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Combined effects of temperature and hypoxia shape female brooding behaviors and the early ontogeny of the Chilean kelp crab *Taliepus dentatus*

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ABSTRACT: The ecophysiology of marine ectotherms is regulated by the interaction of temperature with environmental drivers, such as dissolved oxygen (DO). The combination of low levels of DO and temperature in the ocean affects physiological and behavioral responses, especially in early life history traits of marine species. Here, we aimed to investigate the combined effect of ecologically relevant values of temperature and DO on female brooding behavior as well as on the early ontogeny of the Chilean kelp crab *Taliepus dentatus*. In a laboratory experiment, after acclimation and mating of females and males in constant temperatures (11 or 14°C), we exposed brooding females to 1 of 2 temperatures (11 or 14°C) and 1 of 2 DO levels (normoxia or cycling hypoxia). We tested the effects of these 4 treatments on embryo and larval sizes, embryo developmental time, female brooding behavior (i.e. embryo ventilation), larval hatching (i.e. number of hatched larvae), Zoea 1 survival to starvation, and swimming speed. We found a negative effect of temperature on the size of early embryos, but no interactions were detected in embryo size during development. High temperature and low DO increased female brooding behavior and larval size, reduced the number of hatched larvae, and affected larval swimming speed. Embryo development time and larval survival were negatively affected by temperature. These results suggest that an increasing frequency of hypoxic events, combined with ocean warming, might have important consequences on marine invertebrate brooders, affecting female fecundity, larval performance and, potentially, their dispersal ability even well within their optimal thermal range.

KEY WORDS: Environmental drivers · Egg size · Larval size · Offspring size–performance relationship · Larval swimming speed · Brachyuran crabs · Life history traits

1. INTRODUCTION

Water temperature and dissolved oxygen are critical factors determining physiological responses, survival and growth of adults, embryos and larvae, and influencing geographical distribution of species (Pörtner & Farrell 2008, Stuart-Smith et al. 2017). Many studies have shown that the synergistic effect of temperature with other environmental drivers included, but not limited to, dissolved oxygen (Cancino et al.

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2003, Bopp et al. 2013, Del Rio et al. 2019), limits the performance and narrows the thermal tolerance of adults, eggs and larvae (Woods & Moran 2008, Verberk & Bilton 2013, Carreja et al. 2016, Leiva et al. 2018). Species inhabiting coastal waters generally experience high temporal and spatial variability in several abiotic factors, including temperature and dissolved oxygen (Mann & Lazier 1996, Brierley & Kingsford 2009), and are well adapted to these heterogenous and multivariate environmental landscapes (Kroeker et al. 2016). Extreme events, however, are predicted to be more intense, more frequent, and longer lasting as a consequence of current global change, and this might exceed species-specific temperature and oxygen thresholds on a regular basis (Vaquer-Sunyer & Duarte 2008). The increasing variability in water temperatures interacting with ocean stratification, ventilation, and subsequent deoxygenation, even in shallow coastal waters (Keeling et al. 2010, Shepherd et al. 2017), is becoming a fertile area of research (Breitburg et al. 2018, Limburg et al. 2020). In most of the large upwelling ecosystems, such as the Humboldt Current System, sea surface temperature has been, on average, cooling off over the past 2 decades as a result of increased intensity and poleward expansion of upwelling winds (Schneider et al. 2017, Aguirre et al. 2018). These changes in upwelling could bring up changes in coastal upwelling circulation (Aiken et al. 2011) and increase the occurrence of coastal hypoxic events (Breitburg et al. 2018), further challenging coastal marine species. The episodical penetration of hypoxic and anoxic conditions into shallow coastal waters of eastern boundary upwelling ecosystems, that cause hypoxic and anoxic nearshore events (Grantham et al. 2004, Weidberg et al. 2020), can increase as a result of global change (Schneider et al. 2017). Thus, understanding the response of species to a changing environment needs deeper knowledge of the combined effects of multiple environmental factors on individuals' physiological and reproductive performance (Lange & Marshall 2017) and the consequent capacity to buffer, acclimate and adapt (Angilletta 2009, Somero 2010, Bozinovic & Pörtner 2015).

Early embryos and reproductive adults are among the most sensitive life stages in the ocean (Pörtner & Farrell 2008). In the marine environment, the capacity to brood embryos is typically constrained by oxygen availability and temperature, especially in species with aggregated egg masses (Fernández et al. 2006, Leiva et al. 2018). Crabs, for example, show active parental care in the form of egg mass ventilation to provide a uniform oxygenation of the entire mass of embryos enhancing reproductive success (Fernández & Brante 2003). This behavior is positively related to temperature (Wheatly 1981, Brante et al. 2003) and the oxygen concentration in water (Braga Goncalves et al. 2015, Olsson et al. 2016). There is evidence that temperature and hypoxia in egg masses affect offspring size (Woods & Moran 2008), embryo development (Vasquez et al. 2015), synchronic development (Fernández et al. 2002), embryo metabolic activity (Rudin-Bitterli et al. 2016), as well as survival and sibling cannibalism (Brante et al. 2013). Moreover, embryo mortality, hatching success, and larval deformity have been related to temperature and oxygen changes in several species of fish (Strand et al. 2004, Geist et al. 2006). Low oxygen conditions can trigger premature hatching, especially when oxygen is limited during the late stages of embryonic development (Latham & Just 1989). Since brooding and embryo performance can have great consequences on larval and adult traits associated with fitness, understanding the consequences of interacting abiotic stressors on the reproductive strategy of marine brooders is crucial (Fernández et al. 2020).

Here, using laboratory experiments, we addressed the complex interaction between oxygen and temperature on female brooding behavior during embryo development and on embryo and larval traits associated with fitness (embryo size, embryo developmental time, larval size, larval hatching, larval survival and larval performance) of the kelp crab *Taliepus dentatus*. This species was chosen as model species because it exhibits an extended latitudinal distribution (from central Peru to Southern Chile; Fagetti & Campodonico 1971), experiencing widely different temperature regimes (Baldanzi et al. 2018) and exposure to hypoxic conditions (Levin et al. 2009). There is good information indicating that exposure to different temperatures across the latitudinal gradient positively affects maternal investment during the warmer summer season (Baldanzi et al. 2018), and experimental studies have characterized the thermal tolerance of all larval stages, i.e. Zoea I and II and Megalopa (Storch et al. 2009, 2011).

Specifically, we hypothesized that (1) *T. dentatus* females invest in bigger embryos as temperature decreases, measured as early-stage embryo size; (2) there is a negative relationship with temperature and developmental time, particularly under hypoxic conditions; (3) ventilation of the embryo mass increases under high temperature and hypoxic conditions, negatively affecting the number of larvae and positively affecting larval size; and (4) larval performance is positively affected by temperature and negatively

affected by the hypoxia conditions experienced during embryo development.

2. MATERIALS AND METHODS

2.1. Model species

Females of *Taliepus dentatus* brood embryos year-round with no clear seasonality in reproduction, showing highly variable clutch sizes, ranging from 10 000 to nearly 100 000 eggs per female (Fagetti & Campodonico 1971, Baldanzi et al. 2018). The length of the developmental period is temperature dependent, and last nearly 4 wk at temperatures of 14–15°C (Fagetti & Campodonico 1971; the present study). *T. dentatus*, like any majoid brachyuran crabs, shows an abbreviated larval development, with only 2 Zoea and 1 Megalopa stages (Fagetti & Campodonico 1971), and a developmental time of about 20 d at 15°C (S. Baldanzi unpubl. data). During the pelagic stage, larvae of *T. dentatus* may be exposed to different conditions than those experienced by the mothers during the adult phase.

2.2. Study site and field data collection

The study was conducted in Las Cruces (33.5° S, 71.6° W), central Chile, located in between 2 well-documented sites (Punta Toro and Curaumilla) where topographic features intensify the upwelling of cold water near the shore (Wieters et al. 2003, Narváez et al. 2004). The wave-exposed coast at the study site is influenced by upwelling of coastal waters produced by the seasonal intensification of equatorward winds which bring cold, nutrient-rich and low-oxygen waters to the subsurface (Thiel et al. 2007). Thus, the episodic penetration of hypoxic and anoxic conditions into shallow coastal waters of eastern boundary upwelling ecosystems, that cause hypoxic and anoxic nearshore events (Weidberg et al. 2020), can increase as a result of global warming (Levin et al. 2009).

To characterize the hydrographic conditions to which female and larvae are exposed, temperature and dissolved oxygen data were retrieved from a data logger deployed at our sampling site in 2015 at 20 m depth (HOBO U26 by Onset, www.onsetcomp.com) programmed to record both variables every 10 min, on a hard bottom substrate surrounded by the macroalga *Lessonia trabeculata.* We analyzed winter and summer mean temperatures and oxygen values for the time period of 1 yr (October 2015 to October 2016). The average temperature calculated for austral summer (21 December 2015 to 20 March 2016) and austral winter (20 June to 22 September 2016) was 13.85 and 11.97°C, respectively. Thus, 11 and 14°C were chosen as experimental low and high temperatures. These temperatures also represent the annual averaged sea surface temperatures normally found in southern and central Chile, respectively (Tapia et al. 2014). Oxygen concentration at the sampling site showed well-oxygenated waters at this depth during the winter months, but rapid fluctuations during the summer–autumn transition were observed, when oxygen concentrations dropped below 2.8 mg l^{-1}, which is considered to be biologically significant hypoxic conditions (Vaquer-Sunyer & Duarte 2008, IPCC 2019). These events lasted from a few minutes to several hours, with the highest duration being 4 h d^{-1} during summer months, when upwelling events are more frequent (Tapia et al. 2014). Therefore, we decided to apply 2 dissolved oxygen treatments, referred to as normoxia ('N', 8.99 mg l^{-1} of O_2) and cycling hypoxia ('H', 2.8 mg ml^{-1} of O_2) (field data are available in Table S1 in the Supplement at www.int-res.com/articles/suppl/m646p093_supp.xlsx). The cycling hypoxic treatment was set as 20:4 h of N:H (Fig. 1). We considered the concentration of 2.8 mg l^{-1} of dissolved oxygen to be representative of hypoxic conditions, as it corresponds to the upper limit of the oxygen minimum zone (Vaquer-Sunyer & Duarte 2008).

Hypoxic experimental conditions were set to 2.8 mg l^{-1} (32 % of O_2 concentration), accounting for different oxygen partial pressure (pO_2) at 11 and 14°C (Boutilier et al. 1984). The oxygen concentration of 2.8 mg l^{-1} corresponds to a pO_2 of ~6.7 kPa at 11°C and ~7.1 kPa at 14°C at a mean salinity of 34 PSU and an atmospheric pressure of 1013 mbar.

2.3. Animal collection, acclimation and experimental setup

A total of 20 ovigerous females carrying late-stage embryos and 20 adult males of *T. dentatus* were collected by SCUBA divers at Las Cruces during March–April 2017. Crabs were randomly assigned to acclimation temperatures, resulting in a mean ± SD size (carapace width) of females in the 11°C treatment of 36.43 ± 1.99 mm (n = 10) and 37.36 ± 2.10 mm (n = 10) at 14°C. During a simultaneous field survey conducted at Las Cruces (see methods and detailed results in Baldanzi et al. 2018), we found no relationship between *T. dentatus* female size and embryo

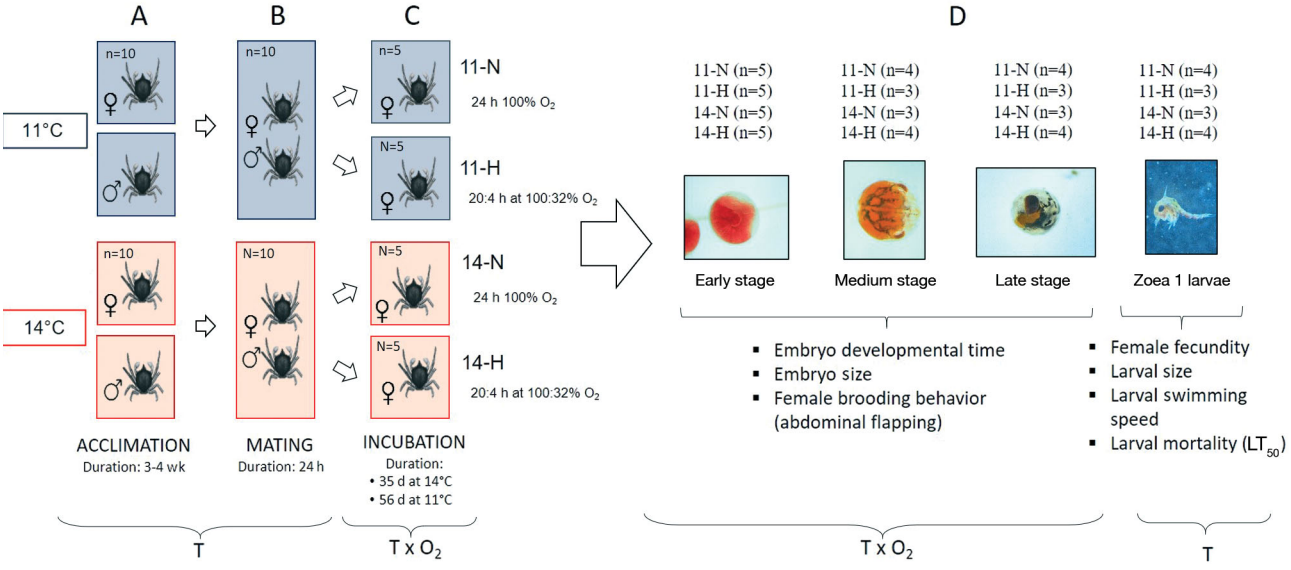

Fig. 1. Schematic drawing of the experimental setup. (A–C) The 2 temperature treatments (11 and 14°C) at which *Taliepus dentatus* females and males were allowed to (A) acclimate, (B) mate and (C) incubate embryos, and the oxygen conditions (as the ratio normoxia:cycling hypoxia) to which females were exposed during embryo incubation. The different treatments applied during the experiments are shown (T: temperature; T × O_2: temperature by O_2 interaction). The 4 treatment combinations 11-N, 11-H, 14-N, and 14-H indicate the temperature (11 or 14°C) and the oxygen conditions (N: normoxia; H: cycling hypoxia). (D) Embryo development during incubation until larval release. For each stage, the measurements taken are listed (LT_{50} is the time at which 50% of the larvae in the experiment died; see Section 2). n is the number of individual females used in the experiment (males were used in 1:1 ratio). During embryo development, there was a reduction in the number of ovigerous females (see Section 2)

volume. Thus, there is no potential confounding effect between female size and embryo volume. The average size of the males was 42.36 ± 1.56 mm (n = 10) and 43.34 ± 1.78 mm (n = 10) in the 11 and 14°C experimental treatments, respectively.

After collection, animals were immediately transported to the laboratory. Ten females were randomly assigned to a maintenance tank of approximately 100 l in volume and kept at a temperature of 11 ± 1.2°C, and another group of 10 females were maintained in a similar tank at 14 ± 0.9°C. During acclimation and subsequent experiments (see next paragraph), a constant photoperiod of 12 h light:12 h dark was set using artificial light. Groups of 10 males were also randomly assigned to 35 l acclimation tanks at either 11 or 14°C. Crabs (females and males) were acclimated until females released larvae, cleaned their abdomen and were ready for a new reproductive cycle (total acclimation time: 3 to 4 wk). Temperature-controlled tanks were semi-closed, flow-through systems with a constant stream of oxygen, allowing seawater circulation within the aquaria at oxygen saturation and experimental temperatures. Water in the tanks was completely renewed every 2 d to prevent accumulation of algal debris and animal waste. All crabs were fed ad libitum with fronds of the kelp *Lessonia* spp. and small opened mussels *Perumytilus purpuratus*.

Once females had released larvae in the acclimation tanks, they were observed for the next 24 h to ensure that all larvae had been released. Then, 'ready to mate' females (i.e. females with a completely emptied abdomen) were individually placed in the mating aquaria, each of which contained 1 male randomly selected from the acclimation tank and assigned to each mating aquarium (1:1 female to male ratio). We repeatedly observed pre-copulatory behavior typical of majiid crabs (Moyano & Gavio 2012), but did not have confirmed information about mating. However, we removed the males after observing the presence of a new clutch in the female abdomen, usually after 24–48 h of having the male and the female in the tank. Females of majoid crabs can accumulate sperm in their spermatheca (Diesel 1989), and the presence of males was needed solely to stimulate females to release new fertilized eggs (as observed in several majoid crabs, Moyano & Gavio 2012) under acclimation conditions. From each temperature treatment (11 and 14°C), 5 females carrying recently deposited eggs were randomly and individually assigned to either normoxic or cycling hypoxic treatment, thus generating all 4 combinations of temperature and oxygen conditions (11-N, 11-H, 14-N, and 14-H; see Fig. 1). Before placing females in experimental aquaria for

the hypoxic conditions, the first embryo samples were collected (named early-stages embryos). A nitrogen stream-flux connected to an automated control unit (Iks Aquastar; iks ComputerSystems) was used to regulate the oxygen concentration to constant levels within experimental tanks after accounting for temperature and atmospheric pressure. The flow-through system allowed seawater to circulate within the aquaria, maintaining constant temperature and reducing the effect of N_2 on the pCO_2 levels in the water during the hypoxic exposure. Preliminary measurements of pH in the aquaria during 4 h of N_2 bubbling showed steady pH values of 7.8 ± 0.02. The daily cycle of hypoxia was implemented starting the day after the early embryos were collected up until larval hatching, when the incubation experiments were also terminated. A schematic of the semi-closed, flow-through system is shown in Fig. A1 in the Appendix.

2.4. Embryo staging and offspring size

Embryo development was monitored every 48 h in order to identify the 3 different embryo stages (see our Fig. 1D), following Vargas (1995): early stage (homogeneous yolk mass), medium (eye spot appearance) and late stage (complex eyes and active heartbeat). To measure embryo volume at each stage, 3 samples of a minimum of 20 embryos each were carefully collected from each female clutch using forceps. Embryos were placed in Petri dishes filled with seawater and immediately photographed using a stereoscope (Leica M205 C) equipped with a digital camera (Leica MC170 HD, 5 megapixels). Photographs were taken at a constant magnification of 0.78× using the automatic exposure provided by the software (Leica Application Suite v. 4.7.1). Embryo volume was estimated based on diameter measured in photographs and assuming the shape of a sphere (see Baldanzi et al. 2018). Mean embryo volume was then calculated from the 3 subsamples and expressed as mm^3 for each female.

To measure larval (Zoea 1) size, 3 subsamples of a minimum of 20 viable larvae per female per treatment were randomly chosen, placed in Petri dishes and individually photographed under a stereoscope. Images were taken at a constant magnification of 3.3×, and larval carapace length (from the bottom of the tail to the bottom of the rostrum) was then measured. Zoea 1 volume was also calculated using the formula of a sphere (Bueno & López-Urrutia 2012), considering carapace length as the diameter. Images were analyzed using the software ImageJ (US National Institutes of Health, http://rsb.info.nih.gov/ij/).

2.5. Embryo developmental time, female brooding behavior and number of hatched larvae

Embryo developmental time was calculated as the total time (in days) between the first embryo appearance in a mother's abdomen and the time of larval hatching (when mothers have completely emptied their abdomen). Once embryos had reached the late stage, female brooding ventilation behavior was quantified following modified procedures from Baeza & Fernández (2002) and Brante et al. (2003). Preliminary tests were performed to confirm that females perform little embryo ventilation behavior during early embryo stages, as reported in other species, including the family Epialtidae (Fernández et al. 2000, Brante et al. 2003). We videotaped 3 females per treatment for 4 h and recorded almost no ventilation during early development. Females carrying late-stage embryos were recorded for 4 consecutive hours at the same time of the day (between 18:00 and 22:00 h) using a webcam (Logitech camera, HD720) placed in front of the aquarium (maximum distance: 15 cm). During the recordings, females were not offered food, as feeding could alter brooding behaviors (Fernández et al. 2020). Females assigned to the cycling hypoxic treatment were recorded during the 4 h of hypoxic exposure. From the 4 h recordings, a 1 h time block was randomly selected and visually analyzed. Following Brante et al. (2003), we counted the total number of abdominal flapping and expressed as number of flaps per hour to quantify this behavior.

The total number of larvae released by each female (number of hatched larvae) was calculated following the same procedure used to estimate clutch size described by Brante et al. (2003) and Baldanzi et al. (2018). From the larval mass of each female (excluding 20 used for larval size, 100 for survival and 5 for swimming behavior, see Section 2.6), we collected 3 subsamples of about 0.05 g, counted all larvae under a stereoscope and oven-dried them at 80°C for 24 h. The remaining larval mass was also oven-dried. The dry weight of the larval mass (LDW) and of the 3 subsamples were weighed using a precision balance to the nearest 0.0001 g. The number of hatched larvae for each female was then estimated from the linear extrapolation of the mean number of larvae per gram in the 3 subsamples and the dry weight of the entire larval mass per female LDW.

2.6. Larval swimming performance and survival to starvation

To measure larval swimming performance, a 2D video was recorded during the first 24 h after larval release (all videos were recorded in daylight). Five larvae from each female replicate and from each treatment (see number of females in Fig. 1) were haphazardly chosen and placed individually in 10 × 10 cm glass containers filled with 100 ml of pre-filtered (75 µm) seawater. Larvae were individually acclimated for 3 h at the experimental temperature treatments (at normoxic conditions) before video recording and then maintained under the same conditions and a 12:12 photoperiod for the 24 h video recording. Larvae were maintained separated according to the O_2 concentration treatment experienced by the female. Using a digital camera (Video Flex 7000 series, Ken-*a*-vision) placed perpendicularly and at 35 cm above the containers, 3 min videos (at 29 frames s^{-1}) were recorded at 5 time intervals (3, 6, 9, 12 and 24 h) for each larva. A total of 350 video clips resulted from the different treatments, females (14 females and 70 larvae) and times, but some larvae died during the experiment, reducing the number of videos actually recorded. Video clips were analyzed using the tracking feature of Blender (version 2.78c) video editing software, using default settings except for frame limits (0 to 5000 frames) and correlation rate (50–70%). The software automatically tracked larval movements providing their 2D coordinate locations (in pixels) at each time frame. Coordinates were converted to minimum Euclidian distances in mm and divided by elapsed time to obtain speed.

To measure larval survival, a subsample of 100 larvae from each female was chosen haphazardly, separated into 5 replicates (n = 20) and placed into five 350 ml containers with pre-filtered (75 µm) seawater. Larvae were maintained unfed in temperature-controlled chambers under the same temperature and light conditions as their original treatments. Larvae were maintained separated according to the O_2 concentration treatment experienced by the female. All starved larvae were exposed to normoxic conditions during this experiment, ensuring daily water replacement and air exchange. Larval mortality was checked daily by counting live and dead larvae (which were then removed) in each container, and survival curves were generated by plotting larval mortality over time for the duration of the experiment (data not shown; see Section 3 for the duration of the experiments). A larva was considered dead when no movement was observed after it was gently poked using a glass micropipette. The experiment ended once all larvae died. Survivorship curves were approximated using a sigmoidal fit (Miller et al. 2009) to calculate the LT_{50} for each treatment and female. In this manner, independent estimates of LT_{50} were obtained for each treatment (total n = 14). LT_{50} is defined as the time at which 50% of the larvae in the experiment died and was estimated by fitting a sigmoidal curve using the following equation:

$$S = 1 - \frac{1}{1 + \exp\left(-\frac{t - LT_{50}}{b}\right)} \quad (1)$$

where S is the lethal fraction of larvae, t is the total time of the experiment (days) and b is the best-fit coefficient determined by the curve-fitting model (Miller et al. 2009).

2.7. Statistical analyses

While all females carried embryos after being in contact with males, not all females kept their embryos. From the initial 20 individuals, 14 females carried their embryos until hatch and released viable larvae, resulting in the following unbalanced female replication per treatment: 11-N (n = 4), 11-H (n = 3), 14-N (n = 3), and 14-H (n = 4).

To test for differences in the volume of early-stage embryos (all under normoxia), we conducted a 1-way ANOVA with temperature as fixed factor (2 levels, 11 and 14°C). Embryos within each replicate female were averaged before analysis. We did not consider the oxygen treatment here because females were not exposed to O_2 concentration treatments at this stage.

The combined effect of temperature and O_2 concentration (factorial fixed factors) on embryo volume was measured twice over time on each female, once at the medium and once at the late stage of development. These 2 measures represent repeated observations on the same females and, therefore, a repeated-measures ANOVA (as mixed model) was used to analyze results, with the factorial treatment structure (oxygen, temperature) as between-subject factors and development stages (early, late) as the within-subject factor (Kuhel 1994). To simplify the model, measurements of embryo volume within broods were averaged within female before analysis.

The effect of temperature and oxygen (fixed factors) on the volume of Zoea 1 larvae, number of hatched larvae, female ventilation, embryo developmental time and larval survival (LT_{50}) were tested with 2-way ANOVAs. In the case of Zoea 1 and following the approach used above, volumes were averaged within fe-

males before the analysis. Pairwise comparisons were performed for the volume of Zoea 1 larvae, number of hatched larvae, female ventilation, embryo developmental time and larval survival (LT_{50}), by using the function ls_means of the R package lmerTest.

The 5 measurements of larval swimming speeds collected during the first 24 h after larval hatching were analyzed using a repeated-measures ANOVA with the orthogonal treatments as fixed factors and the time dependent structure in the error term (repeated measures). Since many larvae died during the time period, observations of different larvae were averaged within female, per time interval and the analysis conducted on this simplified design, which retained the original replicates (females) and time structure. Three variance–covariance structures were explored for the error term, including autoregressive model with homogeneous and heterogeneous variances and compound symmetry, and the structure with the lowest Akaike information criterion (AIC) was selected, which was the homogeneous autoregressive structure. Results for fixed effects were insensitive to the choice of covariance matrix. Larval speed was log transformed to meet the normality assumption.

All analyses were performed using R (R Studio, v. 1.1.463; R Core Team 2019) and the packages lmerTest and ggplot2 (Kuznetsova et al. 2017).

3. RESULTS

3.1. Embryo and larval sizes

Temperature had a significant effect on the early stage of the embryos (Table 1A), with bigger volumes at the lower temperature (Fig. 2A). There was a significant effect of stage on the volume of the embryos (bigger volume at late stages), and the interaction terms were found to be non-significant (Table 1B, Fig. 2B). For the Zoea 1 larvae, a significant interaction between temperature and O_2 concentration was found (Table 1C, Fig. 2C). Pairwise tests on the interaction temperature × cycling hypoxia showed that the volume of Zoea 1 in the 14-H combination

Table 1. ANOVAs of the volumes of (A) early-stage embryos, (B) medium- and late-stage embryos, and (C) Zoea 1 larvae of *Taliepus dentatus*. Temp: temperature; Stage: developmental stage (early, medium or late). The df values are df of factor, df of residuals. Values in **bold**: significant results ($p < 0.05$)

Source	df	*F*	MS	p
(A) Early stage				
Temp	1, 12	8.317	0.0006	**0.0137**
(B) Medium and late stages				
Temp	1, 20	2.3662	0.0003	0.1397
Oxygen	1, 20	0.364	0.0003	0.5531
Stage	1, 20	84.693	0.001	**0.0001**
Temp × Oxygen	1, 20	2.333	0.0003	0.1423
Temp × Stage	1, 20	2.3994	0.0003	0.1371
Oxygen × Stage	1, 20	0.0706	0.0001	0.7932
Temp × Stage × Oxygen	1, 20	1.2521	0.0001	0.2964
(C) Zoea 1 larvae				
Temp	1, 10	13.639	0.0818	**0.0042**
Oxygen	1, 10	5.2928	0.0317	**0.0442**
Temp × Oxygen	1, 10	11.115	0.0666	**0.0076**

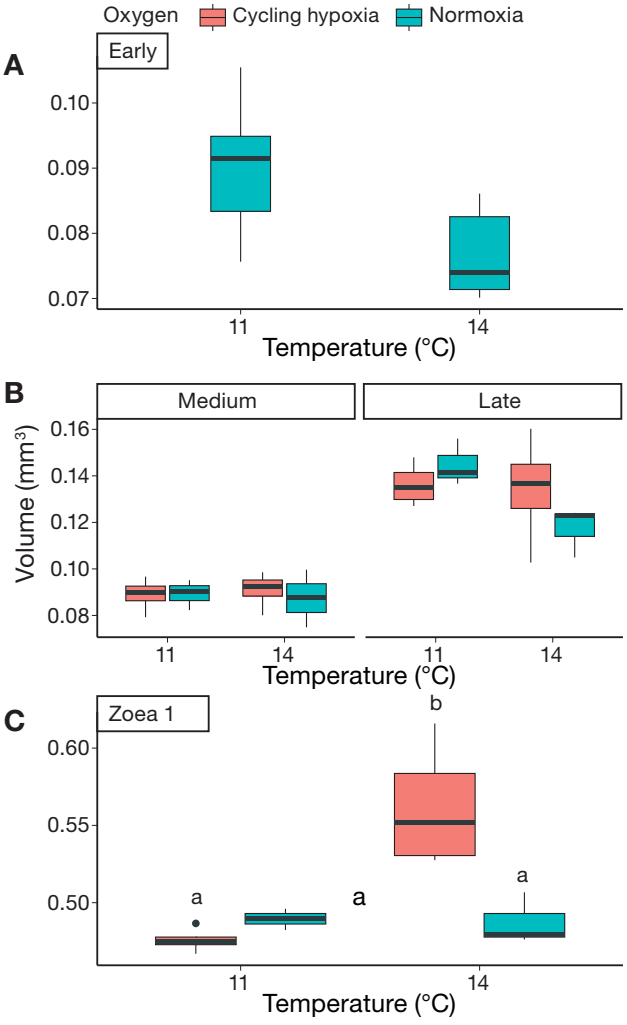

Fig. 2. Effects of temperature and cycling hypoxia on (A) early-stage embryos under normoxia, (B) medium- and late-stage embryos, and (C) Zoea 1 larvae of *Taliepus dentatus*. (A) only shows the effect of temperature (see Section 2). Box: interquartile range; black bar within box: median; whiskers: maximum and minimum values; black dots: outliers. In (C), the letters indicate pairwise comparison among groups ($p < 0.05$)

was significantly bigger than in the rest of the treatments (14-H vs. 11-H: p = 0.0026; 14-H vs. 11-N: p = 0.0137; 14-H vs. 14-N: p = 0.0116).

3.2. Embryo developmental time

Temperature and oxygen affected the embryo developmental time. At 14°C, the embryos developed faster than at 11°C (Table 2A, Fig. 3A), but cycling hypoxia slowed down the development. Conversely, at 11°C, cycling hypoxia accelerated the development compared to normoxic exposure.

3.3. Female brooding behavior

Flapping frequency increased with temperature, in both normoxic and hypoxic treatments (Fig. 3B). Under hypoxic conditions, females always ventilated their embryos more compared to normoxic treatments, with particularly high rates in the 14-H experimental condition (almost double the rate of 11-H).

3.4. Number of hatched larvae

The significant interaction between temperature and dissolved oxygen (Table 2, Fig. 3C) is explained by the lack of differences between O_2 treatments at 11°C (11-N vs. 11-H: p = 0.4605), while at 14°C, the number of hatched larvae was significantly smaller under cycling hypoxia than at normoxia (Tukey HSD post hoc test; p = 0.0001).

3.5. LT_{50} and swimming performance

Starved larvae showed a maximum survival of approximately 11 d, with no significant influence of the oxygen conditions experienced by the embryos (Table 2D, Fig. 3D). A marginally significant effect of

Table 2. Two-way ANOVA of (A) embryo developmental time (EDT), (B) female brooding behavior (abdominal flapping), (C) larval hatching and (D) larval mortality (the time at which 50% of the larvae in the experiment died, or LT_{50}) for *Taliepus dentatus*. Temp: temperature. The df values are df of factor, df of residuals. Values in **bold**: significant results (p < 0.05)

Source	df	*F*	MS	p
(A) EDT				
Temp	1, 10	599.28	1263.5	**0.0001**
Oxygen	1, 10	5.589	0.02	**0.028**
Temp × Oxygen	1, 10	83.832	0.072	**0.0001**
(B) Abdominal flapping				
Temp	1, 10	54.320	5092.1	**<0.0001**
Oxygen	1, 10	18.039	18.039	**0.0017**
Temp × Oxygen	1, 10	3.272	306.7	0.1005
(C) Larval hatching				
Temp	1, 10	688.47	0.818	**0.0001**
Oxygen	1, 10	19.31	0.230	**0.0014**
Temp × Oxygen	1, 10	42.88	0.051	**0.0001**
(D) LT_{50}				
Temp	1, 10	5.0668	0.0548	**0.0481**
Oxygen	1, 10	3.0109	0.0325	0.1134
Temp × Oxygen	1, 10	0.0006	0.0006	0.057

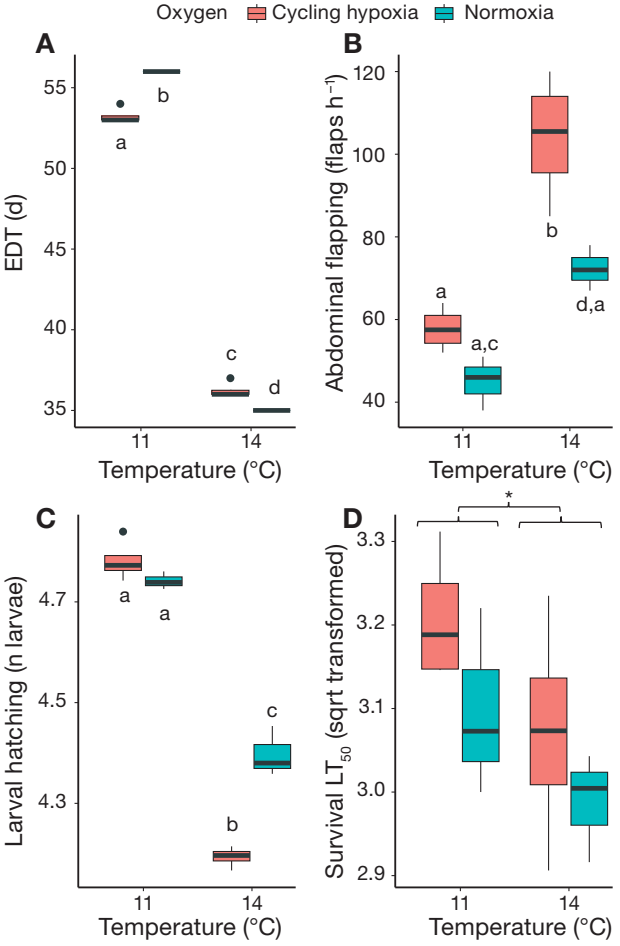

Fig. 3. Effects of temperature and cycling hypoxia on (A) embryo developmental time (EDT), (B) female ventilation (abdominal flapping frequency), (C) larval hatching (number of hatched larvae), and (D) larval survival (the time at which 50% of the larvae in the experiment died, or LT_{50}) of *Taliepus dentatus*. Box: interquartile range; black bar within box: median; whiskers: maximum and minimum values; black dots: outliers. Letters indicate pairwise comparison among groups (p < 0.05). *Significant difference between the 2 temperatures (p < 0.05)

temperature emerged, with lower LT_{50} of unfed larvae at higher temperatures (Table 2, Fig. 3D). Analysis of larval performance showed a significant interaction between time and temperature (Table 3), indicating that larvae performed differently over time depending on the temperature and oxygen conditions to which they were exposed (Fig. 4). In particular, larvae at 14°C showed an increasing speed during the first 12 h (although not consistently during cycling hypoxia), while larvae at 11°C showed a decreasing trend over the same time period (Fig. 4). Regardless of the temperature treatment, all larvae reduced their speed to similar values towards the end of the experimental time (i.e. 24 h) under unfed conditions (Fig. 4). Cycling hypoxia showed a significant interaction with temperature (Table 3), suggesting that the hypoxic conditions experienced by the embryos during development affected larval performance differently (negatively at 11°C and positively at 14°C; Fig. 4).

Table 3. Repeated-measures ANOVA of *Taliepus dentatus* larval swimming performance. Temp: temperature. Values in **bold**: significant results ($p < 0.05$)

Source	df	*F*	MS	p
Time	4	4.5852	0.3759	**0.0013**
Temp	1	0.0380	0.0031	0.8455
Oxygen	1	9.6782	0.7934	**0.0020**
Time × Temp	4	5.2099	0.4271	**0.0005**
Time × Oxygen	4	0.2111	0.0173	0.9321
Temp × Oxygen	1	10.901	0.8936	**0.0011**
Time × Temp × Oxygen	4	0.2147	0.0176	0.9301

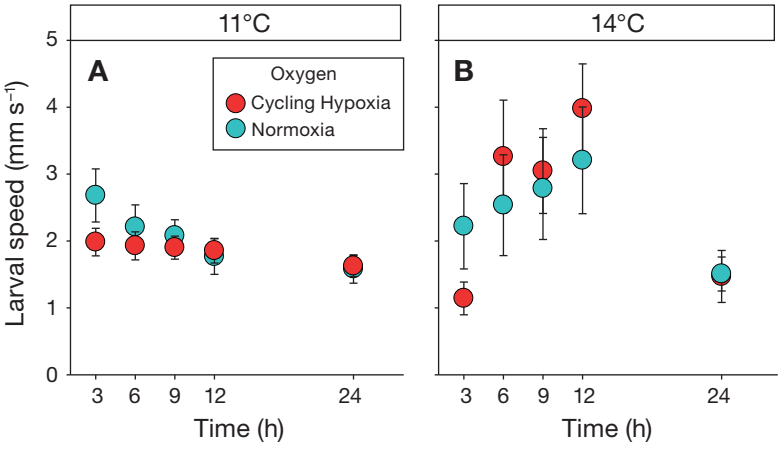

Fig. 4. *Taliepus dentatus* larval swimming performance (i.e. speed, mean ± SD) after hatching, at (A) 11°C and (B) 14°C under cycling hypoxia and normoxia

4. DISCUSSION

In marine systems, the interactions among environmental factors determining the eco-physiological niche where animals live are complex and often poorly understood (Roman et al. 2019, Tittensor et al. 2019). Such interactions become particularly critical in upwelling ecosystems that exhibit highly dynamic environments in terms of temperature and dissolved oxygen (Vaquer-Sunyer & Duarte 2008). Our results contribute to the understanding of the complex relationship between animal physiology and the environment, with possible consequences on tradeoffs and population dynamics (Roman et al. 2019, Tittensor et al. 2019). Specifically, the present study showed (1) a temperature-induced change in the size (volume) of early embryos, (2) a change in the size of Zoea 1 induced by the interaction of low oxygen concentration and high temperature during development, (3) an inverse scaling between the number and the size of Zoea 1 larvae, (4) an interactive effect of temperature and hypoxia on the duration of embryo development, (5) an almost 2-fold increase in female abdominal flapping at the higher temperature and lower oxygen concentration, and (6) an overall effect of temperature and potential carry-over effect of hypoxia on the performance of Zoea 1 larvae, even within their optimal range of temperatures (see Storch et al. 2009).

Generally, during egg deposition and fertilization, females invest differently in their offspring by allocating resources to maximize their own fitness, as well as that of the offspring (Bernardo 1996). This initial mechanism or maternal effect is strongly influenced by the environmental condition experienced by females during reproduction (Moran & McAlister 2009). A negative effect of temperature on offspring size has been shown in a wide variety of taxa, either based on correlations between the environmental temperatures experienced by the mothers and the size of eggs they produce (Marshall & Keough 2007) or theoretically (Kiflawi 2006, Bueno & López-Urrutia 2012). Thus, egg size of marine invertebrates shows high variation, depending on several factors, including seasonal temperatures. For example, a strong negative relationship has been observed between egg size and 3 marine crab species from a tropical upwelling zone (Collin et al. 2018). Urzúa et al. (2012) found that the eggs of the shrimp *Crangon crangon* during winter are, on average, bigger than the ones in summer. Both

works confirmed that water temperature and its variation are strong environmental drivers affecting egg size in the wild, especially in early embryo development. Thus, the temperature mosaic generated by the temporal variability in upwelling conditions are expected to influence the distribution of egg size in large upwelling systems, such as the Humboldt Current System. In the present study, the initial acclimation to temperature (3 to 4 wk) affected female investment in the size of the embryo (early stages), with bigger embryos produced at 11°C. Since the lipid content of early embryos of *Taliepus dentatus* is positively related to latitude (Baldanzi et al. 2018), differential lipid allocation could explain the observed temperature-induced differences in embryo size in the present study (Laptikhovsky 2006, Moran & McAlister 2009). High lipid allocation at the lower temperature, resulting in bigger embryos, may guarantee energy support to cope with the slow developmental rate at such temperatures (Geister et al. 2009). Furthermore, an increased lipid composition at high latitude (cold temperatures) of early-stage embryos of *T. dentatus* was found to be positively associated with genome-wide DNA methylation (S. Baldanzi unpubl. data). This epigenetic regulation of lipid metabolism (Beal et al. 2018) may underpin the temperature-induced, maternally regulated plasticity in embryo size found here, as increasingly reported for marine invertebrate species (Eirin-Lopez & Putnam 2019).

The significant interaction between temperature and oxygen on embryo developmental time suggests that hatching time can vary depending on the oxygen conditions available at each temperature. We found that, at 11°C, embryos of *T. dentatus* showed a faster developmental rate under hypoxic conditions, while under 14°C, hypoxia slowed down the development. This result partially agrees with similar experiments conducted in chinook salmon exposed to comparable levels of temperature and oxygen concentrations used in our study (Del Rio et al. 2019); they found that hypoxia slowed down embryo development at the higher (as we found for *T. dentatus* embryos) as well as the lower temperature. We explain the faster development of embryos in the 14-H combination as a consequence of the natural increase in metabolic rate as embryonic development progresses (Rombough 1988), so that hypoxia might have triggered premature hatching once oxygen became limited at the higher temperature (Del Rio et al. 2019). Nonetheless, given the small sample size of our study, caution must be taken when interpreting this result. Regardless of the limited number of females available at late stages, the embryo developmental time of *T. dentatus* was strongly and negatively affected by temperature, with approximately 2-fold differences in developmental time between temperature treatments. The results agree with theoretical and mathematical models that predict the time of ontogenetic development as a function of body mass and temperature (Gillooly et al. 2001). We cannot exclude, however, that a daily rate of hypoxic exposure higher than the one used in this study or a lower level of dissolved oxygen could cause a delay in embryo developmental time, as found in several studies investigating the combined effect of temperature and hypoxia on the embryo developmental time of fish (Anderson & Podrabsky 2014, Del Rio et al. 2019) and marine invertebrates (Cancino et al. 2003, Gallardo et al. 2019).

Small changes in hypoxic conditions (only 4 h d^{-1} of chronic exposure) caused substantial variation in several response variables, particularly at the higher temperature and especially for those associated with the mothers (i.e. brooding behavior). This suggests that changes in temperature alone may not be a good predictor of offspring size, offspring performance or number of hatched larvae and brooding behavior under an ecologically relevant scenario. In fact, prolonged exposure of ovigerous females to cycling hypoxic conditions during embryo development affected the successive larval stage enough to affect larval size (i.e. big vs. small larvae). Contrary to an expectation of a negative effect of temperature on the size of offspring, we found an increase of larval size with increasing temperature under cycling hypoxic conditions. One interpretation of this combined effect of high temperature and hypoxia on larval size is that exposure of brooding females to such conditions triggers a substantial increase in the brooding behavior enhancing oxygenation of the embryo masses, ultimately affecting both the size and number of larvae. Female brooding behavior in decapods has important implications in the final reproductive output and it is triggered by environmental conditions that females experience during embryo development (Baeza et al. 2016, Fernández et al. 2020). Active oxygenation of egg masses by decapod females (and marine brooders in general), a brooding behavior which is triggered by environmental cues (Fernández et al. 2002), is generally positively related to offspring size and overall female fitness (Kolm & Ahnesjö 2005, Klug & Bonsall 2014). An important result of our study is the association between the number of hatched larvae and larval size. We found a decrease in the number of hatched larvae as larval size increased at high temperature

and low oxygen concentration. This can be interpreted as a combined effect of hypoxia and high temperature on embryo mortality, which caused a reduced number of embryos during late stages, ultimately affecting the number of hatched larvae. Hatching is a process that increases aerobic energy demand (Polymeropoulos et al. 2016), and under hypoxic conditions, the supply of oxygen is further reduced. Warmer conditions may increase these effects by acting on embryo metabolism (e.g. higher embryo respiration at higher temperature). For example, hatching success in salmon was negatively affected by high temperature and hypoxia, and this was related to the high mortality as well as to partial hatching of late embryos (Del Rio et al. 2019). Embryo loss was not measured in our study because it was difficult to count dead embryos in the aquarium due to the continuous water flow and mixing during the treatments. Nonetheless, some embryos were observed at the bottom of the aquaria during cleaning, suggesting anticipated embryo mortality as a consequence of hypoxic exposure. Because oxygen availability within a brood is negatively affected by temperature in densely packed clutches (Moran & Woods 2007), the number of eggs that reach maturation can be reduced due to a lack of oxygen (Nebeker et al. 1992, Dick et al. 1998). At high temperature and hypoxic conditions, more within-brood space is available for embryo growth (Braga Goncalves et al. 2015), which may have caused the remaining embryos to grow faster, generating the inverse scaling between the size and the number of new hatched larvae found in our study.

Larval swimming performance (speed) at the beginning of the experiments (3 h after hatching) was similar across treatments, suggesting that neither temperature nor cycling hypoxia during development affected the performance of newly hatched larvae. There was, however, a different performance of larvae over time between the 2 temperature treatments, and (with smaller effect size) an effect of oxygen on larval speed within each temperature treatment. These findings agree with previous works on Zoea 1 activity of *T. dentatus* which showed no differences in several measurements of larval activity (pleopods, maxilliped and abdominal beating) between temperature ranges comparable to the one used in the present study (11–15°C, see Storch et al. 2011). After 24 h of incubation at different temperatures and under unfed conditions, all larvae (except in treatment 11-N) showed a general decrease towards comparable performances among treatments, a result likely due to the increasing effect of starvation, which we found to be particularly strong during the early stages of *T. dentatus* larvae (Fagetti & Campodonico 1971). Temperature and, with minor effects, the oxygen conditions experienced by the embryos shaped the speed of recently hatched larvae, with significantly different trends. These results suggest that the cumulative effect of conditions (i.e. temperature and oxygen) during the brood cycle and the temperature that larvae experience during the first 12 h are crucial to affecting larval activity, such as speed, even within an optimal thermal breadth (Storch et al. 2011). If we assume that more energy was invested in embryos at the lower temperature, larvae that hatched at 11°C were more energetically equipped to survive without food than those at 14°C (Voesenek et al. 2018). This was supported by the negative effect of temperature observed on the survival of unfed larvae of *T. dentatus*. Therefore, higher larval speed at 14°C compared to 11°C during the first 12 h might be related to the need to search for food to withstand metabolic demands at those temperature conditions.

Importantly, we showed that the performance of larvae, although they did not experience directly any change in oxygen concentration, was initially affected, either negatively (treatment 11-H) or positively (treatment 14-H), by the cycling hypoxia experienced by the mothers and embryos during the embryonic development.

Although the overall trends of larval performance during the first 24 h after hatching suggest signs of recovery from embryonic exposure to the hypoxic treatment, our result is still important because it suggests a metabolic link between 2 successive life history stages (embryos and larvae). A change in environmental condition during embryo development in a coastal benthic population may affect the performance of successive pelagic phases and, together with physical and oceanographic forces, influence dispersal, settlement, and recruitment (Swearer et al. 2019). These carry-over or latent effects may have important ramifications for connectivity and population dynamics, affecting individual post-settlement and reproductive success (Pechenik 2006). The environmental conditions experienced by parents during their adult life and the parental phenotype (e.g. female physiology, egg composition, allocation of nutritional reserves, and parental care) may have effects on the phenotype and condition of the dispersing individual (Green 2008, Donelson et al. 2009, Crean et al. 2013).

In conclusion, this study showed that ecologically relevant temperature and low dissolved oxygen

interact in a complex manner, affecting traits associated with females and the early ontogeny of *T. dentatus*. We showed that the interaction of these 2 factors changed the offspring size during ontogeny, inverting an initial trend of larges embryos at the lower temperature. Although we could not statistically consider the change in size during the whole ontogeny (including larval stage), the trends are evident and suggest that an approach based on multi-environmental drivers should be always tested, especially when investigating traits associated with the fitness of mothers and offspring. Given the small sample size of our study, however, caution must be taken when interpreting non-significant results, such as the lack of hypoxia effect on embryo volume at late stages and on larval survival (LT_{50}).

By introducing the important, but often overlooked, effect of maternal environmental conditions on life history trajectories, our results shed light on the complex interaction between maternal allocation, maternal behavior and offspring traits. This individual-based physiological approach can contribute to creating a more accurate mechanistic framework when investigating the biophysical constraints affecting larval dispersal and recruitment (Kearney & Porter 2009, Swearer et al. 2019). Further studies should focus on exploring such latent effects, e.g. by investigating the response of later larval stages, recruits, and juveniles to the maternal environmental conditions, including the molecular mechanisms responsible for these effects (e.g. epigenetic mechanisms). We have also provided evidence that female brooding behavior, triggered by changes in hypoxia and temperature during embryo development, played a crucial role in shaping the larval size/number relationship and, ultimately, larval performance. This highlights the need to adopt ecologically relevant, multistressor approaches to increase the reliability of results and their ecological application in a dynamic ecological landscape such as the coastal ecosystem.

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Appendix

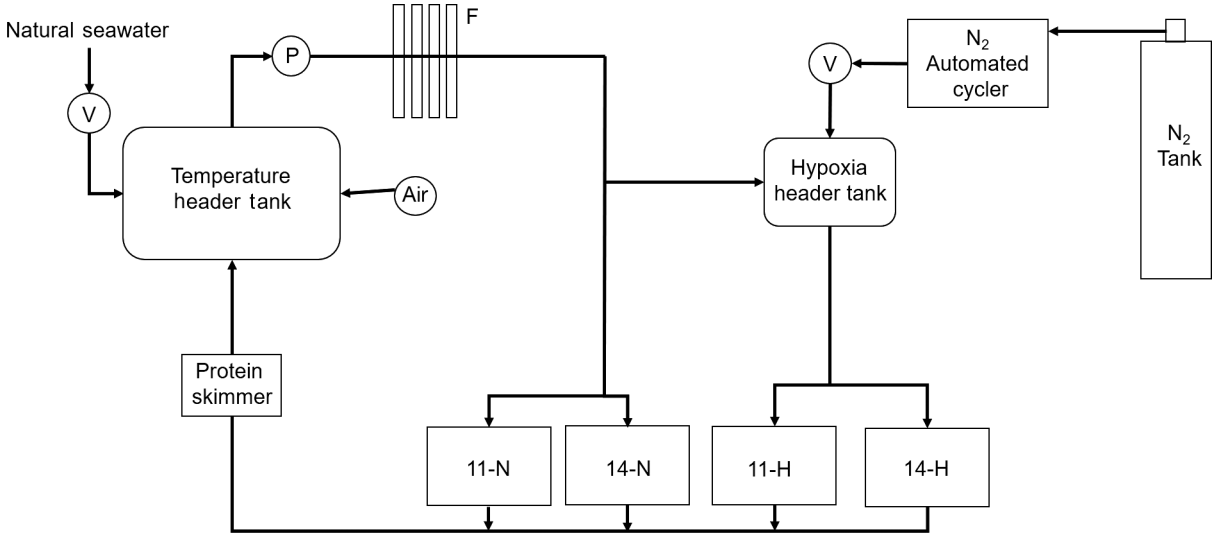

Fig. A1. Schematic of the semi-closed, flow-through system used for the experiments on the effect of temperature and cycling hypoxia on the early ontogeny of the kelp crab *Taliepus dentatus*. Natural seawater is collected by an underwater pump adjacent to the marine station (Estación Costera de Investigaciones Marinas) and distributed to the laboratory facilities. In the laboratory, a semi-closed, flow-through system allows control over the seawater temperature within the aquaria. A flux of nitrogen gas (N_2) was applied to a header tank to control the dissolved oxygen concentration within the aquaria. This setting allowed us to perform a fully factorial experimental design with the 4 different treatment combinations of 11-N, 11-H, 14-N, and 14-H, indicating the temperature (11 or 14°C) and the oxygen conditions (N: normoxia; H: cycling hypoxia). See Section 2 for further details. V: valve; P: pump; F: filtering system (20 to 1 µm)

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