200-μm mesh size WP2 net equipped with a closing

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[1] www.idronaut.it

system and non-filtering 1 L cod-end, from 7 to 12 m (estuarine) and 10–15 m (upwelling) depth strata. Each time, the content of the cod-end was gently transferred to a temperature-controlled container containing aquarium bubblers to keep containers well oxygenated over the duration of transport to the laboratory. Within 2 h of collection, mature and visibly healthy adult copepod females were sorted out under a stereomicroscope and transferred in groups of 10 females to 200 ml beakers. Copepods were incubated in natural seawater collected during sampling with the aim of conducting the experiments to estimate phenotypic plasticity in parental and offspring traits as related to the habitat-specific variations in environmental conditions (**Supplementary Figure 2**). Laboratory temperature was maintained at a controlled temperature (14 ± 1°C at the estuarine and 15 ± 1°C at the upwelling habitat), which corresponded to the temperature conditions experienced by the various cohorts in the natural habitats. Given that we infer that the parental and offspring traits are all based on the adult females makes a more scattered sampling in the estuarine area more acceptable.

Copepod traits

The assessed traits in the adult females were the following: body size (BS), mean egg production rate (EPR), egg size, and ingestion rate (IR). For BS estimations, up to 45 copepod females grouped in three batches were immediately preserved in 90% ethanol for further measurements of body size (cephalothorax length, mm). Body length was converted to body mass with the *A. clausi* length–dry weight regressions (Uye, 1982) and to body carbon (BC) assuming that the C content was 45% of dry weight (Kiørboe and Nielsen, 1994). The body mass was then considered to estimate specific reproduction rates. Mean egg production rates (EPR) were estimated based on three groups of 30–40 copepod females, individually incubated at *in situ* temperature for 17–20 h in 200 ml clean crystallizing dishes filled with sieved (<200 μm) natural seawater. Eggs produced over this period by each group were counted under a stereomicroscope, standardized to daily duration (24 h), with the mean EPR expressed as the egg fem^{-1} d^{-1} (± SD). Eggs (30) produced by each female group were randomly sorted and preserved (90% ethanol), and their size (i.e., diameter, μm) was measured using an Olympus IX-51 inverted microscope within two weeks of preservation to diminish ethanol effects on egg size (Moksness and Fossum, 1992). Assuming spherical-shaped eggs and a conversion factor of 0.14×10^{-6} μg C μm^{-3} ratio (Huntley and Lopez, 1992), the diameter of the egg size was converted to mass (μg C). Specific EPR was calculated as the EPR/BC quotient × 100% ($\text{EPR}_{\%BC}$).

In parallel, ingestion rates (IR) were estimated through the bottle incubation method (Kiørboe et al., 1982) and expressed in μg C fem^{-1} d^{-1}. Additional copepod (4–5) females were pipetted into three 660-ml borosilicate bottles containing ambient water filled with < 200 μm natural food assemblages. These bottles alongshore another three control bottles without animals (i.e., blank bottles) were placed on a plankton wheel rotated r at 1.2 rpm for 14–16 h at the same temperature as the EPR was estimated. Subsamples were preserved at the beginning (T_0) and at the end of the incubation period (T_f) from experimental and blank bottles. Ingestion rate (IR) (μg C fem^{-1} d^{-1}) was calculated following Frost (1972) and modified by Marín et al. (1986). With data of EPR (μg C fem^{-1} d^{-1}) and IR (μg C fem^{-1} d^{-1}), the egg production efficiency (EPE) was calculated as the EPR/IR quotient.

Defining terms

Due to the abundance of data and multiple analyses across various cohorts, we provide a definition of the measurements and this related interpretation in this study. In this study, we refer to estuarine and upwelling *A. tonsa* populations in the absence of genetic evidence supporting the occurrence of various genotypes. Cohorts of the respective population were sampled in subsequent chronological order, which implies that the latter cohorts inherently contain genetic information in response to environmental conditions experienced by earlier cohorts. However, cohorts, especially those from the estuarine habitat but also those from the upwelling location considering habitat environmental variability, were sampled and correspond to discrete subgroups within a highest-level nominal group or population. Habitat drivers and copepod traits were characterized in terms of mean values and their variability, with reaction norms on different cohorts of both *A. tonsa* populations. For each cohort, habitat conditions and traits were assessed only at the adult stages, which referred to different cohorts between measurements. There are two measures of variability within and among cohorts. The first one is a measure of variations, i.e., the variability experienced by a single cohort. The second is similar to that experienced across generations or among different cohorts, ultimately characterizing each population. We also define the term "trade-off" as a significant correlation between two traits (Zera and Harshman, 2001; Ortega-Mayagoitia et al., 2018).

Data analysis

Habitat environmental and copepod data were examined for requirements of parametric tests, such as normal distribution (Lilliefors test, $p < 0.01$) and homogeneity of variance

(Levene's tests). Variations in nominal environmental drivers and copepod traits were assessed regarding two variability factors: habitats/populations (groups) and cohorts (subgroups). To evaluate changes in a nominal variable among subgroups in relation to a single value of the highest-level nominal variable (the groups), we utilized a nested design ANOVA (Error type III) in which cohorts were nested within their respective habitat/population. To evaluate the potential effects of plasticity on trait differences between habitats, we conducted a linear mixed effects model in which environmental drivers and habitat were considered as fixed effects and cohorts as random effects. Plasticity effects on trait variations were indicated by a significant interaction between environmental drivers and habitat. The correlation or coupling between environmental variability with identified drivers and biological variables was explored in both habitats, first through the Principal Component Analysis (PCA). The numerical relationships of specific $EPR_{\%BC}$ and EPE with environmental drivers were explored with Pearson's correlation tests, such as plastic responses expressed as a mean reaction norm that had a significantly non-zero slope, and were compared between populations (Stearns, 1992). Mechanistic relationships between traits and specific environmental features can yield insights into ecological speciation among populations (Chen and Hare, 2008). Ocean Data View and SURFER packages provided graphical outputs, whereas statistical analyses were performed in STATISTICA package10.

Results

Intrahabitat/population variability

The distribution of environmental conditions (temperature, salinity, pH_T, and food concentration) showed normal distribution and homogeneity of variance in both habitats. Habitat drivers varied widely among cohorts of the estuarine (**Figure 1**) and upwelling (**Figure 2**) population, while also exhibiting fluctuations within cohorts (**Supplementary Figure 3**). The mean temperature range at the estuarine habitat was 12°C–17°C (**Figure 2A**), in which each cohort experienced mean thermal fluctuations of 3.5°C without significant changes among cohorts (**Table 2**). A warmer mean temperature range (14°C–17°C) was observed in the upwelling habitat (**Figure 2A**). The significant changes in temperature were observed among cohorts of the upwelling population (**Table 2**), which in general experienced narrower (2.6°C) thermal fluctuations. Salinity varied between 31.5 and 34 psu at the estuarine (**Figure 1B**) and between 34.6 and 35.1 psu at the upwelling habitat (**Figure 2B**). Cohorts of the estuarine population experienced larger salinity fluctuations, however, salinity varied significantly among cohorts of both populations, especially of the upwelling population (**Table 2**). There was a surprising similarity in the ranges of pH_T variations experienced by cohorts of the estuarine (**Figure 1C**) and upwelling (**Figure 2C**) populations. The significant pH_T variations occurred among cohorts of both populations (**Table 2**) though pH_T ranges were wider for estuarine cohorts. Outliers were detected in food concentration data of the upwelling habitat (Grubbs' test, $> 400\ \mu g\ C\ L^{-1}$). After removing outlier values, food varied between 0 and 350 $\mu g\ C\ L^{-1}$ in the estuarine (**Figure 1D**) and upwelling (**Figure 2D**) habitat. Variations in food concentration were significant among cohorts of the upwelling population (**Table 2**).

The distribution of parental and offspring traits was normally distributed, but egg size failed the test for homogeneity of variance. The expression of parental and offspring traits displayed important variations within the estuarine (**Figure 1**) and upwelling (**Figure 2**) populations. With respect to body size, it varied between 0.85 and 0.99 mm within the estuarine (**Figure 1E**) and between 0.80 and 0.99 mm within the upwelling (**Figure 2E**) population. Variations in BS were significant only among cohorts of the upwelling population (**Table 2**), although BS fluctuations within cohorts were similar between populations. Removing the 0 values (likely related to the incubation of non-egg producing C5 stage with a similar size range as the adults) and outliers (> 70 egg $fem^{-1}\ d^{-1}$), the EPR range was 1–57 egg $fem^{-1}\ d^{-1}$ in the estuarine (**Figure 1F**) and 2–25 egg $fem^{-1}\ d^{-1}$ in the upwelling (**Figure 2F**) population. There were significant EPR variations among cohorts of both populations (**Table 2**), and larger fluctuations were observed within and among estuarine cohorts. The egg size (ES) varied between 71.3 and 86.2 μm, and 76.5 and 89.5 μm among the cohorts of the estuarine (**Figure 1G**), and the upwelling (**Figure 2G**) population, respectively. Although changes in the ES within cohorts were similar between populations, significant differences in BS were observed among cohorts of the upwelling population (**Table 2**). IR varied between 2 and 7 $\mu g\ C\ fem^{-1}\ d^{-1}$ among the three cohorts assessed within the estuarine population (**Figure 1H**) and between 3 and 5 $\mu g\ C\ fem^{-1}\ d^{-1}$ among two cohorts of the upwelling population (**Figure 2H**). IR varied significantly only among cohorts of the estuarine population (**Table 2**).

Significant effects of isolated environmental drivers together with interactions between environmental drivers and habitat explained a relatively lower (28%) to moderate (51%) proportion of the variations accounted for in the expression of copepod traits, according to the linear mixed-effects model (**Table 3**). The interactions between salinity, food, and habitat were important for changes observed in body size. With the exception of food, the interactions between habitat and assessed environmental drivers were significant for EPR variation, whereas only the interaction between habitat and salinity was relevant for changes in egg size. Significant correlations (i.e., trade-offs) between morphometric and physiological traits were detected only in

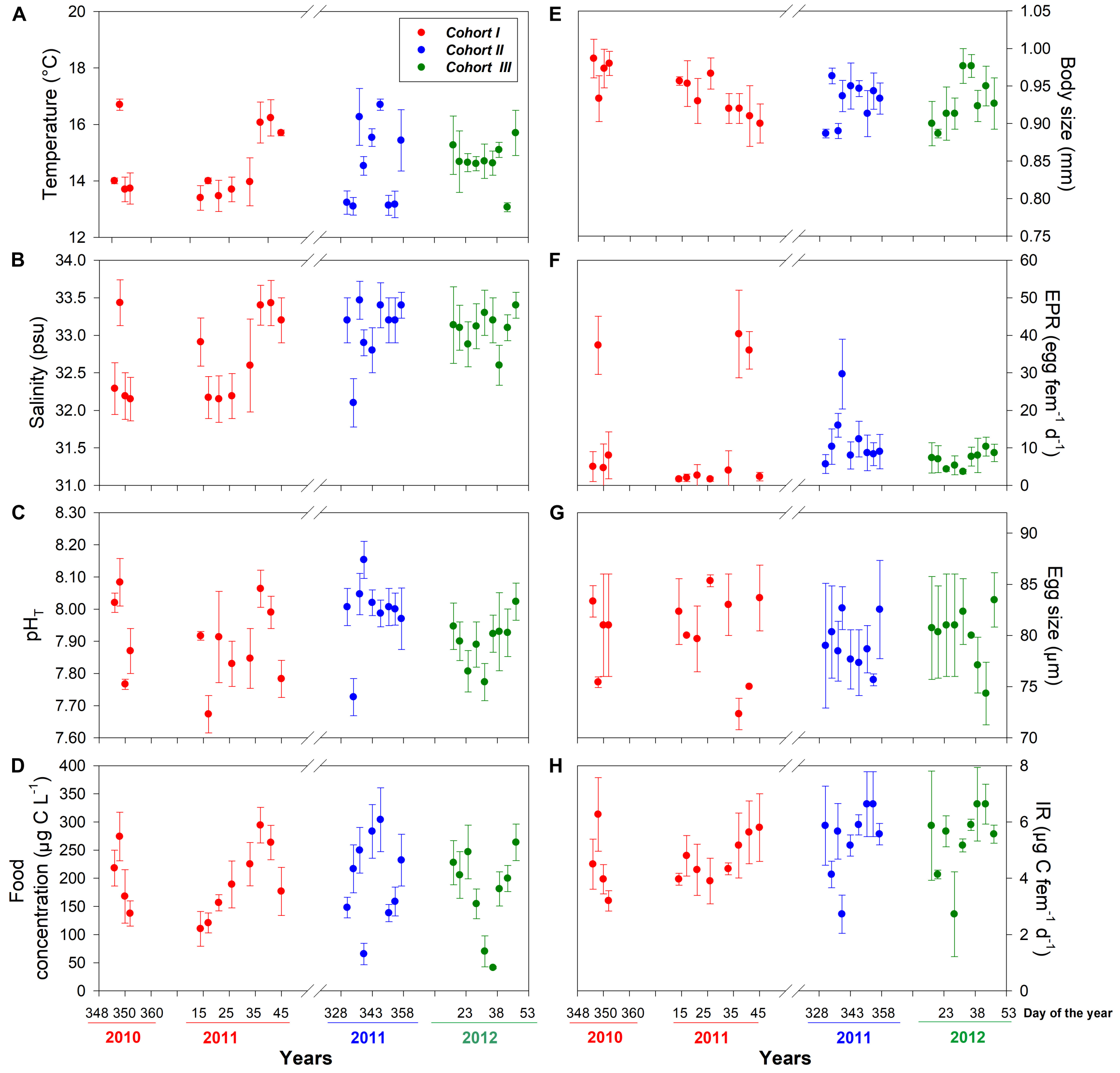

FIGURE 1
Interdaily mean (± SD) values of environmental drivers **(A–D)** and copepod traits **(E–H)** assessed for three different cohorts at the estuarine habitat. Sampling was conducted during the seasonal occurrence and reproductive period of the local *A. tonsa* population.

the estuarine population (Figure 3). Female size was inversely related to EPR (Figure 3A), and EPR was negatively related to eggs (Figure 3B).

Interhabitat/population variability

An average and ranges of environmental conditions and copepod traits were plotted in box plots for comparison between habitats and populations (estuarine and upwelling) (Figure 4). There were significant differences between the two habitats in all assessed environmental conditions except for pH_T (Table 2). The ranges of temperature and salinity were wider, and mean values were significantly higher in the upwelling than estuarine habitat (Figures 4A,B). In contrast to temperature and salinity, pH_T ranges largely overlapped (Figure 4C) with no significant difference in mean pH_T between the habitats (Table 2). Food concentration ranges also overlapped between habitats (Figure 4D), although the mean food concentration in the estuarine (< 150 µg C L^{-1}) was lower than in the upwelling habitat (> 200 µg C L^{-1}) (Table 2). There were significant differences in the expression of parental and offspring traits between the populations, except for EPR (Figure 4). The body size of adult estuarine females was significantly larger than that of their upwelling counterparts (Table 2), though exhibiting a narrower BS range (Figure 4E). Despite mean EPR values not varying significantly between the estuarine and upwelling populations (Table 2), the EPR range was

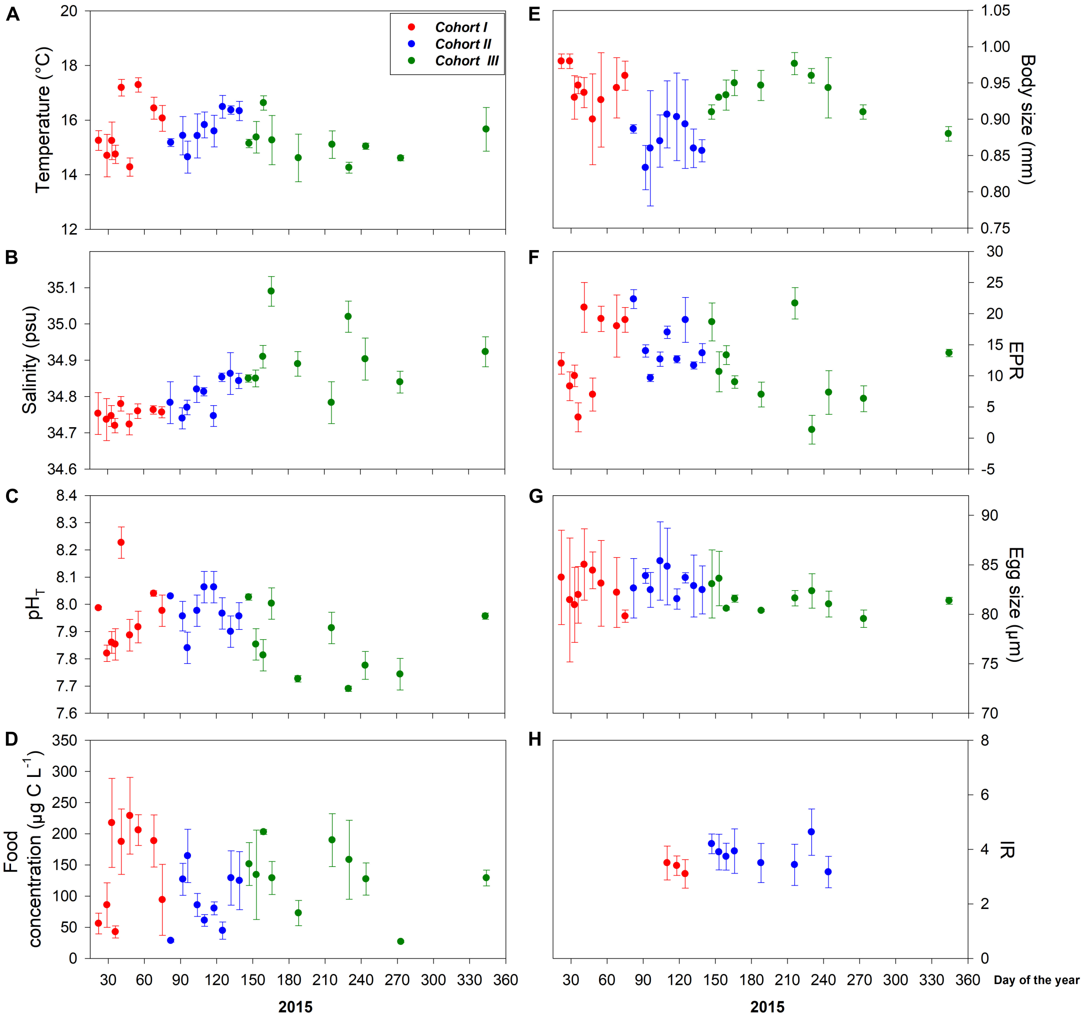

FIGURE 2

Interdaily mean (± SD) values of environmental drivers **(A–D)** and copepod traits **(E–H)** assessed for three different cohorts at the upwelling habitat. Local *A. tonsa* population reproduces year-round under upwelling-dominant environmental conditions, which were strongly disrupted during samplings due to El Niño 2015.

wider within estuarine females (Figure 4F). Among similar ranges of egg size (Figure 4G), the mean value of egg size produced by upwelling females was significantly larger than that produced by estuarine females (Table 2). The IR of estuarine females was significantly higher than that of their upwelling counterparts (Table 2) also exhibiting a wider IR range (Figure 4H).

The association or coupling among nominal environmental drivers and between those with copepod traits was assessed for each habitat with a Principal component analysis (PCA) (Figure 5). Upon assessed abiotic and biotic variables, this analysis accounted for a higher explained variability in the estuarine ($R^2 = 69$) than in the upwelling ($R^2 = 64$) habitat. This analysis also revealed a stronger coupling among environmental conditions and between environmental conditions with copepod traits in the estuarine (Figure 5A) than in the upwelling habitat (Figure 5B). Featured variables were salinity, pH$_T$, EPR, and ES in the estuarine habitat, and salinity, pH$_T$, BS, and EPR in the upwelling habitat (Supplementary Table 1).

Habitat-specific drivers on the reproduction

Reproduction rates (EPR) were standardized by the body mass of females in carbon units (EPR expressed as a percentage of body mass) (Figure 6). The significant changes in EPR$_{\%BC}$ were observed among cohorts of both populations ($F_{4,168} = 14$, $p < 0.0001$) (Figure 6A). Such variability in the EPR$_{\%BC}$ was higher among cohorts of the upwelling population, which produced significantly ($F_{1,168} = 16$, $p < 0.0001$) more carbon

TABLE 2 Comparison of environmental drivers and copepod traits regarding two variability factors: cohorts (3 levels) and habitats (2 levels).

						Among cohorts *Post hoc*		**Between habitats *Post hoc***
Variable	**Factor**	**d.f.**	**MS**	**F**	***p*-value**	**Estuarine**	**Upwelling**	**Habitat**
Temperature	Cohorts	4,168	1.4	1.2	< 0.0001	I = II = III	I = II > III	E < U
	Habitat	1,168	36	32	< 0.0001			
Salinity	Cohorts	4,168	1.5	11	< 0.0001	I<II = III	I < II < III	E < U
	Habitat	1,168	162	1191	< 0.0001			
pH_T	Cohorts	4,168	0.1	8.0	< 0.0001	I = III < II	I = II > III	E = U
	Habitat	1,168	0.01	0.1	0.8			
Food	Cohorts	4,168	> 10000	2.3	0.1	I = II = III	I = III > II	E > U
	Habitat	1,168	> 10000	38	< 0.0001			
Body size	Cohorts	4,168	0.02	14	< 0.0001	I = II = III	I = III > II	E > U
	Habitat	1,168	0.01	8.5	0.004			
EPR	Cohorts	4,168	169	2	0.1	I = II > III	I = II > III	E = U
	Habitat	1,168	231	3	0.1			
Egg size	Cohorts	4,168	160	1.2	0.3	I = II = III	I = II > III	E < U
	Habitat	1,168	290	23	< 0.0001			
IR	Cohorts	3,118	8.2	6.8	0.0003	I = II > III	II = III	E > U
	Habitat	1,118	24.0	20.3	< 0.0001			

Cohorts were nested within their respective habitats. Environmental and biological traits passed tests of normal distribution and homogeneity of variance. *Post hoc* test (Tukey) denotes the comparison among cohorts and between populations.

TABLE 3 Results of the linear mixed effects model.

Trait	**Factor**	**Effect(F/R)**	**SS**	**d.f.**	**MS**	**F**	***P***
Body size	Cohort	Random	0.08	4	0.02	15.7	< 0.0001
	Habitat × Environ Sal	Fixed	0.02	2	0.008	5.7	0.004
	Habitat × Environ Food	Fixed	0.01	2	0.005	4.1	0.02
	Error		0.22	162	0.001		
					R^2 = 0.38		
EPR	Cohort	Random	1012.0	4	253.0	5.2	0.001
	Habitat	Fixed	349.8	1	349.8	7.2	0.01
	Temperature	Fixed	751	1	751	15.1	0.0001
	pH	Fixed	1905	1	1905	39.2	< 0.0001
	Habitat × Environ Temp	Fixed	765.5	2	382.7	7.9	0.0005
	Habitat × Environ Sal	Fixed	394.1	2	197.0	4.0	0.02
	Habitat × Environ pH	Fixed	2193.6	2	1096.8	22.6	< 0.0001
	Error		7777.5	160	48.6		
					R^2 = 0.51		
Egg size	Habitat × Environ Sal	Fixed	89.7	2	44.8	3.9	0.02
	Error		1826.0	160	11.4		
					R^2 = 0.28		
IR	Cohort	Random	20.3	3	6.8	7.2	0.0002
	Food	Fixed	1.7	1	1.7	3.3	0.04
	Habitat × Environ Temp	Fixed	6.6	2	3.3	3.5	0.03
	Habitat × Environ Sal	Fixed	18.8	2	9.4	10.0	0.0001
	Habitat × Environ Food	Fixed	10.2	2	5.1	5.4	0.006
	Error		103.2	110	0.9		
					R^2 = 0.46		

Only significant terms were included after straightforward analyses in which the adjusted correlation coefficient (R^2) did not vary. EPR and IR correspond to egg production and ingestion rate, respectively.

eggs at a similar body size than their estuarine counterparts (Figure 6B). In addition, the egg production efficiency (EPE), corresponding to the EPR/IR ratio was significantly correlated (i.e., reaction norms), with environmental conditions in both habitats (Figure 7). These correlations demonstrate the impact (i.e., mitigating/aggravating) of habitat conditions and potential differences in the tolerance (i.e., slope) to environmental drivers between populations (Table 4). Temperature exerted a

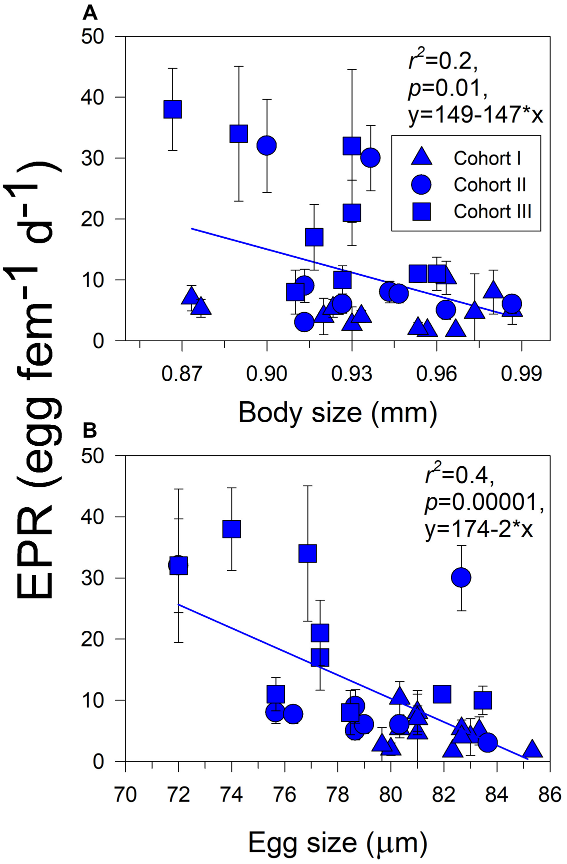

FIGURE 3
Correlations (i.e., trade-offs) between parental **(A)** and offspring **(B)** traits with reproductive trait in the estuarine *A. tonsa* population. Dots represent the average (± SD) value of each trait at a given sampling survey.

mitigating (i.e., positive) effect on the EPE of both populations (**Figure 7A**), though the EPE of the estuarine population was less tolerant to thermal changes (**Table 4**). Salinity showed contrasting effects on EPE, mitigating in the estuarine, and aggravating (i.e., negative) in the upwelling population (**Figure 7B**). The pH_T exerted mitigating effects on the EPE of both populations (**Figure 7C**), and the estuarine population was less tolerant (slope = 0.8) than the upwelling (slope = 0.12) population to pH changes. Food concentration was only related to EPE in the estuarine population, where it exerted a positive effect (**Figure 7D**).

Discussion

This study comprehensively captured patterns of variability in the expression of parental and offspring traits within and among cohorts of *A. tonsa* populations sourced from latitudinal distant habitats located in contrasting climatic provinces alongshore the SEP. Understanding the variation in phenotypic plasticity within the species in the wild conferring the acclimation and adaptation to climate change provides central insights for evolutionary ecology (Porlier et al., 2012; Sasaki and Dam, 2020; Barley et al., 2021). One of the aspects is variability across different habitats as it provides cues on different habitat-specific variability that shapes different phenotypes. The information gathered in this study allowed us to compare the habitats' variability manifested as mean values and environmental temporal heterogeneity, environmental–biological coupling, and its relationship with patterns of phenotypic plasticity in parental and offspring traits. In understanding and interpreting these results, contrasting life strategies related to the timing of occurrence to cope with seasonal (estuarine) or synoptic (upwelling) temporal variability affecting habitat conditions can be considered. Under such selective pressure(s), we focused on parental traits that largely predict tactical variation in offspring fitness among populations concerning the environmental quality or variability (Morrongiello et al., 2012 and references therein). The estuarine habitat located in the seasonal ocean seascape exhibited lower temporal variability in habitat drivers among cohorts of the local

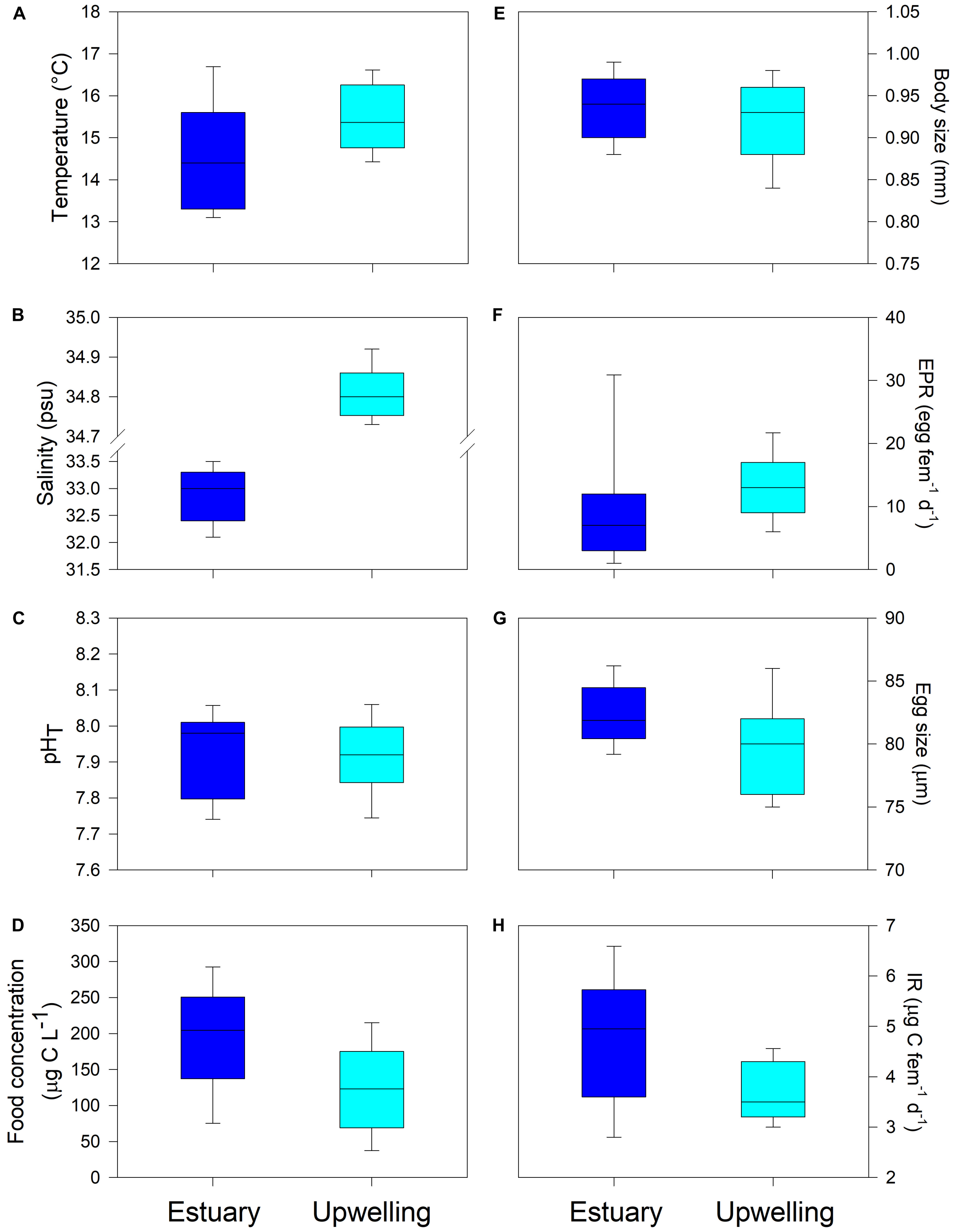

FIGURE 4
Comparison of box plots of environmental drivers **(A–D)** and copepod traits **(E–H)** between examined estuarine and upwelling populations. Statistical results are provided in Table 2.

population. The relationship between habitat variability and related traits was higher in the estuarine habitat, suggesting how the extent of habitat variability impacts phenotypic plasticity. The phenotypic divergence was translated to cohort-related patterns of trait trade-offs regulating reproduction and population-specific tolerance

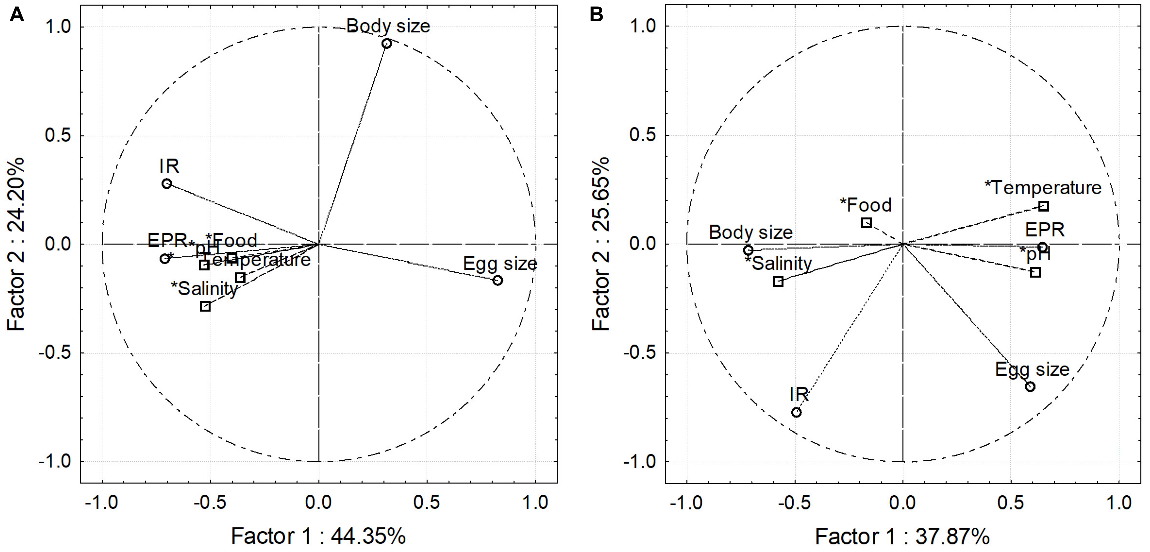

FIGURE 5
Environmental–biological coupling in the estuarine **(A)** and upwelling **(B)** habitat explored through Principal Component Analysis. Besides explaining a higher proportion of the observed variance, PCA in the estuarine habitat also indicates biological cues, especially physiological ones correlated among them.

to environmental changes. Current findings provide novel evidence of how divergent phenotypes might sustain the population of *A. tonsa* across variable coastal provinces of the SEP.

Characterization of cohorts and habitat variability

The assessment of discrete cohorts is well supported in the population inhabiting the seasonal estuarine habitat. Temperate populations exhibit seasonal–biological dynamics (Uye, 1982), and the estuarine population was sampled seasonally across different spring–summer periods from 2010 to 2012. At a mean habitat temperature of 14.6 ± 1.2°C, the local *A. tonsa* population should complete its ontogenetic development within 17–18 days and thus, several recruitment events can occur in the estuarine habitat during the seasonal reproductive cycle over approximately four months between the austral spring (September to October) and summer (December to February) period. In contrast, the upwelling population that reproduces year-round (Hidalgo and Escribano, 2007) was sampled throughout 2015. As with the most wild populations, age distribution among copepod cohorts might be stable or change over time (i.e., true cohort) (Landry, 1978; Durbin and Durbin, 1981). Copepod populations, including *A. tonsa*, grow in a temperature-dependent way in the permanent upwelling habitat (Hidalgo and Escribano, 2007). Furthermore, species of the genus *Acartia* can complete each molt-to-molt phase of the life cycle at a constant time (i.e., isochronal development), which depends on habitat temperature. At a mean temperature of 15.6 ± 0.8°C, the upwelling *A. tonsa* population should complete its life cycle within 17 days (Miller et al., 1977). Given the high likelihood of collecting females belonging to the same cohorts, we assigned Cohort I to those females collected at the beginning of 2015. Although upwelling-driven hydrographic variability prevailed throughout the study period, El Niño 2015 also impacted hydrographic conditions in the upwelling habitat during the fall–winter. In this sense, El Niño 2015 shifted the subsurface temperature range from 14.0°C to 15.3°C, prevailing because of the upwelling dynamics, to warmer (> 16°C) water under El Niño which separated the year into three different hydrographic regimes, each lasting around 2–3 months (Aguilera et al., 2019). By analyzing accumulated (over 4 consecutive sampling days) changes in the body size of reproducing females, we detected significant changes after periods of roughly 100 days matching the above described hydrographic regimes (**Figure 2E**). Although females belonging to subsequent cohorts (true cohorts) might have overlapped during consecutive samplings, the integrated analysis of changes in female body size allowed us to distinguish among different female cohorts, each exhibiting specific plasticity patterns related to a specific thermal experience. Indeed, females sampled during El Niño 2015 were relatively smaller than females sampled before and after El Niño.

Both estuarine and upwelling habitats were characterized by interdaily variability of the suite of environmental conditions, such as temperature, pH, salinity, and food availability (Henson et al., 2017). We acknowledge that our sampling might have underestimated higher frequency variability (i.e., hours-day)

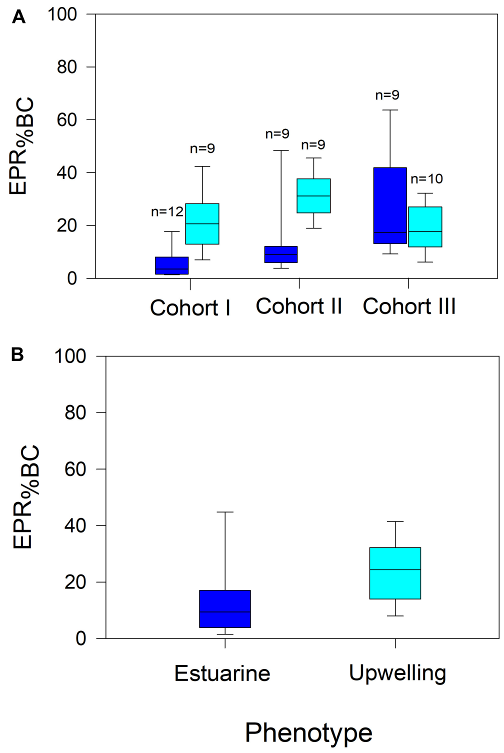

FIGURE 6
Among cohorts **(A)** and between populations **(B)** comparison of specific EPR ($EPR_{\%BC}$). Significant $EPR_{\%BC}$ variations were found among cohorts of both populations, in which upwelling females produced comparatively more eggs per body carbon than estuarine females. n denotes the number of observations for each cohort.

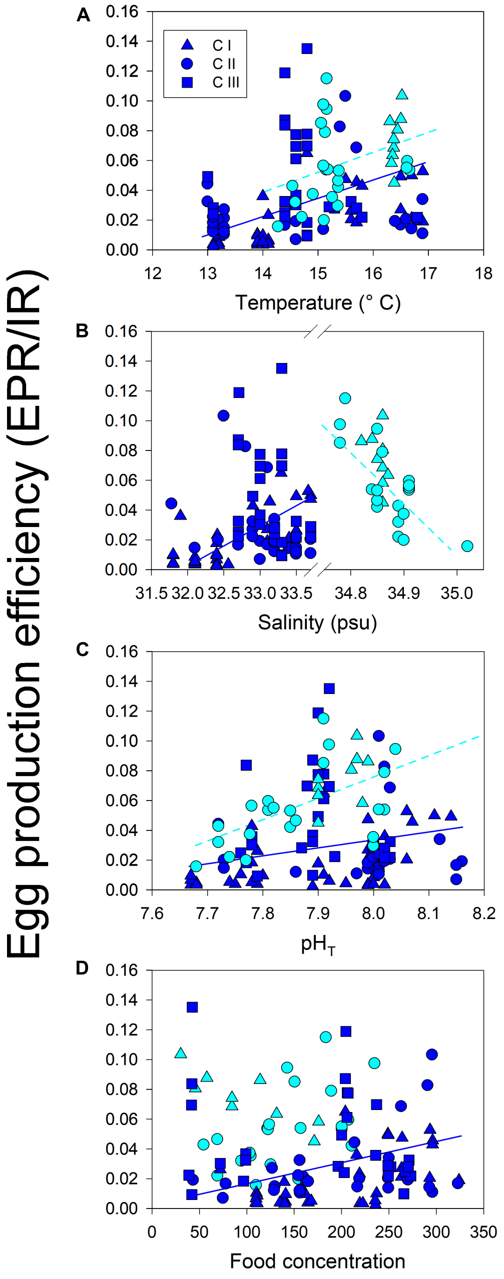

FIGURE 7
Phenotypic plasticity of EPE (EPR/IR quotient) to sampling temperature **(A)**, salinity **(B)**, pH_T **(C)** and food **(D)** in each habitat. Plastic responses were expressed as a mean reaction norm that had a significantly non-zero slope. The slope denotes the EPE tolerance to the respective driver.

affecting physical–chemical conditions (see e.g., Hofmann et al., 2011. Bednarsšek et al., 2022). For short life-cycle copepods (i.e., month-months), this high-frequency variability might be critical in shaping the biological plasticity and tolerance to changing habitat conditions (Gaitán-Espitia et al., 2017; Vargas et al., 2017; Sasaki and Dam, 2020) and needs to be further assessed. In addition, there were clear differences in the temporal characterization of environmental variability between habitats. The estuarine habitat variability was characterized only during spring–summer periods, while the variability at the upwelling location was characterized throughout the annual cycle. This difference in habitat characterization might likely have underestimated the range of both environmental conditions and phenotypic plasticity within the estuarine population. However, the seasonal occurrence and biological productivity of the estuarine population are likely restricted to spring-summer periods, like the other mid-high latitude copepod species (Uye, 1982). In addition, copepod vertical migrations can extend the range of environmental conditions experienced within and among cohorts. Contrary to large-sized species, the vertical distribution of *A. tonsa* in coastal provinces of the SEP is restricted to the upper vertical layers. Within this range, constrained in the estuarine habitat by the surface freshwater lens (Osma et al., 2020), tidal currents (Vargas et al., 2003) in the estuarine habitats, and shallow oxygen-poor water in the upwelling habitat (Escribano et al., 2009); habitat and cohorts' characterization were assessed in this study at a mean

TABLE 4 Significant correlations (i.e., phenotypic plasticity) between EPE and environmental drivers for each population.

Habitat	**Estuarine**			**Upwelling**		
Driver	***r***	***p*-value**	**equation**	***r***	***p*-value**	**equation**
Temperature	0.4	0.001	$y = -1.14 + 0.12 \times x$	0.4	0.03	$y = -1.15 + 0.01 \times x$
Salinity	0.5	0.0001	$y = -11 + 0.3 \times x$	−0.6	0.0003	$y = 6.6 - 0.19 \times x$
pH_T	0.2	0.03	$y = -5.6 + 0.8 \times x$	0.6	0.001	$y = -1.1 + 0.14 \times x$
Food	0.2	0.05	$y = 0.46 + 0.001 \times x$			

The slope of the regressions denotes the sensitivity of EPE to the respective driver. *r* denotes the Pearson correlation coefficients.

depth likely representing the most prevalent environmental conditions affecting cohorts' traits (**Supplementary Figure 2**). In the case of seasonal observations in the estuarine habitat, a similar thermal range (4°C) was reported during the seasonal reproductive occurrence of *A. tonsa* and *A. hudsonica* in Narragansett Bay (41° N) (Sullivan and McManus, 1986), and *Oithona nana* in temperate (39° S) coastal waters of Argentina (Temperoni et al., 2011). The subsurface (10 m depth) thermal range (14°C–17°C) observed in the upwelling habitat was consistent with the reported annual cycle achieved in the same site at a higher (i.e., weekly) frequency (Hidalgo et al., 2005), and a subtropical (20° S) upwelling loci in the northern Benguela upwelling system (Bode et al., 2014). In this sense, laboratory temperature was 1°C above and below the lower and upper edge, respectively, of the thermal range in the estuarine habitat, and 1°C below the upper edge in the upwelling habitat. However, these cases corresponded to only 14% (estuarine) and 6% (upwelling) of incubations, which might have introduced a negligible effect on estimations of copepod traits. All this suggests that the conducted estuarine characterization might have covered environmental variations experienced within generations. This is important when we consider the importance of fluctuating selection on genetic polymorphism of the local population (Sasaki and Dam, 2020).

Intrapopulation variability in traits and plasticity

There were significant variations in the expression of parental and offspring traits within cohorts of the estuarine population (**Supplementary Figure 3**) and among cohorts of the upwelling population (**Table 2**). Significant effects of single drivers on the expression of trait suggest there is an effect of plasticity, while the effect of habitat–environmental driver interactions indicates such plasticity diverged between populations (**Table 3**). In terms of environmental conditions, members of the same cohort experienced greater variations in the estuarine habitat, conditions that tended to prevail across the different cohorts. In turn, environmental conditions seem to be relatively more homogeneous during the development of a single cohort in the upwelling population, which, however, were environmentally decoupled from other cohorts. The divergent pattern in phenotypic plasticity between both *A. tonsa* populations can be related to genetic polymorphism among cohorts (Sasaki and Dam, 2020) and habitat variability (Pigliucci, 2005; Reed et al., 2010). For example, temperature and food abundance modulate phenotypic changes in the body size/weight of copepods (McLaren, 1963; Huntley and Lopez, 1992). Temperature and food abundance varied across the cohorts alongshore the body size of copepods in the upwelling habitat (**Figure 3**). In the upwelling seascape, changes in the body size of copepods have been related to temperature increases either experimentally (Escribano and McLaren, 1999) or in the field due to El Niño (Ulloa et al., 2001; Escribano et al., 2004). However, plasticity in morphometric traits was not related to habitat–temperature but habitat–salinity interaction, whereas physiological traits did relate to habitat-specific temperature conditions. Morphometric traits are settled in the early ontogeny of copepods in a tight relationship with habitat temperature (Miller et al., 1977), which might not necessarily correspond to the sampling temperature. Furthermore, the narrow temperature range associated with either seasonal thermal changes or vertical migrations over a restricted (30 m) depth range shaping the phenotypic plasticity (and genetic diversity) of the upwelling *A. tonsa* population was disrupted during this study by drastic thermal changes associated with El Niño 2015. Along with explaining the lack of correlation between variations in the expression of traits and habitat temperature, El Niño 2015 experienced among cohorts of the upwelling population could have changed the relative allele frequency and favored the selection of different alleles, for example, related to the phenotypic responses (Sasaki and Dam, 2020). Changes in the body size further translate to and influence the expression of reproductive traits (Durbin et al., 1983; Roff, 2002). In fact, EPR and egg size also varied significantly among cohorts in the upwelling population (**Table 2**). As the environment experienced by earlier generations can transgenerationally interact with the future generations, this can predetermine the phenotype, and thus related plasticity against future conditions (Donelson et al., 2018 and references therein); these findings suggest a reduced capacity in adult females for sensing habitat cues early in the season period, to better anticipate or fit their own and offspring traits through anticipatory maternal effects (Marshall et al., 2008). In the case of the estuarine population, the expression of body and egg size remained constant among cohorts while physiological EPR and IR traits varied among cohorts (**Figure 2** and **Table 2**). The expression of these physiological traits is also strongly dependent on the food-driven phenotypic changes through food composition and quality (Kleppel and Burkart, 1995; Jónasdóttir et al., 2009). Although we did not evaluate changes in food quality, there were significant variations in food abundance among cohorts of the estuarine population, responding to the observed temporal and alongshore changes in the estuarine habitat (Aguilera et al., 2013; Osma et al., 2020). The relatively larger phenotypic plasticity observed in the expression of physiological traits might have rendered a reduced tolerance to environmental drivers (see below) in the estuarine population.

Variability in traits and plasticity between populations

The significant differences in the expression of parental and offspring traits were found between examined *A. tonsa* populations (**Figure 4** and **Table 2**), overall, indicating variations in parental phenotypic plasticity (Burgess and Marshall, 2014). Adult females from the estuarine habitat were significantly larger than their upwelling counterparts, although the EPR of each population was similar. However, standardized by body size, the $EPR_{\%BC}$ of upwelling was higher than that of estuarine females (**Figure 6**). This suggests that the upwelling population is allocating a greater proportion of their energy intake to reproduction. Furthermore, since female body size is positively correlated with the number of eggs produced (Durbin et al., 1983; Hopcroft and Roff, 1998), observed differences in body size and $EPR_{\%BC}$ can have strong implications for population growth rates and ecosystem processes (Bassar et al., 2010; Gianuca et al., 2016). The slower growth in the colder estuarine environment, with no increment in fecundity related to the body size and lower gross reproductive output, might represent a handicap for the estuarine population (Stearns, 1989, 1992). This seems to be in part countered by a stronger phenological regulation manifested in higher fecundity in smaller females, as suggested by the trade-off between body size and EPR observed consistently among cohorts of the estuarine population (**Figure 3A**). The EPR varied within upwelling females without any obvious phenological cycles. Females from the upwelling habitat produced larger eggs (**Table 2**), which varied (trade-off) inversely with EPR in estuarine females (**Figure 3B**). Larger eggs in upwelling females cannot be explained phenologically as the upwelling females have a relatively smaller body size. Alternatively, the offspring size could evolve to reflect the optimal compromise between the positive fitness effects of increased size on offspring survival and the reduced/increased number of offspring produced (Smith and Fretwell, 1974; Brockelman, 1975). Increased EPR in smaller estuarine females might be related to elevated IR, which was phenotypically mediated by food variations in the estuarine habitat. Variation in habitat conditions can indeed determine phenotype variations among populations as demonstrated by the linear mixed-effects model (**Table 3**). In terms of fluctuations and correlations among points of a given variable, and between points of different variables in each habitat, the estuarine habitat seemed less variable with lower heterogeneity in the environmental conditions (**Figure 5**). In the estuarine habitat, the environmental drivers strongly covary, which was also reflected in strongly coupled environmental–biological variables (**Figure 5**), suggesting a significant role of habitat variability on phenotypic plasticity. Our current results in spatial patterns of variability in environmental conditions and reproduction-related traits agree that divergent phenotype variations, for example, parental anticipatory effects, take place in response to different environmental pressures alongshore climate-spatial gradients (Marshall et al., 2008; Burgess and Marshall, 2014).

Tolerance to habitat drivers between populations

Significant correlations found between EPE (EPR/IR quotient) and environmental conditions might correspond to the effect of simultaneous drivers on EPE (**Figure 7**). Building upon different and discrete cohorts of both *A. tonsa* populations, mechanistic relationships between EPE and environmental drivers can also reflect ecological specialization and local adaptation, for example, disentangling individual mechanisms that can be assessed later through multifactorial experimental approaches (Boyd et al., 2018). Furthermore, significant non-zero slopes indicate phenotypic plasticity in EPE and variation in the tolerance (sensitivity) to environmental changes. Both populations exhibited roughly the same tolerance to temperature which exerted positive effects on the EPE of both populations (**Table 4**). The mean temperature was higher at the upwelling habitat, whereas wider ranges and drastic thermal fluctuations were observed among cohorts of the estuarine population. Thermal fluctuations can strongly modulate selection on plasticity and thermal tolerance (Stillman, 2003; Sasaki and Dam, 2020), in which the mean temperature experienced by a population corresponds to tolerance, whereas phenotypic plasticity should evolve in response to temperature variability (Stevens, 1989). Salinity exerted population-specific effects on the EPE. The positive effect on the estuarine population might be related to the high natural variability of salinity in estuarine habitats (Kinne, 1966). In fact, elevated EPE levels were evident among cohorts of the local *A. tonsa* population when high salinity values prevailed. In contrast, the salinity range and fluctuations typically prevailing at the upwelling habitat were dramatically disrupted in timing and magnitude by the 2015 El Niño-related hydrographic shifts (Aguilera et al., 2020). Under upwelling influence, salinity in the upper water column varies over a narrow (0.01 psu) range forced by upwelling-driven replacement of water masses with slightly different salinity values (Silva et al., 2009). Larger ($>$ 0.4 psu) salinity shifts were observed in the upwelling habitat due to El Niño (Aguilera et al., 2020) together with other unpredictable hydrographic changes significantly affecting the distribution and physiology of upwelling populations (Escribano et al., 2004; Riascos et al., 2009; Aguilera et al., 2019). Interestingly, both populations showed relatively similar tolerance to salinity and pH_T. In the case of pH_T, this promoted a positive effect on EPE of both populations, such that elevated EPE levels were evident when high pH_T prevailed. The significant changes in pH_T were observed within and among cohorts of both populations. However, phenotypic plasticity in physiological traits was higher in the estuarine population. These findings suggest that higher phenotypic plasticity can render the population less tolerant to changes in the environmental drivers (Stillman, 2003; Sasaki and Dam, 2019), which could decrease its overall adaptive variability (Leung et al., 2020).

Other potential factors structuring phenotypic plasticity

Our study is limited in the way that it only considers the impact of habitat variability in structuring phenotypic plasticity across two divergent habitats. However, we strongly emphasize that habitat-related parameters are only one aspect, while genetic structure, local adaptation, and gene flow include additional parameters that simultaneously impact the overall determination of the level of plasticity. A comprehensive interpretation of all observed parameters provides novel evidence of how divergent phenotypes might sustain the population of *A. tonsa* across variable coastal provinces of the SEP. Preliminary results based on (COI) mtDNA sequence data indicate the occurrence of different clades alongshore the SEP with higher haplotype diversity in the southern population, and the presence of a common haplotype with reduced frequency in the upwelling population (pers. work in progress). Ocean clines delimiting coastal provinces alongshore the SEP tend to act as effective dispersal barriers for populations of benthic species (Cardenas et al., 2009 and references therein). Comparatively, pelagic copepods exhibit higher dispersal potential that erodes ocean clines and manifested ecological speciation (Chen and Hare, 2008), preventing selection and local adaptation across large spatial scales in the presence of ocean connectivity. Despite evident differences in mean values and ranges of environmental conditions between the estuarine and upwelling habitat, patterns of phenotypic plasticity in the tolerance to environmental variations tended to overlap between populations. This might suggest there was or is a south-to-north gene flow which can posit some antagonist influence in the phenotypic plasticity and adaptive genetic differentiation between *A. tonsa* populations. Common garden experiments would help to clarify the role of habitat variability, genetic structure, and adaptations on phenotypic plasticity to fluctuations in other combined habitat drivers.

Conclusion

Our results underline the role of habitat-related variability between the estuarine and upwelling habitat toward shaping the distribution of the expression of trait and subsequent plasticity. The significant differences in patterns of environmental variability and phenotypic plasticity were found between both *A. tonsa* populations, in parental and offspring morphological and physiological traits, traits trade-offs regulating reproduction, and sensitivity to environmental drivers. Such differences in plasticity and tolerance related to temperature, pH and other ocean drivers are concerning if considering the species role in trophodynamics and biogeochemical cycles, and that both upwelling (Sydeman et al., 2014) and El Niño (Cai et al., 2021) intensity are projected to increase in the SEP due to global warming. Despite the absence of genetic evidence supporting the occurrence of various genotypes, our findings strongly indicate divergent *A. tonsa* phenotypes to be currently distributed across specific climate and ecogeographic provinces alongshore the SEP.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: doi: 10.1007/s12237-013-9615-2 and doi: 10.1594/PANGAEA.925450.

Author contributions

VA performed field campaigns and experiments. VA and NB analyzed and interpreted the data and led the writing of the manuscript. Both authors contributed critically to the drafts and gave final approval for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.925648/full#supplementary-material

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