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The impact of hypoxia and increasing temperature on parental care in the brachyuran crab Romaleon setosum

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Abstract

Protecting embryos provides multiple benefits but also entails costs and constraints. In aquatic environments, aggregating metabolically active tissues limits oxygen acquisition, as evidenced by studies reporting oxygen depletion at the center of embryo aggregations across taxa and aggregation types among marine invertebrates. However, the effects of concurrent ocean warming and deoxygenation on species that protect and aggregate their embryos remain poorly understood. We investigated the effects of temperature and hypoxia on oxygen-provisioning behaviors, oxygen conditions within embryo aggregations, and the metabolic costs of oxygen supply in the brachyuran crab *Romaleon setosum*. We conducted a laboratory experiment manipulating temperature (16 and 20°C) and oxygen conditions (normoxia, > 80% air saturation; and 5-day hypoxia, 17–18% air saturation). Behaviors were video recorded, while oxygen levels (percentage air saturation) within embryo masses were continuously monitored using microsensors. Metabolic costs were estimated from oxygen consumption in respiration chambers for brooding and non-brooding females, as well as embryos (to estimate oxygen consumption of the embryo mass). Oxygen levels within embryo aggregations remained low under environmental normoxia despite increased oxygen-provisioning behaviors as temperature increased, resulting in elevated metabolic costs for brooding females. Notably, no compensatory behavioral responses were observed under ambient hypoxia and increasing temperature, suggesting metabolic constraints on oxygen provision to embryo aggregations. These findings indicate potential consequences for embryo development and clutch size under ongoing ocean warming and deoxygenation. More broadly, our results highlight the need to improve our understanding of how interacting environmental stressors affect reproduction in marine invertebrates across diverse life-history patterns.

Introduction

The ocean is changing rapidly. Temperature is a key environmental stressor undergoing global changes (Abraham et al. 2013; Cheng et al. 2025). Ocean acidification poses a major threat to marine species worldwide (Kroeker et al. 2013), and hypoxia is expanding globally, increasingly affecting coastal waters (e.g., Gilbert et al. 2010). Approximately 90% of the excess heat generated by anthropogenic activity has been absorbed by the ocean, resulting in an increase of 0.68–1.01°C in sea surface temperature (von Schuckmann et al. 2020; Cheng et al. 2025). Rising temperature, together with the slowdown of ocean circulation and enhanced bacterial respiration associated with the expansion of eutrophic zones, are the main mechanisms driving ocean deoxygenation (e.g., Schmidtko et al. 2017; Wei et al. 2021; Pasquier et al. 2024). It is estimated that dissolved oxygen content in the global ocean has declined by 1–3% over the past 5 decades (Schmidtko et al. 2017; Pasquier et al. 2024). Recent projections for the year 2100 suggest that, under different emission scenarios, (a) ocean temperature may increase by 0.43–4.07°C (IPCC 2023), and (b) surface dissolved oxygen concentrations could decrease by 1–13% (Kwiatkowski et al. 2020) with stronger intensification in coastal regions (e.g., Chan et al. 2008; Gilbert et al. 2010).

The effects of global environmental change on marine life are clear and pervasive, spanning levels of biological organization from molecules to ecosystems (e.g.,Vaquer-Sunyer and Duarte 2011; Harvey et al. 2013; Llabrés et al. 2013; Gobler and Baumann 2016). The influence of individual and multiple stressors is, however, complex and varies among biological variables, life-history stages, and taxa. Moreover, our understanding of the influence of global stressors is strongly biased toward specific processes, taxa, biological traits, and life-history stages. For instance, a substantial body of research documents the narrow environmental tolerances of planktonic early life-history stages to global stressors, resulting in altered development and reduced survival, among other effects (e.g., Vaquer-Sunyer and Duarte 2011; Harvey et al. 2013; Llabrés et al. 2013; Gobler and Baumann 2016). In contrast, evidence on the effects of climate change on embryonic development and early life-history stages in species that protect and brood their offspring remains limited. (e.g., Fernández and Brante 2003; Cancino et al. 2003, 2010; Brante et al. 2003; Lucey et al. 2015). Improving our understanding of how environmental stressors affect the broad diversity of reproductive strategies in marine organisms remains a key research priority, particularly for stressors that have been comparatively neglected (e.g., hypoxia; Sampaio et al. 2021).

Marine invertebrates exhibit a wide diversity of reproductive patterns, ranging from species that spawn gametes into the water column, thereby investing relatively little in each offspring, to those that provide active parental care to their brood (Strathmann and Strathmann 1982). Between these extremes are species that enclose their embryos within elaborate capsules or gelatinous masses, which provide protection against predators during development along with additional benefits (e.g., Pechenik 1982; Perron and Corpuz 1982). Protecting embryos in the ocean necessarily involves aggregating metabolically active tissues in an environment where oxygen acquisition is strongly constrained relative to terrestrial systems (Strathmann and Strathmann 1995). A variety of strategies have evolved to overcome the challenges of supplying oxygen to aggregated embryos. While some species rely on simple diffusive exchange, others exhibit active behaviors that enhance oxygen delivery to the aggregation (e.g., Perron and Corpuz 1982; Cohen and Strathmann 1996; Baeza and Fernández 2002). Nevertheless, oxygen limitation and even extreme hypoxia have been reported in gelatinous embryo masses and capsules of marine gastropods (e.g., Chaffee and Strathmann 1984; Strathmann and Chaffee 1984; Strathmann and Strathmann 1995; Lardies and Fernández 2002; Moran and Woods 2007; Cancino et al. 2010) as well as in embryo masses of Brachyuran crabs, despite females adjusting their brooding behaviors to meet embryonic oxygen demands (Baeza and Fernández 2002; Brante et al. 2003). Hypoxic conditions in aggregations during embryonic development have important consequences, including reduced developmental and growth rates in crab embryos (Fernández et al. 2003) and constraints on clutch size in gastropods (Lardies and Fernández 2002; Fernández et al. 2006b). Despite this evidence, the influence of globally changing environmental factors on oxygen conditions within embryo aggregations, maternal strategies for oxygen provisioning, and development of aggregated embryos remains insufficiently explored. This gap persists despite limited evidence suggesting that embryo aggregation in aquatic systems may be particularly sensitive to temperature and hypoxia (e.g., Brante et al. 2003; Cancino et al. 2003, 2010).

This study addresses this knowledge gap by examining the effects of two globally changing variables, temperature and hypoxia, on an embryo-aggregating species, *Romaleon setosum*, which belongs to a taxonomic group considered highly vulnerable to environmental stressors (Crustaceans; Vaquer-Sunyer and Duarte 2008; Llabrés et al. 2013). Specifically, we analyze oxygen conditions within embryo aggregations and changes in female behaviors associated with oxygen provision. We hypothesize that oxygen conditions within embryo aggregations decrease as temperature and hypoxia increase, whereas behaviors associated with oxygen provision to the aggregations increase, but not in an additive manner because they are constrained by the females' metabolic capacity. Consequently, the metabolic costs associated with oxygen provision are expected to mirror the behavioral responses to temperature and hypoxia.

Materials and methods

Model species and study area

The hairy edible crab *Romaleon setosum* (Molina 1782) inhabits coastal waters along the southeastern Pacific, from Guayaquil (2°S, 79°W) to the Taitao Peninsula in southern Chile (46°S, 75°W). The species exhibits a broad bathymetric distribution, with records at depths greater than 100 m, but it is most common between 5 and 45 m depth (Retamal 1981). It inhabits reefs as well as gravelly or sandy bottoms that provide refuges such as rocks, shells, or macroalgae (Wolff and Cerda 1992). *R. setosum* is a key predator and also forms part of the diet of other species (Wolff and Cerda 1992). In central and southern Chile, ovigerous females have been reported throughout the year, with higher proportions from late winter to early spring (Maldonado 2019).

The coastal area of central and northern Chile is strongly influenced by coastal upwelling, which brings cold, oxygen-depleted, and nutrient-rich waters from the open ocean onto the continental shelf. This process fuels high levels of primary and secondary production that support many of the world's most important fisheries (e.g., Thiel et al. 2007). Hypoxia is becoming increasingly evident in the global ocean, and particularly in coastal waters, both in Chile and worldwide in part due to eutrophication and increased upwelling-favorable wind stress associated with climate change (e.g., Chan et al. 2008; Aguirre et al. 2018; De La Maza and Farías 2023). Recent studies showed that in the southeast Pacific: (a) the frequent intrusion of hypoxic waters onto the continental shelf appears to be linked to global ocean deoxygenation (De La Maza and Farías 2023); (b) as coastal upwelling has intensified, hypoxic events have become more frequent and severe (De La Maza and Farías 2023); (c) shallow (ca. 14–15 m depth) and mesophotic (40–60 m depth) reefs of central–northern Chile are frequently exposed to hypoxic conditions; dissolved oxygen concentrations below 2.5 mg L^{-1} for periods ranging from several hours to 9 days have been registered in shallow waters (Muñoz et al. 2025; S Navarrete pers comm) and (d) hypoxic events occur primarily during spring and summer (De La Maza and Farías 2023), coinciding with the main reproductive season of most benthic marine invertebrates.

Experimental design and treatments

A laboratory experiment was conducted to test the hypothesis by manipulating temperature (2 levels: 16 and 20°C) and oxygen condition (2 levels: normoxia, > 80% air saturation; and hypoxia, ~ 17–18% O_2 air saturation, equivalent to 1.3 to 1.4 mg L^{-1} O_2). Rather than focusing on long-term projected environmental scenarios, we examined severe conditions relevant to the reproductive season. Experimental temperatures fall within the annual thermal variability of the study area (Brante et al. 2003), but higher than those typically recorded during the embryo-carrying period in the study area (mean ± SD = 12.64 ± 1.1°C at 10–15 m depth; pers. comm.). They may therefore influence behaviors associated with the regulation of environmental conditions for embryonic development under present-day conditions. The hypoxic treatment (17 to 18% air saturation) and its duration (5 days) were defined based on records of extreme hypoxic events occurring in zones inhabited by this species at 15 m depth in the study area (Muñoz et al. 2025; S Navarrete pers comm).

A factorial design was applied, exposing organisms to the 4 experimental treatments. To evaluate behavioral patterns and the costs associated with oxygen provision to embryos, female reproductive condition was considered, including both ovigerous and non-ovigerous females. Female crabs of *R. setosum* were collected by SCUBA diving at depths ranging from 10 to 20 m in Las Cruces and transported to the laboratory in coolers lined with seawater-moistened fabric to prevent damage to the animals. Females of similar body size were selected (mean carapace width ± SD = 110 ± 5.6 mm). Upon arrival at the Marine Laboratory of Las Cruces, crabs were maintained for 2 days in tanks supplied with recirculating seawater directly pumped from the natural environment (mean temperature during the study period 12.64 ± 1.1°C) and under continuous aeration, with a controlled 12:12 h light:dark photoperiod. Females were fed ad libitum with mussels (*Mytilus chilensis*) obtained from a local market. The study was conducted between August and December 2024 and 2025.

The developmental stage of the embryonic mass of each ovigerous female was determined under a stereomicroscope, following the classification of Vargas (1995). In the case of ovigerous females, only those carrying embryos developed up to stage III (approximately 45 days of development) were included, ensuring that the embryo mass could reach the late developmental stage at the time of measurements (stage IV; embryos with developed eyes, heart, and appendages, and approximately 60 days of development). Females carrying late-stage embryos were selected because they exhibit the highest frequency of behaviors associated with oxygen provision (Baeza and Fernández 2002; Brante et al. 2003). From the total number of crabs collected, 48 females meeting the size and reproductive condition criteria were selected: 24 ovigerous females and 24 non-ovigerous females. Females were randomly assigned to the experimental treatments, with 6 replicates per treatment (temperature, oxygen condition).

After 2 days in the laboratory, females were sequentially exposed to the 2 experimental factors to minimize the combined stress of increasing temperature and hypoxia, which would not occur under normoxic conditions. Previously identified females were randomly assigned to the experimental

temperatures and placed individually in aquaria (42 × 32 × 16 cm) with low seawater flow. The aquaria were positioned within 400-L trays containing seawater maintained at the experimental temperatures using thermostatically controlled baths. Small temperature fluctuations were recorded in the aquaria (mean ± SD = 16 ± 1°C and 20 ± 1°C). After 48 h of exposure to the experimental temperatures, the second factor was manipulated by randomly assigning females to either normoxic or hypoxic conditions. Oxygen concentrations in the aquaria were regulated by the controlled injection of air, CO_2, and nitrogen (N_2) bubbles using an IKS Aquastar system (IKS ComputerSysteme GmbH, Karlsbad, Germany) equipped with temperature, pH, and oxygen sensors that were previously calibrated. The control system adjusted oxygen partial pressure by integrating temperature-dependent variations in oxygen solubility. Mean air saturation under normoxia was 100 ± 2% O_2 saturation, whereas hypoxic conditions averaged 17 ± 2% at 16°C and 18.3 ± 4% at 20°C. These conditions were maintained for 5 days, after which female behavior, oxygen conditions within the embryo mass, and female oxygen consumption were measured.

Quantification of parental care–related behaviors

To monitor and quantify behaviors associated with parental care, individuals were transferred to 20-L aquaria at the end of the 5–day exposure to experimental conditions and video-recorded individually for 4 consecutive hours. The aquaria were previously prepared with gravel and a shelter (Baeza and Fernández 2002) and maintained under the conditions corresponding to each treatment. Video recordings were conducted during the daytime, and from the total recording period, 1 h was randomly selected for analysis, monitoring behavior only when the female was positioned in front of the camera. The first hour of recording was discarded to avoid potential stress caused by handling.

The primary behaviors associated with oxygen provision included abdominal flapping (Baeza and Fernández 2002) and active contractions of the embryo mass, which occur during flapping. In addition, complementary parental care behaviors that may indirectly contribute to embryo mass oxygenation, either by enabling females to assess oxygen conditions (insertion of pereopods into the embryo mass) or by enhancing water flow through the embryo mass (abdominal elevation; Baeza and Fernández 2002), were recorded. These behaviors were also recorded in non-ovigerous females (controls) following Baeza and Fernández (2002). For all behaviors, the total time during which each behavior was performed was quantified (s). The frequency of movement was estimated for all behaviors, with slight differences in the approach depending on the behavior. For flapping, abdominal elevation (standing), achieved through extension of the pereopods and typically observed before and during flapping, and for insertion of pereopods, frequency was recorded simply as the number of events h^{-1}). In addition, because abdominal flapping and embryo mass contractions occur as repeated movements over a given time interval, interspersed with resting periods, their frequency was calculated as the number of events s^{-1} during the periods in which the behavior occurred, thereby reflecting behavioral intensity. To evaluate the effects of the treatments on the behaviors analyzed, generalized linear models (GLMs; McCullagh 2019) were used. Reproductive condition (ovigerous vs. non-ovigerous) was not included in the analyses because the behaviors evaluated are generally not exhibited by non-ovigerous females, resulting in

consistently zero values. Model selection was based on comparisons among alternative models using Akaike's Information Criterion (AIC; Akaike 1974). Total behavioral time (s), as well as the intensity of flapping and embryo mass contractions, showed right-skewed distributions with the presence of zeros; therefore, models with a Tweedie distribution and a logarithmic link function were used (Bonat and Kokonendji 2017). Pereopod insertion and standing frequencies were analyzed using GLMs with negative binomial distribution and a logarithmic link function, as these variables corresponded to count data and showed overdispersion (McCullagh 2019). Flapping frequency was analyzed using the same model structure, but with a zero-inflated negative binomial distribution (McCullagh 2019).When significant interaction effects between factors were detected, Tukey post hoc tests were applied.

Assessment of oxygen condition in the embryo mass

To assess oxygen conditions at the center of the embryonic mass, expressed as percentage of air saturation, measurements were conducted simultaneously with the behavioral recordings described above and only in females carrying embryos. The optical fiber does not affect embryo ventilation behavior in the study species (Baeza and Fernández 2002). Measurements were performed by positioning a high-resolution optical fiber (PSt7-0_2, PreSens Precision Sensing GmbH, Regensburg, Germany) at the center of the embryo mass. The oxygen sensor was inserted through a small hole previously made in the third abdominal segment and secured using cyanoacrylate adhesive and dental wax. Prior to insertion, the sensor was calibrated by bubbling N_2 (0% air saturation) and air (100% air saturation), while maintaining the experimental temperature constant.

Air saturation percentage was recorded continuously on a computer every 5 s during the same 4–h period used for behavioral recordings. To avoid potential effects associated with handling, the first hour of measurements was discarded. Using the remaining 3 h of recording, (a) the mean percentage air saturation within the embryonic mass for each female and (b) the percentage of time embryos were exposed to hypoxia (air saturation levels below 17–18%) within the embryo mass, were calculated. To analyze the effects of temperature, oxygen condition, and their interaction on mean percentage air saturation, a GLM with a Gaussian distribution and identity link was used. Additionally, the percentage of time during which hypoxic conditions were recorded at the center of the embryo mass was estimated and compared among treatments using a GLM with a quasibinomial distribution and logit link (McCullagh 2019).

Estimation of the metabolic cost of parental care

After the exposure period to the experimental conditions (5 days), and prior to the manipulations conducted to assess behavior and oxygen conditions within the embryonic mass, female oxygen consumption was measured. These measurements were performed to evaluate the effects of temperature and oxygen condition on oxygen consumption, which may serve as an indicator of the metabolic cost associated with oxygen-provisioning behaviors, using ovigerous females carrying late-

stage embryos and control females (without embryos). To estimate the metabolic cost of embryo-bearing females, it was necessary to subtract embryonic oxygen consumption; therefore, oxygen consumption of the embryos was also measured.

To evaluate female oxygen consumption, experimental organisms were placed individually in a closed respirometry chamber (11 L) submerged in a tank (25 × 25 × 25 cm^3) containing filtered seawater conditioned to the 4 experimental treatments. Prior to insertion, the sensor was calibrated following the procedure described above. The evolution of oxygen was assessed using microsensors (PSt7-10, PreSens Precision Sensing GmbH, Regensburg, Germany) connected to a computer for continuous data recording. The sensor was positioned at a fixed point through a T-type connector, which was connected to a 5-mm C-Flex silicone tubing. Water flowed through the tubing across the respirometry chamber and then to the sensor using a 4-channel peristaltic pump (Cole-Parmer Instrument Company, Vernon Hills, IL, USA), thereby creating a closed water recirculation system. Under normoxic conditions, oxygen levels were recorded for 1 h or until a 20% decrease in air saturation was reached, whichever occurred first. Air saturation within the respirometer was never below 80%, ensuring that female oxygen consumption was not affected (Brill et al. 2015). Under hypoxic conditions, oxygen levels were recorded for 1 h or until air saturation reached 10%, whichever occurred first.

To estimate oxygen consumption of late-stage embryos, and thus subtract embryonic consumption from that recorded for embryo-bearing females, an aliquot of embryos (approximately 1 g) was removed from the embryo mass of 6 ovigerous females per treatment. Each sample was placed on a small mesh inside a microchamber (3 mL) containing 2 mL of filtered seawater adjusted to the corresponding treatment conditions. Measurements lasted approximately 20 min, during which oxygen levels within the microchamber were recorded using microsensors (Polymer optical fiber; PreSens Precision Sensing GmbH, Regensburg, Germany). During measurements, a magnetic stirrer was placed beneath each mesh to homogenize oxygen conditions within the microchamber. Finally, embryos were weighed (g) and oxygen consumption was estimated per unit time and mass for each embryonic aliquot (μmol g^{-1} min^{-1}).

At the end of the measurements, experimental females were anesthetized using clove essential oil. Wet mass (g) was then measured using an analytical balance. In ovigerous females, the embryo mass was subsequently removed and its wet mass recorded. Females and embryo masses were then dried at 80°C for 48 h in a drying oven to obtain dry mass (g). Using the dry mass of both the embryo mass and the females, oxygen consumption was estimated for: (a) the embryo mass, based on oxygen consumption per gram of embryos and the dry mass of the ovigerous mass; and (b) females, based on oxygen consumption per gram and total female dry mass (ovigerous and non-ovigerous). For ovigerous females, the difference between total oxygen consumption of the ovigerous female and that of her corresponding embryo mass for each experimental treatment was calculated, following protocols used in similar studies (e.g., Baeza and Fernández 2002). Finally, oxygen consumption of ovigerous females was compared with that of non-ovigerous females, and observed differences were attributed to the metabolic cost of oxygen-provisioning behavior.

Female oxygen consumption, expressed as O_2 mg^{-1} min^{-1}, was analyzed as a function of temperature, oxygen condition, and reproductive status, considering their interactions. A GLM with a Gamma distribution and log link function was used (McCullagh 2019; Zuur and Ieno 2021). When appropriate, post hoc comparisons among levels of the experimental factors were performed. All statistical analyses were conducted in R (R Core Team 2024), GLMs were fitted using functions from stats and glmmTMB (Brooks et al. 2017), depending on the distribution of each response variable. Estimated marginal means and Tukey post hoc comparisons were performed using the *emmeans* (Lenth 2016) and *multcomp* (Bretz et al. 2008) packages. In general, model selection was based on the nature of the response variables and the distribution of the data, while considering the assumptions inherent to each analytical approach.

Results

Experimental oxygen conditions affected mean oxygen availability at the center of the embryo mass, measured as percent air saturation (Table 1A). Mean air saturation was significantly higher in the center of the embryo mass under normoxia than under hypoxia, reflecting the experimental conditions, and was significantly lower at the higher temperature under normoxic conditions (Table 1A; Fig. 1a). No differences in mean air saturation at the center of the embryo mass were detected between temperatures under experimental hypoxia.The proportion of time that oxygen levels fell below 18% air saturation in the center of the embryo mass was substantially higher under hypoxia than under normoxia, independent of temperature (Table 1B; Fig. 1b). Under normoxic conditions, however, embryos experienced oxygen levels below the 18% air saturation threshold for an average of 7.23 ± 7.03% and 26.1 ± 16.3%) of the time at 16°C and 20°C, respectively. Despite a nearly 4–fold increase in the time spent under hypoxic conditions in the center of the embryo mass between 16 and 20°C, differences between temperatures were not statistically significant, likely reflecting high variability among individuals. No significant interaction between factors was detected for either variable analyzed.

Table 1

Generalized linear models (GLMs) results used to evaluate the effects of temperature, ambient oxygen condition, and their interaction on (A) the mean percentage of air saturation at the center of the embryo mass (Gaussian GLM); (B) the mean percentage of time that embryos remained below 18% oxygen saturation at the center of the embryo aggregation (quasibinomial GLM); (C–F) the mean total time allocated to flapping, contraction of the embryo mass, pereiopod movements, and standing behaviors (Tweedie GLM); (G–H) the frequency (negative binomial GLM) and intensity of flapping (Tweedie GLMs); (I) intensity of embryo-mass contraction (Tweedie GLMs); and (J-K) the frequency of pereiopod movements and standing behavior (negative binomial GLMs), in ovigerous females of *Romaleon setosum*. Frequency measures events h^{-1} and intensity the number of events during the time each behavior occurred. Statistically significant effects are shown in bold ($P < 0.05$). Residual deviance values are 2945.5 for oxygen availability and 69.4 for percentage of time below 18% air saturation

Response variable	**Treatment**	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	**Residual degrees of freedom**
A. Oxygen availability (% air saturation)	Temperature	-20.118	7.007	-2.871	**0.00944**	20
	Experimental oxygen condition	-45.537	7.007	-6.499	**0.00000246**	
	Interaction	19.323	9.909	1.95	0.06532	
B. Percentage of time below 18% air saturation	Temperature	1.5133	1.1090	1.3650	0.18753	20
	Experimental oxygen condition	6.1510	1.8098	3.3990	**0.00285**	
	Interaction	0.05942	3.8072	0.016	0.9877	
Behavior	**Treatment**	**Estimate**	**Std, Error**	**z value**	**P(>|z|)**	**Residual degrees of freedom**
C. Total time flapping	Temperature	0.9141	0.6246	1.463	0.143	18
	Experimental oxygen condition	-1.0347	0.7971	-1.298	0.194	
	Interaction	-0.8174	1.0378	-0.788	0.431	
D. Total time of contraction of the embryo mass	Temperature	2.8411	0.9004	3.155	**0.001603**	18
	Experimental oxygen condition	1.0827	1.0471	1.034	0.301128	
	Interaction	-4.5963	1.4121	-3.255	**0.001134**	
E. Total time of use of pereiopods	Temperature	1.9612	0.761	2.577	**0.00996**	18
	Experimental oxygen condition	-0.2581	0.9854	-0.262	0.79333	

Response variable	Treatment	Estimate	Std. Error	t value	Pr(>|t|)	Residual degrees of freedom
	Interaction	-3.2709	1.367	-2.393	**0.01672**	
F. Total time standing	Temperature	2.06381	0.68282	3.022	**0.00251**	18
	Experimental oxygen condition	0.03025	0.85263	0.035	0.9717	
	Interaction	-1.38246	1.03369	-1.337	0.18109	
G. Flapping intensity	Temperature	1.5545	0.4507	3.449	**0.000563**	18
	Experimental oxygen condition	1.1127	0.4838	2.3	**0.021455**	
	Interaction	-1.2753	0.5542	-2.301	**0.021389**	
H. Flapping frequency	Temperature	2.2593	0.8255	2.737	**0.0062**	19
	Experimental oxygen condition	-0.1462	0.8712	-0.168	0.8667	
	Interaction	-2.0018	1.1792	-1.698	0.0896	
I. Intensity of contraction of the embryo mass	Temperature	0.27236	0.60631	0.449	0.65328	18
	Experimental oxygen condition	-0.05423	0.6769	-0.08	0.936143	
	Interaction	-1.45184	1.04055	-1.395	0.16294	
J. Frequency of use of pereiopods	Temperature	1.5315	0.797	1.922	**0.0547**	19
	Experimental oxygen condition	-0.7985	0.9188	-0.869	0.3848	
	Interaction	-1.2618	1.2116	-1.042	0.2977	
K. Standing frequency	Temperature	0.8348	0.6321	1.321	0.18664	19
	Experimental oxygen condition	0.2249	0.6754	0.333	0.73916	
	Interaction	-0.4781	0.8994	-0.532	0.59502	

The behaviors evaluated showed a consistent general pattern; however, high variability often prevented the detection of statistically significant effects of experimental factors (e.g., mean total time flapping or

standing). In fact, a 3– to 6–fold increase in mean total flapping time was observed under normoxia in comparison to hypoxia, with the largest contrast observed at 20°C (Fig. 2a). However, mean total time devoted to oxygen-provisioning movements (flapping) did not differ significantly among factors (Table 1; Fig. 2a). Similar, although significant trends, were detected for the other behaviors. A significant interaction between temperature and oxygen condition (Table 1c–f) was detected for the mean time using the pereopods and contracting the embryo mass (Table 1; Fig. 2b–c). Post hoc analyses showed that the total time devoted to embryo-mass contractions and pereopod use increased significantly (1–to 7–fold) with temperature under normoxia, whereas no temperature-related changes were observed under hypoxia (Table 1; Fig. 2). No effect of experimental factors were detected on mean total time adopting the standing posture (however, nearly a 5–fold increase in mean total time standing at the highest temperature under normoxia was observed, as indicated by significant post hoc analysis; Fig. 2d). Except for brief occurrences of abdominal elevation in 2 treatments (Fig. 2d), the other behaviors were not performed by non-ovigerous females, regardless of temperature or experimental oxygen condition.

Figure 2 Mean total time allocated to behaviors directly and indirectly related to oxygen provision in ovigerous females of *Romaleon setosum* at two experimental temperatures (16 and 20°C) under normoxic and hypoxic ambient conditions. The behaviors analyzed were (a) flapping, (b) contraction of the embryonic mass, (c) use of pereiopod, and (d) standing position (broken lines indicates the mean behavior recorded in non-ovigerous females). Bars represent mean ± SE. Different letters indicate significant differences among treatments based on Tukey post hoc multiple comparisons ($P < 0.05$)

The frequency of brooding female behaviors followed the general pattern described above in relation to the experimental conditions, although in some cases high variability prevented the detection of statistically significant differences (Table 1; Fig. 3). A significant interaction between experimental factors was detected in the 2 behaviors directly associated with oxygen provision: flapping frequency and intensity (Table 1G & H). Under normoxia, mean flapping frequency increased markedly with temperature, whereas no change was observed under hypoxia (Table 1H; mean ± SE: hypoxia 16°C = 18 ± 12.3, hypoxia 20°C = 23.4 ± 6.2, normoxia 16°C = 20.8 ± 13, normoxia 20°C = 199.6 ± 97.2). A similar pattern was observed for mean flapping intensity (number of events per unit time during which the behavior occurred), with a 4–fold increase with temperature under normoxia (from 0.09 to 0.42 events s^{-1}), whereas no differences were detected between temperatures under hypoxia (0.27 and 0.36 events s^{-1} at 16 and 20°C, respectively; Table 1G; Fig. 3a). Notably, although total time spent flapping under hypoxia was very low, when the behavior did occur its intensity fell within the range observed under normoxia (Fig. 3a). The intensity of embryo-mass contractions was not affected by the experimental factors (Table 1I; Fig. 3). Similarly, the frequency of the standing posture–one of the behaviors indirectly associated with oxygen provision–was not influenced by the experimental conditions (Table 1K; Fig. 3). Standing was the only behavior observed in non-ovigerous females, although its frequency was very low (Fig. 3). The frequency of pereopod use was significantly higher at 20°C than at 16°C (Table 1).

The pattern of female oxygen consumption showed clear differences under hypoxic and normoxic conditions in relation to temperature and the reproductive condition of female crab (Table 3; Fig. 4). Under normoxia, mean oxygen consumption rates at 20°C doubled the recorded estimates at 16°C in ovigerous females, whereas no differences were observed in non-ovigerous females. In contrast, under hypoxia, mean oxygen consumption remained overall low, with no differences detected between ovigerous and non-ovigerous females (Fig. 4).

Table 2
Results of the gamma generalized linear model used to evaluate the effect of experimental combinations of temperature (16 and 20°C) and ambient oxygen condition (normoxia and hypoxia) on oxygen consumption of ovigerous and non-ovigerous females of *Romaleon setosum*. Statistically significant effects ($P < 0.05$) are shown in bold

Treatment	Estimate	Std. Error	z value	Pr(>|t|)	Residual deviance
Reproductive condition	-0.2595	0.06066	-4.278	**0.000107**	80.667
Temperature	-0.13745	0.06066	-2.266	**0.028662**	
Experimental oxygen condition	0.16206	0.06066	2.672	**0.010693**	
Reproductive condition x temperature	0.05531	0.06066	0.912	0.367035	
Reproductive condition x experimental oxygen condition	-0.28626	0.06066	-4.719	**0.0000263**	
Temperature x experimental oxygen condition	-0.21777	0.06066	-3.590	**0.000858**	
Reproductive condition x temperature x experimental oxygen condition	-0.01784	0.06066	-0.294	0.770166	

Discussion

This study contributes to understanding the influence of globally changing factors on behaviors associated with oxygen provision in species that aggregate and protect embryos. Rather than proposing speculative future scenarios for which organisms may lack adaptive responses, our approach was to examine extreme conditions during the reproductive season in the study area, but that have been reported across the species' distributional range. Overall, the main findings are: (a) Low average oxygen levels (< 52% air saturation) at the center of embryo aggregations under normoxic environmental conditions, particularly as temperature increased (reaching values as low as 32%), (b) Under normoxic conditions, ovigerous females responded to the increasing oxygen demand of embryos at higher temperatures by intensifying behaviors associated with oxygen provision; nevertheless, this response did not fully compensate for the increased demand, as evidenced by a 3.5-fold increase in the time embryos spent under hypoxia (1.8 mg L^{-1}) at the highest experimental temperature; and (c) a clear association between the time allocated to oxygen-provision behaviors, their frequency, and female oxygen

consumption, reinforcing previous findings on the costs of reproduction (e.g., Baeza and Fernández 2002; Brante et al. 2003). Importantly, this study provides novel evidence on oxygen-provision behaviors under ambient hypoxia, which differ from those previously described under normoxic conditions: under hypoxic conditions, behaviors associated with oxygen provision were not influenced by temperature. Together, these findings raise important questions about whether metabolic constraints in brooding females of *R. setosum* limit the scaling of behaviors required to maintain adequate oxygen supply, and how rising temperatures and deoxygenation may ultimately influence reproductive patterns in species that rely on active oxygen provision. The study also addresses two biases in research on globally environmental change affecting marine life: (a) deoxygenation, a comparatively neglected stressor (Sampaio et al. 2021), and (b) the sensitivity of early life-history stages in species that aggregate embryos (e.g., Pandori et al. 2019).

The results of this study contribute to the growing body of evidence on oxygen limitation within embryo aggregations of marine invertebrates. Although oxygen levels within the embryo mass under normoxic ambient conditions were variable, the mean percentage of air saturation recorded at the center of the aggregations (mean ± SE = 51.7 ± 7.4% and 31.5 ± 6.2% at 16 and 20°C, respectively) is remarkably consistent with values reported for other taxonomic groups, aggregation types, and forms during late developmental stages. For instance, air saturation in gelatinous aggregations of opisthobranchs ranges between 30 and 50% (Cohen and Strathmann 1996) with similar values reported for species that encapsulate embryos (30–50%, Lardies and Fernández 2002; 40–60%, Fernández et al. 2006b), and even lower levels in nudibranchs (~ 30%; Moran and Woods 2007) under normoxic ambient conditions. Notably, these levels of air saturation are reached at sizes approximately an order of magnitude smaller in species that encapsulate or aggregate embryos in gelatinous masses than in those providing active parental care, such as those examined here (Cohen and Strathmann 1996; Lardies and Fernández 2002; Fernández et al. 2006a). It is important to note that reduced oxygen levels (70–80% air saturation) have been documented even at early developmental stages under ambient normoxic conditions in embryo aggregation of species that rely on diffusion for oxygen provision (e.g., Cohen and Strathmann 1996; Moran and Woods 2007). Differences in oxygen conditions at the center of embryo masses between early and late developmental stages are consistent with increases in embryo oxygen consumption, which can raise 4–to 10–fold over development (e.g., Baeza and Fernández 2002; Moran and Woods 2007). The consistency of oxygen patterns across studies, spanning diverse aggregation types, sizes, and mechanisms facilitating oxygen transport from the periphery to the center of embryo masses–highlights the need for a comparative and integrative analysis.

This study also addresses a much less explored aspect: oxygen dynamics within embryo aggregations under environmental hypoxia, a condition that is increasing globally and is widely reported in coastal areas (e.g., Chan et al. 2008; Gilbert et al. 2010). As expected, our results show that under hypoxic experimental conditions, embryos at the center of the aggregation experience hypoxia nearly 100% of the time. Notably, air saturation levels at the center (5–6%) of the aggregations are substantially lower than those in the surrounding hypoxic water (18%) at both temperatures. These findings suggest that embryos continue to consume oxygen under ambient hypoxia, although at markedly lower rates than

under normoxic ambient conditions. Based on previous studies on *R. setosum* (Baeza and Fernández 2002), oxygen consumption at this air saturation levels would be approximately 6–fold lower than that expected under ambient normoxia (30–50% air saturation at the center of the aggregation). Together, these patterns raise important questions regarding the effects of hypoxia on oxygen-provision behaviors, the associated reproductive costs, and the consequences for embryonic and larval development.

The behaviors analyzed–those directly (flapping) or indirectly (pereopod use, standing position) associated with oxygen provision in brachyuran crabs (Baeza and Fernández 2002)–showed a clear general response, increasing with temperature only under normoxic ambient conditions. This trend was reflected not only in the total time allocated to these behaviors but, in some cases, also in their frequency. In particular, the frequency of pereopod insertion increased with temperature, a behavior associated with sensing the condition of the embryo mass, along with an increase in flapping frequency (number of events over the observation period), consistent with previous studies in brachyurans (Baeza and Fernández 2002; Brante et al. 2003). To better characterize these responses, we introduced a new metric, recognizing that frequency alone does not characterize individual flapping episodes. Both flapping frequency and intensity followed the same pattern, indicating that under normoxic conditions ovigerous females not only invest more time in flapping, but also perform this behavior more frequently and with greater intensity as temperature increases. This general pattern of increased behavioral activity with temperature has been reported previously in Brachyuran crabs (Brante et al. 2003) and may help explain why embryo masses up to 20 times larger than the gelatinous masses of opisthobranchs or gastropod capsules discussed earlier exhibit similar oxygen conditions. However, comparison of oxygen conditions between the two temperatures indicates that the increase in time and frequency of behaviors performed by ovigerous females is insufficient to meet embryonic oxygen demand: mean air saturation within the aggregation is 60% lower at 20 than at 16°C. Moreover, the proportion of time that embryos experience hypoxia (< 18% air saturation) under normoxic ambient conditions is 3.5 times higher at 20°C than at 16°C. These results indicate that, although females adjust oxygen-provision behaviors in response to embryonic requirements, such adjustments are not sufficient to compensate for increased demand. This raises important questions as to whether metabolic constraints in brooding females limit the scaling of these behaviors required to maintain adequate oxygen supply, and how rising temperatures and deoxygenation may ultimately influence reproductive patterns in species that rely on active oxygen provision.

A distinctive contribution of this study is the analysis of behavioral patterns under ambient hypoxia, which differ markedly from those previously reported under normoxia (e.g., Baeza and Fernández 2002; Fernández et al. 2002; Brante et al. 2003) and remain poorly explored in other types of embryo aggregations (but see Lardies and Fernández 2002; Fernández et al. 2006b; Cancino et al. 2003). Interestingly, neither the time allocated to most oxygen-provision behaviors nor their frequency is affected by temperature under ambient hypoxia. These findings raise the question of why flapping frequency and duration do not increase under environmental hypoxia. The observed patterns in flapping frequency contrast with previous studies showing that females carrying early-stage embryos substantially increased flapping frequency in response to experimentally induced hypoxia within the

embryo mass (i.e., injection of hypoxic water), while remaining in a normoxic ambient (Fernández et al. 2002). These results have been interpreted as evidence that females can sense oxygen conditions within the aggregation–or metabolites produced by embryos under hypoxia–and exhibit compensatory oxygen-provision behaviors (Fernández et al. 2002). A similar response would therefore have been expected in the present study. The absence of such compensatory responses under ambient hypoxia suggests that metabolic constraints limit the upregulation of parental care behaviors associated with oxygen provision.

The patterns of oxygen consumption in females indicate that they can withstand periods of hypoxia that sporadically occur in coastal environments without impairing aerobic metabolism: oxygen consumption rates do not differ between normoxia and hypoxia in non-ovigerous females at the temperatures analyzed. In contrast, maternal metabolic investment per reproductive event increases with temperature under normoxic conditions, consistent with the observed behavioral responses. The agreement between these findings and previous studies reporting increased oxygen consumption in ovigerous females with temperature suggests that the costs of carrying embryo masses may rise substantially under global warming scenarios, potentially altering the trade-off between investment in embryos and their protection (Brante et al. 2003). Consistent with behavioral patterns, no additional metabolic costs associated with parental care are evident under hypoxia. However, the costs of hypoxia may instead be expressed through reduced embryonic developmental success, which was not addressed in this study.

Although the consequences of hypoxia for embryonic development were not addressed in this study, embryos generally appear to possess adaptations that allow development in aggregations at oxygen levels substantially lower than those of the normoxic waters they inhabit (Cohen and Strathmann 1996; Lardies and Fernández 2002; Fernández et al. 2006a). Nevertheless, several effects have been documented: (a) reduced metabolic rates as oxygen partial pressure decreases; (b) slower developmental rates in aggregated embryos or larvae compared to isolated ones at similar stages and temperatures (e.g., Brante et al. 2003; Moran and Woods 2007); and (c) delayed development of central embryos relative to peripheral ones, observed both in thin ribbons of nudibranchs and in small gelatinous masses that rely on passive diffusion (Chaffee and Strathmann 1984; Strathmann and Strathmann 1995), as well as in larger masses of species that actively provide oxygen (Fernández et al. 2003). Importantly, under normoxic conditions, the degree of developmental asynchrony between peripheral and central embryos is much lower in species that exhibit oxygen-provision behaviors than in those that rely on passive diffusion (Chaffee and Strathmann 1984; Strathmann and Strathmann 1995; Cohen and Strathmann 1996; Baeza and Fernández 2002). However, under hypoxic conditions, the consequences for development may differ. Indeed, increased mortality, developmental delays, and changes in the proportion of encapsulated embryos that successfully develop have been reported, depending on oxygen levels and the duration of hypoxic experimental exposure (Lardies and Fernández 2002; Fernández et al. 2006b; Cancino et al. 2003). Under constant conditions of 75% air saturation in seawater, with intracapsular air saturation fluctuating between 25 and 50%, development in *Acanthina monodon* is not affected (Lardies and Fernández 2002), again highlighting embryonic tolerance to oxygen limitation. However, when ambient oxygen decreases to a constant 50% air saturation, a large proportion of embryos fail to develop (Fernández et al. 2006b). Similarly, constant exposure to 50% air saturation

affects intracapsular developmental rates in *Chorus giganteus*, although this effect disappears when hypoxia is interspersed with periods of normoxia in the surrounding water (Cancino et al. 2003). Taken together, these lines of evidence, along with the results of this study, highlight the importance of advancing our understanding of embryonic development, developmental asynchrony, and embryo mortality across different types and forms of embryo aggregations in an ocean that, across much of the planet, is both warming and becoming deoxygenated.

Declarations

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Authors contributions Miriam Fernández conceived the original idea, participated in the planning of the experiments, provided overall guidance and supervision, and prepared the first draft of the manuscript. Eloísa Garrido Moller, Karina Perez-Pezoa, Pierina Virinella and Beatriz Esnard Hernández participated in the planning of the experiment, experimental set up and data collection. Eloísa Garrido Moller and Karina Perez-Pezoa conducted statistical analysis. All authors contributed to earlier versions of the manuscript and approved the final version of the manuscript.

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Data availability Data and statistical codes have not yet been provided, but if this paper is accepted, they will be available on reasonable request.

Competing interests The authors declare no conflicts of interest.

Ethical approval The study received approval from the ethics committee of the Pontificia Universidad Católica de Chile (ID 210622021).

References

1. Abraham JP, Baringer M, Bindoff NL, Boyer T, Cheng LJ, Church JA, Conroy JL, Domingues CM, Fasullo JT, Gilson J, Goni G, Good SA, Gorman JM, Gouretski V, Ishii M, Johnson GC, Kizu S, Lyman JM, Macdonald AM, Minkowycz WJ, Moffitt SE, Palmer MD, Piola AR, Reseghetti F, Schuckmann K, Trenberth KE, Velicogna I, Willis JK (2013) A review of global ocean temperature observations: implications for ocean heat content estimates and climate change. Rev Geophys 51:450–483. https://doi.org/10.1002/rog.20022

2. Aguirre C, García-Loyola S, Testa G, Silva D, Farías L (2018) Insight into anthropogenic forcing on coastal upwelling off south-central Chile. Elem Sci Anth 6:59. https://doi.org/10.1525/elementa.314
3. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723. https://doi.org/10.1109/TAC.1974.1100705
4. Baeza JA, Fernández M (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. Funct Ecol 16:241–251. https://doi.org/10.1046/j.1365-2435.2002.00616.x
5. Bonat WH, Kokonendji CC (2017) Flexible Tweedie regression models for continuous data. J Stat Comput Simul 87:2138–2152. https://doi.org/10.1080/00949655.2017.1318876
6. Brante A, Fernández M, Eckerle L, Mark F, Pörtner H-O, Arntz W (2003) Reproductive investment in the crab *Cancer setosus* along a latitudinal cline: egg production, embryo losses and embryo ventilation. Mar Ecol Prog Ser 251:221–232. https://doi.org/10.3354/meps251221
7. Bretz F, Hothorn T, Westfall P (2008) Multiple comparison procedures in linear models. In: Brito P (ed) COMPSTAT 2008. Physica-Verlag HD, Heidelberg, pp 423–431. https://doi.org/10.1007/978-3-7908-2084-3_35
8. Brill RW, Bushnell PG, Elton TA, Small HJ (2015) The ability of blue crab (*Callinectes sapidus*, Rathbun 1886) to sustain aerobic metabolism during hypoxia. J Exp Mar Biol Ecol 471:126–136. https://doi.org/10.1016/j.jembe.2015.06.003
9. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400. https://doi.org/10.32614/RJ-2017-066
10. Cancino JM, Gallardo JA, Torres FA (2003) Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus*. Mar Biol 142:133–139. https://doi.org/10.1007/s00227-002-0925-3
11. Cancino JM, Gallardo JA, Brante A (2010) The relationship between temperature, oxygen condition and embryo encapsulation in the marine gastropod *Chorus giganteus*. J Mar Biol Assoc UK 91:727–733. https://doi.org/10.1017/S0025315410001335
12. Chaffee C, Strathmann RR (1984) Constraints on egg masses. I. Retarded development within thick egg masses. J Exp Mar Biol Ecol 84:73–83. https://doi.org/10.1016/0022-0981(84)90231-4
13. Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, Peterson WT, Menge BA (2008) Emergence of anoxia in the California Current Large Marine Ecosystem. Science 319:920. https://doi.org/10.1126/science.1149016
14. Cheng L, Abraham J, Trenberth KE, Reagan J, Zhang H-M, Storto A, von Schuckmann K, Pan Y, Zhu Y, Mann ME, Zhu J, Wang F, Yu F, Locarnini R, Fasullo J, Huang B, Graham G, Yin X, Gouretski V, Zheng F, Li Y, Zhang B, Wan L, Chen X, Wang D, Feng L, Song X, Liu Y, Reseghetti F, Simoncelli S, Chen G, Zhang R, Mishonov A, Tan Z, Wei W, Yuan H, Li G, Ren Q, Cao L, Lu Y, Du J, Lyu K, Sulaiman A, Mayer M, Wang H, Ma Z, Bao S, Yan H, Liu Z, Yang C, Liu X, Hausfather Z, Szekely T, Gues F (2025) Record

high temperatures in the ocean in 2024. Adv Atmos Sci 42:1092–1109. https://doi.org/10.1007/s00376-025-4541-3

15. Cohen CS, Strathmann RR (1996) Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. Biol Bull 190:8–15. https://doi.org/10.2307/1542671
16. De La Maza L, Farías L (2023) The intensification of coastal hypoxia off central Chile: long term and high frequency variability. Front Earth Sci 10:929271. https://doi.org/10.3389/feart.2022.929271
17. Fernández M, Brante A (2003) Brood care in brachyuran crabs: the effect of oxygen provision on reproductive costs. Rev Chil Hist Nat 76:157–168. https://doi.org/10.4067/S0716-078X2003000200003
18. Fernández M, Pardo L, Baeza J (2002) Patterns of oxygen supply in embryo masses of brachyuran crabs throughout development: the effect of oxygen availability and chemical cues in determining female brooding behavior. Mar Ecol Prog Ser 245:181–190. https://doi.org/10.3354/meps245181
19. Fernández M, Ruiz-Tagle N, Cifuentes S, Pörtner H-O, Arntz W (2003) Oxygen-dependent asynchrony of embryonic development in embryo masses of brachyuran crabs. Mar Biol 142:559–565. https://doi.org/10.1007/s00227-002-0965-8
20. Fernández M, Calderón R, Cifuentes M, Pappalardo P (2006a) Brooding behaviour and cost of brooding in small body size brachyuran crabs. Mar Ecol Prog Ser 309:213–220. https://doi.org/10.3354/meps309213
21. Fernández M, Pappalardo P, Jeno K (2006b) El efecto de la temperatura y la disponibilidad de oxígeno sobre el desarrollo intracapsular de *Acanthina monodon* (Gastropoda: Muricidae). Rev Chil Hist Nat 79:155–167. https://doi.org/10.4067/S0716-078X2006000200002
22. Gilbert D, Rabalais NN, Díaz RJ, Zhang J (2010) Evidence for greater oxygen decline rates in the coastal ocean than in the open ocean. Biogeosciences 7:2283–2296. https://doi.org/10.5194/bg-7-2283-2010
23. Gobler CJ, Baumann H (2016) Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. Biol Lett 12:20150976. https://doi.org/10.1098/rsbl.2015.0976
24. Harvey BP, Gwynn-Jones D, Moore PJ (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. Ecol Evol 3:1016–1030. https://doi.org/10.1002/ece3.516
25. IPCC (2023) Summary for Policymakers. In: *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, Lee H, Romero J (eds)]. IPCC, Geneva, pp 1–34. https://doi.org/10.59327/IPCC/AR6-9789291691647.001
26. Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso J-P (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob Change Biol 19:1884–1896. https://doi.org/10.1111/gcb.12179

27. Kwiatkowski L, Torres O, Bopp L, Aumont O, Chamberlain M, Christian JR, Dunne JP, Gehlen M, Ilyina T, John JG, Lenton A, Li H, Lovenduski NS, Orr JC, Palmieri J, Santana-Falcón Y, Schwinger J, Séférian R, Stock CA, Tagliabue A, Takano Y, Tjiputra J, Toyama K, Tsujino H, Watanabe M, Yamamoto A, Yool A, Ziehn T (2020) Twenty-first century ocean warming, acidification, deoxygenation, and upper-ocean nutrient and primary production decline from CMIP6 model projections. Biogeosciences 17:3439–3470. https://doi.org/10.5194/bg-17-3439-2020
28. Lardies MA, Fernández M (2002) Effect of oxygen availability in determining clutch size in *Acanthina monodon*. Mar Ecol Prog Ser 239:139–146. https://doi.org/10.3354/meps239139
29. Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw 69:1–33. https://doi.org/10.18637/jss.v069.i01
30. Llabrés M, Agustí S, Fernández M, Canepa A, Maurin F, Vidal F, Duarte CM (2013) Impact of elevated UVB radiation on marine biota: a meta-analysis. Glob Ecol Biogeogr 22:131–144. https://doi.org/10.1111/j.1466-8238.2012.00784.x
31. Lucey NM, Lombardi C, DeMarchi L, Schulze A, Gambi MC, Calosi P (2015) To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification? Sci Rep 5:12009. https://doi.org/10.1038/srep12009
32. Maldonado ST (2019) Parámetros reproductivos en la jaiba comercial *Romaleon setosum* (Molina, 1782) en el centro-sur de Chile. Undergraduate thesis, Universidad Austral de Chile.
33. McCullagh P (2019) *Generalized linear models*, 2nd edn. Routledge, New York. https://doi.org/10.1201/9780203753736
34. Molina JI (1782) *Saggio sulla storia naturale del Chili*. Tommaso d'Aquino, Bologna.
35. Moran AL, Woods HA (2007) Oxygen in egg masses: interactive effects of temperature, age, and egg-mass morphology on oxygen supply to embryos. J Exp Biol 210:722–731. https://doi.org/10.1242/jeb.02702
36. Muñoz R, Tapia FJ, Sobarzo M (2025) Temporal variability of bottom hypoxia in open and semi-enclosed coastal areas in an upwelling region. Prog Oceanogr 233:103435. https://doi.org/10.1016/j.pocean.2025.103435
37. Pandori LLM, Sorte CJB (2019) The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. Oikos 128:621–629. https://doi.org/10.1111/oik.05886
38. Pasquier B, Holzer M, Chamberlain MA, Matear RJ, Bindoff NL (2024) Deoxygenation and its drivers analyzed in steady state for perpetually slower and warmer oceans. J Geophys Res Oceans 129:e2024JC021043. https://doi.org/10.1029/2024JC021043
39. Pechenik JA (1982) Ability of some gastropod egg capsules to protect against low-salinity stress. J Exp Mar Biol Ecol 63:195–208. https://doi.org/10.1016/0022-0981(82)90178-2
40. Perron FE, Corpuz GC (1982) Costs of parental care in the gastropod *Conus pennaceus:* age-specific changes and physical constraints. Oecologia 55:319–324. https://doi.org/10.1007/BF00376919
41. Retamal MA (1981) *Catálogo ilustrado de los crustáceos decápodos de Chile*. Universidad de Concepción, Concepción.

42. R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
43. Sampaio E, Santos C, Rosa IC, Ferreira V, Pörtner H-O, Duarte CM, Levin LA, Rosa R (2021) Impacts of hypoxic events surpass those of future ocean warming and acidification. Nat Ecol Evol 5:311–321. https://doi.org/10.1038/s41559-020-01370-3
44. Schmidtko S, Stramma L, Visbeck M (2017) Decline in global oceanic oxygen content during the past five decades. Nature 542:335–339. https://doi.org/10.1038/nature21399
45. Strathmann RR, Chaffee C (1984) Constraints on egg masses. II. Effect of spacing, size, and number of eggs on ventilation of masses of embryos in jelly, adherent groups, or thin-walled capsules. J Exp Mar Biol Ecol 84:85–93. https://doi.org/10.1016/0022-0981(84)90232-6
46. Strathmann RR, Strathmann MF (1982) The relationship between adult size and brooding in marine invertebrates. Am Nat 119:91–101. https://doi.org/10.1086/283892
47. Strathmann RR, Strathmann MF (1995) Oxygen supply and limits on aggregation of embryos. J Mar Biol Assoc UK 75:413–428. https://doi.org/10.1017/S0025315400018270
48. Thiel M, Macaya E, Acuña E, Arntz W, Bastias H, Brokordt K, Camus P, Castilla J, Castro L, Cortes M, Dumont C, Escribano R, Fernández M, Gajardo J, Gaymer C, Gómez I, González A, González HE, Haye P, Vega JMA (2007) The Humboldt Current System of northern and central Chile. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) *Oceanography and marine biology: an annual review*, vol 45. CRC Press, Boca Raton, pp 195–345.
49. Vaquer-Sunyer R, Duarte CM (2008) Thresholds of hypoxia for marine biodiversity. Proc Natl Acad Sci USA 105:15452–15457. https://doi.org/10.1073/pnas.0803833105
50. Vaquer-Sunyer R, Duarte CM (2011) Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. Glob Change Biol 17:1788–1797. https://doi.org/10.1111/j.1365-2486.2010.02343.x
51. Vargas LM (1995) Descripción del desarrollo embrionario de Cancer polyodon Poepping, 1836 (Crustacea: Decapoda: Brachyura) y efectos de la temperatura, bajo condiciones de laboratorio. Undergraduate thesis, Universidad Católica del Norte.
52. von Schuckmann K, Cheng L, Palmer MD, Hansen J, Tassone C, Aich V, Adusumilli S, Beltrami H, Boyer T, Cuesta-Valero FJ, Desbruyères D, Domingues CM, García-García A, Gentine P, Gilson J, Gorfer M, Haimberger L, Ishii M, Johnson GC, Killick R, King BA, Kirchengast G, Kolodziejczyk N, Lyman J, Marzeion B, Mayer M, Monier M, Monselesan DP, Purkey S, Roemmich D, Schweiger A, Seneviratne SI, Shepherd A, Slater DA, Steiner AK, Straneo F, Timmermans M-L, Wijffels SE (2020) Heat stored in the Earth system: where does the energy go? Earth Syst Sci Data 12:2013–2041. https://doi.org/10.5194/essd-12-2013-2020
53. Wei Q, Xue L, Yao Q, Wang B, Yu Z (2021) Oxygen decline in a temperate marginal sea: contribution of warming and eutrophication. Sci Total Environ 757:143227. https://doi.org/10.1016/j.scitotenv.2020.143227

54. Wolff M, Cerda G (1992) Feeding ecology of the crab *Cancer polyodon* in La Herradura Bay, northern Chile. I. Feeding chronology, food intake, and gross growth and ecological efficiency. Mar Ecol Prog Ser 89:213–219. https://doi.org/10.3354/meps089213
55. Zuur AF, Ieno EN (2021) Using GLM. Highland Statistics Ltd, Newburgh.

Figures

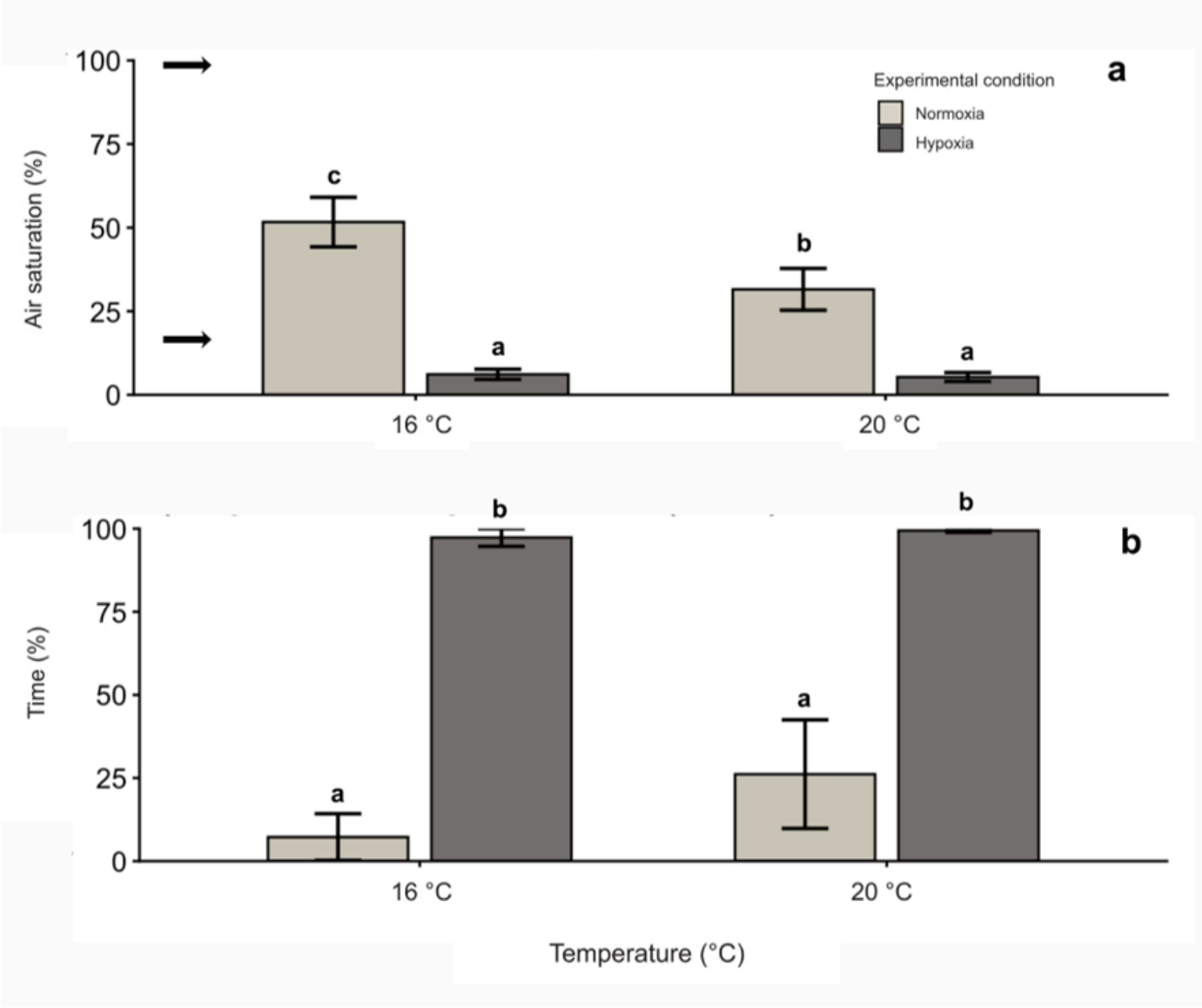


Figure 1

Mean (a) percentage of air saturation and (b) percentage of recording time with <18% air saturation at the center of the embryo mass of ovigerous females of *Romaleon setosum* at two experimental temperatures (16 and 20 °C) under normoxic and hypoxic ambient conditions. Values represent means ± SE. Arrows indicate experimental oxygen conditions. Different letters indicate significant differences among treatments based on Tukey post hoc multiple comparisons ($P < 0.05$)

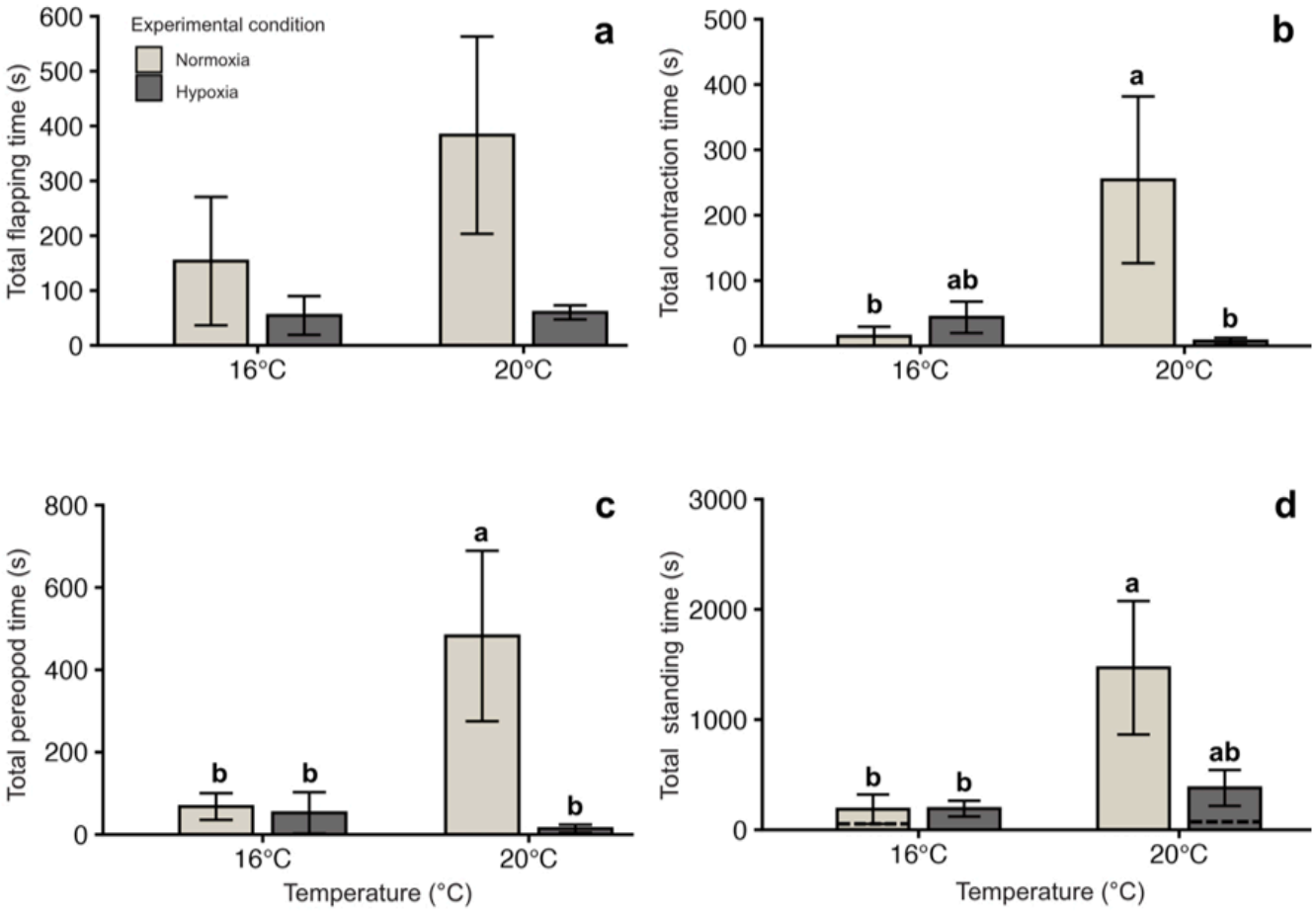


Figure 2

Mean total time allocated to behaviors directly and indirectly related to oxygen provision in ovigerous females of *Romaleon setosum* at two experimental temperatures (16 and 20 °C) under normoxic and hypoxic ambient conditions. The behaviors analyzed were (a) flapping, (b) contraction of the embryonic mass, (c) use of pereiopod, and (d) standing position (broken lines indicates the mean behavior recorded in non-ovigerous females). Bars represent mean ± SE. Different letters indicate significant differences among treatments based on Tukey post hoc multiple comparisons ($P < 0.05$)

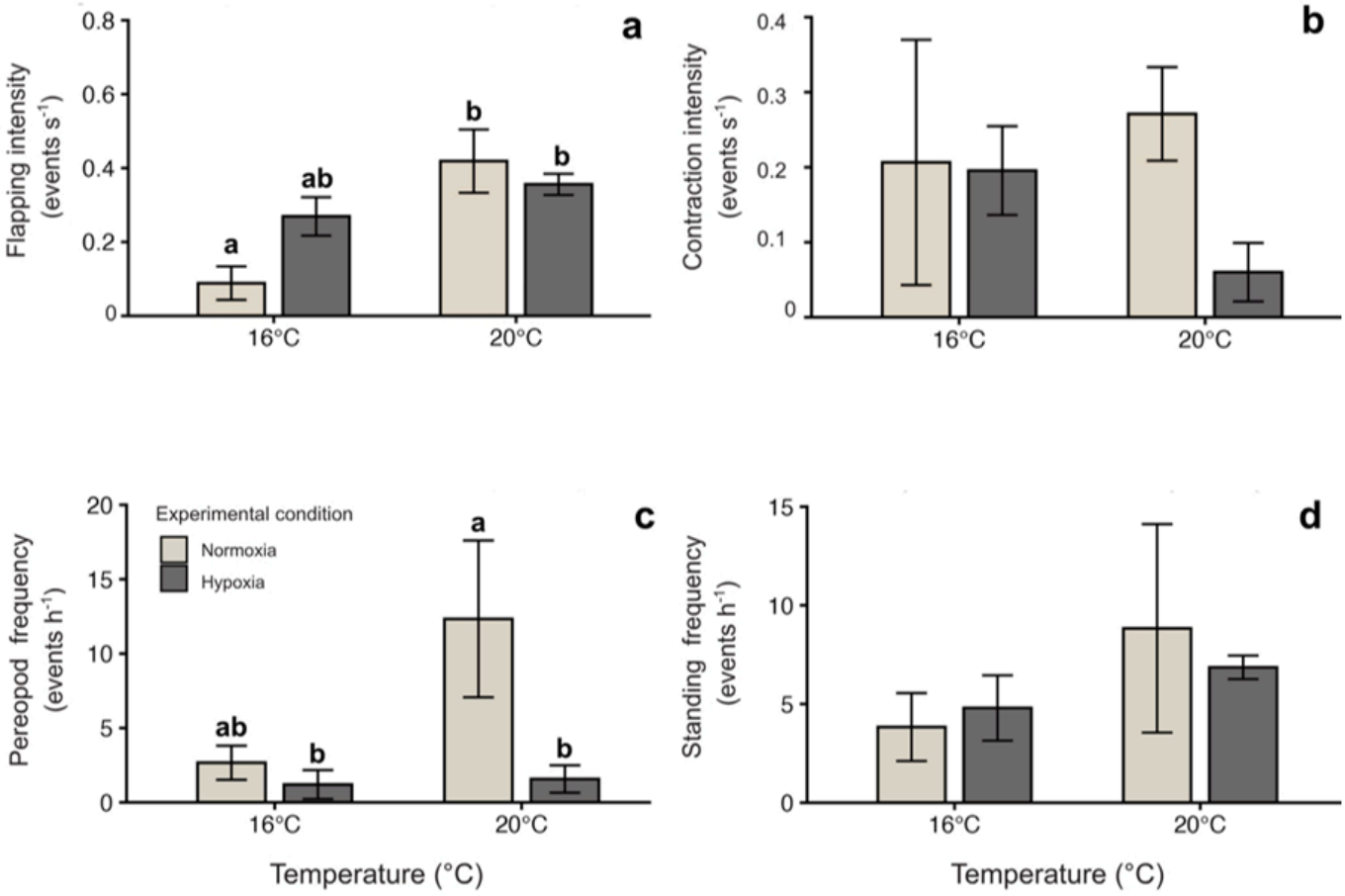


Figure 3

Frequency of occurrence of parental behaviors in ovigerous females of *Romaleon setosum* at two experimental temperatures (16 and 20 °C) under normoxic and hypoxic ambient conditions. For two behaviors, frequency is expressed as number of events during the time in which the behavior occurred, reflecting its intensity: (A) flapping and (B) contraction of the embryo mass. For the remaining behaviors, frequency is reported as the number of events h^{-1} of behavioral observation. Bars represent mean ± SE. Different letters indicate significant differences among treatments based on Tukey post hoc multiple comparisons ($P < 0.05$)

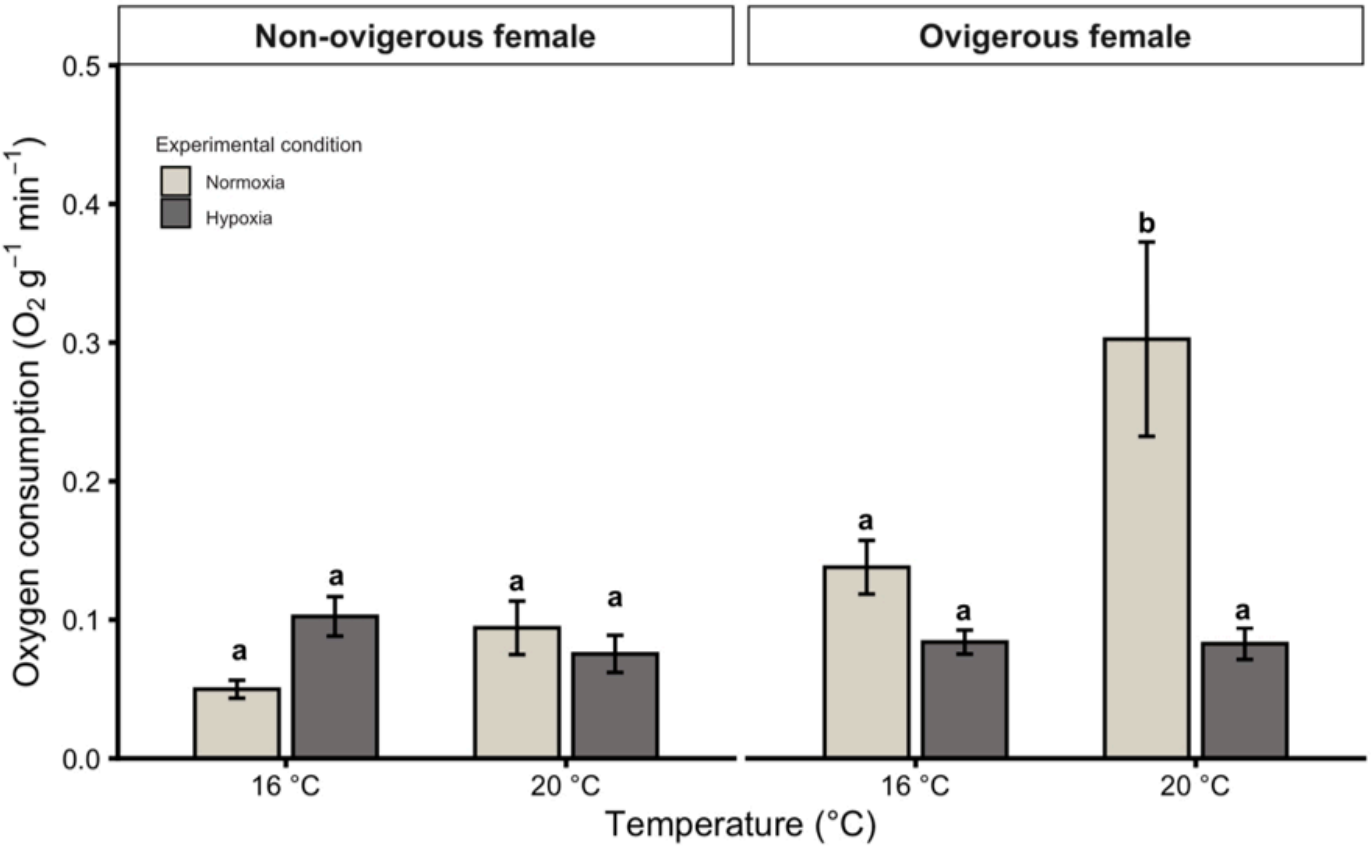


Figure 4

Oxygen consumption rate (μmol O_2 g^{-1} min^{-1}) of ovigerous and non-ovigerous females of *Romaleon setosum* at 16 and 20 °C under normoxic and hypoxic ambient conditions. Bars represent mean ± SE. Different letters indicate significant differences among treatments based on post hoc comparisons ($P < 0.05$)