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Correlated Effects of Ocean Acidification and Warming on Behavioral and Metabolic Traits of a Large Pelagic Fish

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Abstract: Ocean acidification and warming are co-occurring stressors, yet their effects on early life stages of large pelagic fishes are not well known. Here, we determined the effects of elevated CO_2 and temperature at levels projected for the end of the century on activity levels, boldness, and metabolic traits (i.e., oxygen uptake rates) in larval kingfish (*Seriola lalandi*), a large pelagic fish with a circumglobal distribution. We also examined correlations between these behavioral and physiological traits measured under different treatments. Kingfish were reared from the egg stage to 25 days post-hatch in a full factorial design of ambient and elevated CO_2 (~500 µatm and ~1000 µatm) and temperature (21 °C and 25 °C). Activity levels were higher in fish from the elevated temperature treatment compared with fish reared under ambient temperature. However, elevated CO_2 did not affect activity, and boldness was not affected by either elevated CO_2 or temperature. Both elevated CO_2 and temperature resulted in increased resting oxygen uptake rates compared to fish reared under ambient conditions, but neither affected maximum oxygen uptake rates nor aerobic scope. Resting oxygen uptake rates and boldness were negatively correlated under ambient temperature, but positively correlated under elevated temperature. Maximum oxygen uptake rates and boldness were also negatively correlated under ambient temperature. These findings suggest that elevated temperature has a greater impact on behavioral and physiological traits of larval kingfish than elevated CO_2. However, elevated CO_2 exposure did increase resting oxygen uptake rates and interact with temperature in complex ways. Our results provide novel behavioral and physiological data on the responses of the larval stage of a large pelagic fish to ocean acidification and warming conditions, demonstrate correlations between these traits, and suggest that these correlations could influence the direction and pace of adaptation to global climate change.

Keywords: physiology; behavior; temperature; CO_2; climate change; yellowtail kingfish; *Seriola lalandi*

1. Introduction

The oceans are becoming more acidic due to increased uptake of anthropogenic carbon dioxide from the atmosphere [1]. The dissolution of additional CO_2 into oceanic surface waters causes a decrease in oceanic pH through a process known as ocean acidification [2]. There is now an extensive body of literature assessing biological responses to ocean acidification across a wide range of marine species [3–5]. These single-stressor studies provide useful indications of large-scale trends in response to acidification, as well as the mechanisms underlying these responses. However, marine organisms are subject to multiple co-occurring stressors, which might interact in diverse ways. Ocean warming is an ecologically-relevant stressor that co-occurs with acidification [6]; yet, predicting the combined impacts of ocean acidification and warming is not straightforward, as elevated CO_2 and temperature have been found to interact both synergistically and antagonistically on marine organisms [7,8].

Previous studies have investigated the effects of ocean acidification on marine fishes, finding effects on metabolic rate, behavioral performance, reproductive output, otolith growth, and sensory responses in some species, but not others [9–12]. More recently, studies have begun to investigate the interacting effects of ocean acidification and warming on marine fishes, finding they may mitigate, reverse, or enhance the effects of elevated CO_2. For instance, Domenici et al. [13] found that elevated CO_2 and temperature have different and interacting effects on behavioral lateralization in reef fishes, whereas Munday et al. [14] found that elevated CO_2 and temperature have additive effects on metabolic rates in two species of cardinalfishes. Other studies point towards temperature having a greater overall effect on ontogenetic development, swimming ability [15], and the outcome of predator-prey interactions [16] compared with elevated CO_2. Therefore, it is necessary to consider the interacting effect of warming to properly understand the effects of ocean acidification on fishes and predict outcomes for the future.

Among the existing experimental research on the effects of ocean acidification and warming on marine fishes, large pelagic fishes have been relatively understudied. This represents a critical knowledge gap, as large pelagic fishes are both ecologically and economically important. As abundant top predators, they can impact the structure and functioning of the marine ecosystem, and have strong top-down influences on marine food webs [17,18]. They are also a critical food source for millions of people in coastal regions worldwide, and constitute a large proportion of wild-caught fisheries [19]. Furthermore, large pelagic fishes are hypothesized to be more susceptible to ocean acidification and warming due to the relatively stable environments they experience in open waters [20,21] when compared to the highly fluctuating temperature and pH conditions of coastal and shallow water habitats [22,23]. Thus, their absence from the literature represents a critical avenue for climate change research, and motivated our study.

We focused on the larval stages of a large pelagic fish. The larval stage is one of the most vulnerable, yet critically important, stages in the development of marine fishes. Larval fishes are subject to high mortality rates due in part to predation, as well as environmental effects on growth [24]. The dynamics of the larval phase can influence patterns of population replenishment and connectivity in adult populations [25,26]. Additionally, larval fishes are presumed to be more vulnerable to changes in temperature and pH than adults, possibly due to their larger surface area-to-volume ratio, which makes them more susceptible to environment perturbations [27,28]. Therefore, understanding how elevated CO_2 and temperature affect larval pelagic fishes could have broader implications for how adult populations might be affected by climate change.

Here, we tested the effects of elevated CO_2 and temperature on key behavioral and physiological traits during the larval stage in yellowtail kingfish, *Seriola lalandi*, from New Zealand. We used a cross-factored experiment that comprised current-day ambient CO_2 levels (~500 µatm) and average summer temperature for the study location (21 °C), crossed with elevated CO_2 (~1000 µatm) and temperature (25 °C) based on projections for the open ocean by the end of the century under RCP 8.5 [1,29]. The specific traits we focused on were routine activity, boldness, and metabolic performance, and we also examined correlations between these traits.

Routine activity is a commonly examined behavioral trait in ocean acidification studies of larval fishes. Ecologically, activity levels are relevant because increased activity has been shown to increase feeding and growth rates in fishes and other animals, but decrease survivorship due to higher incidences of predation [30–32]. In previous studies, the routine activity of large pelagic fishes has been mostly unaffected by elevated CO_2 levels ranging from 800 to 2100 µatm [33–36]. However, one species, mahi mahi *Coryphaena hippurus*, did exhibit a decrease in swimming duration upon exposure to 1600 µatm CO_2 [37], and a decrease in maximum swimming velocity upon exposure to 1460 µatm CO_2 [34]. By contrast, temperature is well known to increase activity in marine fishes [38,39]. The mechanism for this relationship is not well known, though it has been suggested that temperature influences activity indirectly through its effect on metabolic rate [40,41].

We also measured boldness, or the propensity to take risks, which is another commonly measured behavioral trait in fishes [42–44]. As with increased activity, increased boldness in fishes has been linked to decreased survivorship through higher rates of predation [30,45], and also higher likelihood of being captured by fishing gear [46]. Boldness has not been specifically studied with respect to CO_2 effects on early life stages of large pelagic fishes, but other ocean acidification studies on marine fishes have found boldness to either increase [45] or decrease [47] under elevated CO_2. Temperature has also been shown to increase boldness in marine fishes [38,48]. As with activity, the relationship between temperature and boldness has been suggested to be driven by the direct impact of temperature on metabolic rate [38,49].

Our physiological trait of interest was metabolic performance, which we approximated by measuring maximal and resting oxygen uptake rates, and calculating aerobic scope, or the difference between these two values. Metabolic traits reveal the energetic requirements of organisms, and are therefore assumed to underpin a range of fitness-related traits [50]. For instance, maximal and resting oxygen uptake rates have been correlated with swimming performance and foraging success in fishes [51,52]. Studies to date show mixed effects of elevated CO_2 on maximum and resting oxygen uptake rates in marine fishes [11,53,54]. While few studies have examined the effects of elevated CO_2 on metabolic performance in larval pelagic fishes, Pimentel et al. [37] did find a reduction in oxygen uptake rates of mahi mahi upon exposure to 1600 µatm CO_2. By comparison, temperature has been well established to affect metabolic traits in marine fishes through its effect on rates of biochemical reactions [55]. Furthermore, a recent meta-analysis [11] indicates that elevated temperature generally has a greater effect than elevated CO_2 on metabolic traits of marine fishes.

In addition to documenting mean trends across activity, boldness, and metabolic performance, we also tracked individual fish through all assays to determine whether correlations exist between traits at the individual level. Historically, individual variation has often been treated as noise around the population mean [56]. However, there has been a recent surge of interest in studying individual variation in both behavioral and physiological traits [50,57], and whether these traits are correlated [40,51,56]. It has been proposed that environmental stressors can alter the relationship between behavioral and physiological traits, either revealing or masking these relationships [58]. Importantly, correlations between traits could have implications for the capacity to adapt to environmental change. For instance, if the behavioral and physiological traits of interest are heritable, then correlations between them could either increase or decrease the rate of adaptive evolution, depending on whether the traits are positively or negatively correlated with respect to the fitness landscape [59]. If traits are positively correlated, then selection on one trait will enhance the other, accelerating the rate of adaptation, and vice versa. Thus, examining correlations between behavioral and physiological traits, as well as how those correlations shift under different environmental conditions, can reveal the evolutionary implications of climate change.

2. Materials and Methods

2.1. Study Species, Broodstock, Egg and Larval Maintenance

We chose the yellowtail kingfish, *Seriola lalandi*, because it is one of the few species of large pelagic fishes that can be reliably reared in captivity [60]. The yellowtail kingfish has a circumglobal

distribution in subtropical and temperate waters [61]. Kingfish inhabit open coastal waters, where they form large shoals around deep reefs, pinnacles, or rocky outcrops [62]. Adults can reach up to 1.93 m in length and weigh over 58 kg [63]. Kingfish are an important recreational and commercial fishery in subtropical countries such as Australia, New Zealand, Peru, Chile, USA, South Africa, and Japan, and have been a target for aquaculture in some of these countries as well [61].

This study was conducted at the National Institute of Water and Atmospheric Research (NIWA) Northland Marine Research Centre in Ruakaka, New Zealand. Wild-caught, adult yellowtail kingfish were maintained as broodstock in six 20 m^3 circular tanks (Figure 1). Each tank contained up to six fish, with approximately equal sex ratios in each tank. The fish had been domesticated at NIWA for up to nine years. Filtered (10 µm) seawater was supplied to the tanks at 130 L min^{-1}, and each tank was exposed to an ambient photoperiod and ambient ocean temperatures (maximal seasonal range of 13–24 °C). The broodstock were fed a mixture of pilchard (*Sardinops sagax*) and squid (*Notodarus* spp.). For further details, all broodstock, egg, and larval maintenance protocols followed Watson et al. [15].

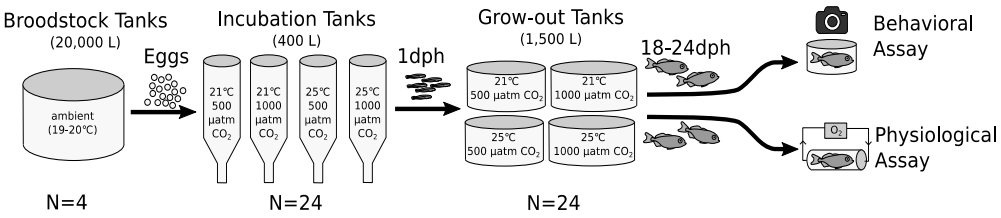

Figure 1. A schematic diagram of the broodstock, egg, and larval maintenance of yellowtail kingfish prior to behavioral and physiological testing. Illustration by Erin Walsh.

The offspring used for this experiment were collected from a spawning event on the night of 23 January 2017. Spawning occurred within the last 2 h of daylight across four broodstock tanks, containing a total of nine females, nine males, and one fish of unknown sex. Eggs were collected from all four tanks to maximize genetic variation. Ambient water temperatures ranged from 19–20 °C in the week prior to spawning, but dropped to 18.2 °C on the night of spawning. Eggs were collected on the morning of 24 January 2017, approximately 12 h after fertilization using an external egg collector as described by Moran et al. [64]. Eggs were collected in approximately equal proportions from each tank and mixed together. They were rinsed with oxygenated seawater for 5 min, disinfected with Tosylchloramide (chloramine-T) at 50 ppm for 15 min, then rinsed a second time in seawater. The eggs were then transferred to 24 conical 400-L incubation tanks at 12:45 h, with an average concentration of 101,778 ± 9860 (SD) eggs per tank.

The incubation tanks were exposed to a 14:10 light to dark photoperiod. Flow-through seawater was supplied at 4 L min^{-1}, and the tanks were aerated with a weighted 4 mm airline. Tanks were at ambient ocean temperature (18.2 °C) at stocking, after which the heating was turned on and tank temperatures rose to either 21 or 25 °C overnight, which resulted in hatching after three or two days, respectively. At 1 day post-hatch (dph), larvae were transferred from incubator tanks to 24 reciprocal 1500 L circular grow-out tanks (black interior, sloped bottoms) at an average concentration of 44,227 ± 2152 (SD) larvae per tank. Tanks were supplied with seawater at a flow rate of 3 L min^{-1} and, as with incubation tanks, exposed to a 14:10 light to dark photoperiod and aerated with a weighted 4 mm airline. Larvae were fed with enriched rotifers up to 4 times per day.

2.2. Carbonate Chemistry

Seawater was pumped continuously from the ocean, sand and particle (5 µm) filtered, and UV (150 mW cm^{-2}) sterilized before reaching large header tanks. Inside the header tanks, foam fractionators removed any additional organic matter, and oxygen diffusers ensured a minimum dissolved oxygen concentration of 100% saturation. Water from the header tanks was then gravity-fed into eight 100 L sumps. These sumps were treated to create a fully crossed 2 × 2 experimental design

of CO_2 and temperature, with CO_2 at either ambient (~500 µatm) or elevated levels (~1000 µatm), and temperature at either an ambient (21 °C) or elevated (25 °C) level. There were two replicate sumps for each treatment, totaling eight sumps. Each sump supplied water to three rearing tanks, meaning that there were six replicate experimental tanks for each CO_2 × temperature treatment throughout the duration of the experiment.

Each sump contained two aquarium pumps; one (HX-6540, Hailea, Guangdong, China) delivered water from the sump to the experimental rearing tanks, while the second (Maxi 103, Aqua One, Ingleburn, NSW, Australia) ensured even mixing of the water within the sump. The second pump was also the site of CO_2 dosing for the elevated CO_2 treatments. A pH computer (Aqua Medic, Bissendorf, Germany) and needle valve were used to slowly dose CO_2 into the pump inlet, which ensured a slow, steady stream of CO_2 that was immediately mixed by the pump impeller.

The temperature and pH_{total} of each rearing tank were measured daily using a pH electrode (SG8 SevenGo Pro, Mettler Toledo, Switzerland). The pH electrode was calibrated using Tris buffers obtained from A.G. Dickson (Scripps Institution of Oceanography, La Jolla, CA, USA, batch number 26). Water samples for carbonate chemistry analysis were collected from all rearing tanks at the start, middle, and end of the experiment, and immediately poisoned with a saturated solution of mercuric chloride at 0.05% of the sample volume. The samples were later analyzed for total alkalinity (TA) at the University of Otago Research Centre for Oceanography (Dunedin, New Zealand). See Watson et al. [15] for full details of the water sample analysis. Salinity was measured for each sample bottle using a YSI Pro30 salinity probe. Temperatures reported by the pH electrode were cross-checked each day with a calibrated 3 decimal point digital thermometer (FSH15-077-8 Digital thermometer, Fisherbrand™ Traceable™ Digital Thermometer, Thermo Fisher Scientific, Waltham, MA, USA).

Carbonate chemistry parameters in each tank were calculated in CO2SYS using the measured values of pH_{total}, salinity, temperature, and TA and the constants K1 and K2 from Mehrbach et al. [65], refit by Dickson & Millero [66] and Dickson for $KHSO_4$ [67]. Seawater carbonate chemistry parameters are displayed in Table 1.

Table 1. Experimental water chemistry. Mean (± S.D.) temperature, salinity, pH_{total}, total alkalinity, and pCO_2 in experiments with yellowtail kingfish (*Seriola lalandi*) eggs and larvae. Water chemistry in broodstock tanks was measured in the week prior to spawning. Temperature, salinity, pH_{total}, and total alkalinity were measured directly, while pCO_2 was estimated from these parameters in CO2SYS.

CO_2 Treatment	Temperature Treatment	Temperature (°C)	Salinity	pH_{total}	Total Alkalinity (µmol.kg^{-1} SW)	pCO_2 (µatm)
Broodstock–ambient	Broodstock–ambient	19.4 (0.4)	35.6 (0.1)	7.906 (0.024)	2329.6 (6.1)	589.4 (38.0)
Control	21 °C	21.1 (0.1)	35.6 (0.1)	7.995 (0.025)	2318.8 (7.2)	462.0 (42.8)
Control	25 °C	24.8 (0.4)	35.6 (0.1)	7.938 (0.011)	2319.9 (7.7)	538.3 (15.6)
Elevated	21 °C	21.1 (0.1)	35.6 (0.2)	7.718 (0.028)	2319.0 (3.8)	959.8 (57.3)
Elevated	25 °C	24.9 (0.4)	35.6 (0.1)	7.700 (0.012)	2320.0 (6.2)	1010.6 (30.4)

2.3. Experimental Design

Behavioral and metabolic traits were assessed from 18–24 dph during daylight hours only (08:00–19:00). Each morning, the fish to be tested that day were sampled randomly from the experimental rearing tanks. The fish were placed individually into labeled sample jars (10 cm diameter, 10 cm height) maintained at the fish's respective treatment conditions. A total of 137 fish were used in the study. Fish were only tested in a single assay, except for 45 individuals that were tracked through both the behavioral and physiological assays to examine correlations between traits. All experiments were conducted in each fish's respective treatment water. After the final assay, fish were euthanized using an overdose of clove oil. Any excess water was removed by blotting with a paper towel, and the fish's mass (0.028 ± 0.008 g; mean ± SD) and standard length (9.74 ± 1.07 mm; mean ± SD) were recorded. Research was carried out under approval of the James Cook University animal ethics committee (permit: A2357) and according to the university's animal ethics guidelines.

2.4. Behavioral Assay

Routine activity and boldness were determined using an open field test [44,68]. The test arena consisted of a round, white plastic bucket (19 cm diameter, 7 cm height) placed inside a white plastic bin (52 cm length, 32 cm width, 34 cm height), which was opaque to minimize visual disturbance for the fish, but allowed light through for filming. A sheet of white corflute was fitted to the top of the plastic bin, with a small circular hole cut into its center, where a video camera (HC-V160, Panasonic Australia, Macquarie Park, NSW, Australia) was placed. To begin a trial, a fish was placed into the center of the arena by gently transferring it with a beaker to minimize stress. The lid was immediately fit to the plastic bin, the camera was turned on, and the fish was filmed for 17 min. At the end of a trial, the fish was removed from the test arena with a beaker, returned to its respective treatment water, and the test arena was rinsed with seawater.

All videos were analyzed blind to treatment using Lolitrack software (v4.1.0 Loligo Systems, Tjele, Denmark), which tracked and quantified the movements of the fish. The first and last minute of each video were discarded to allow for the researcher to enter and exit the arena area. Before each video analysis, a circular arena was drawn within the test arena, with the same central point, but which was 13 cm in diameter, or approximately three body lengths away from the edges of the test arena. This "inner zone" was used to quantify boldness. The open field test has been commonly used in fishes to determine boldness based on the idea that a novel, open field is considered dangerous, and that venturing into the inner zone represents boldness, or the willingness to undertake risk [44,68]. Therefore, we quantified boldness as time spent in the inner zone. The parameters quantified by the software were: total distance moved (cm), average swimming velocity (cm s^{-1}), time active (defined as time spent moving) (s), and time spent in the inner zone (s). Between 29 and 38 individuals were tested per treatment.

2.5. Physiological Assay

Oxygen uptake rates ($\dot{M}O_2$) of fish were determined using intermittent flow respirometry, based on standard respirometry methods [69,70]. Fish were starved for 20 h prior to testing to ensure a post-absorptive state [71]. To measure maximal oxygen uptake ($\dot{M}O_{2Max}$), fish were chased (3 min) in a circular container (20 cm diameter, 9 cm height) and then exposed to air (1 min). This chase protocol was determined in pilot trials to be sufficient for all fish to reach exhaustion. Immediately following the chase and air exposure, fish were gently placed into individual darkened glass respirometry chambers (15 mL total volume including tubing) submerged in a water bath containing the fish's respective treatment water. The water bath received a continuous flow of treatment water to ensure the temperature and CO_2 of the water remained constant. Fish remained in chambers while recovering back to their resting oxygen uptake rates ($\dot{M}O_{2Rest}$) over four hours. Although adult fish typically remain in chambers for 24 h [72], larvae and small juvenile fish recover much more quickly from exhaustive exercise, and are commonly measured for only 2–3 h to minimize stress and the risk of starvation [73–76]. Flush pumps supplied the chambers with clean, well-oxygenated water for 2 min every 8 min, ensuring that O_2 levels within chambers did not fall below 80% air saturation. This flush pattern was controlled using a custom-built timer which turned power to the flush pumps on and off via a programmed timing sequence. The temperature-compensated oxygen concentration (mg L^{-1}) of the water in each chamber was continuously recorded (2 s^{-1}) using oxygen-sensitive REDFLASH dye on contactless spots (2 mm) adhered to a glass tube in line with the chamber, and linked to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) with 2 m fiber-optic cables. Between 11 and 14 individuals were tested per treatment.

Oxygen uptake rates were calculated using linear least squares regression in LabChart version 7.2.5 (ADInstruments, Colorado Springs, CO, USA). Background microbial respiration was subtracted from total chamber respiration to determine the oxygen uptake rate of the fish, as per Rummer et al. [70]. The $\dot{M}O_{2Max}$ was taken to be the highest oxygen uptake rate (over 2 min intervals) and usually occurred

during the first measurement cycle. The $\dot{M}O_{2Rest}$ was estimated as the average of the lowest 10% of values, excluding outliers above or below 2 SD. Aerobic scope (AS) was calculated as the difference between $\dot{M}O_{2Max}$ and $\dot{M}O_{2Rest}$.

2.6. Statistical Analyses

All analyses were conducted using R version 3.1.3 [77]. Linear mixed-effects models (LME, "nlme" package in R) were used to determine the effect of CO_2 and temperature treatment on behavioral and metabolic traits. Total distance traveled (in body lengths) and velocity (in body lengths per second) were standardized by body length to facilitate comparisons between treatments where fish were differently sized. For these dependent variables, the CO_2 and temperature treatments were fixed effects, with time of day as a covariate, allowing for interactions between CO_2 treatment, temperature treatment, and time of day. Time of day was mean-centered to help with the interpretation of model intercepts. For time spent active and time spent in the inner zone, similar linear mixed effects models were used, with the addition of mean-centered mass as a covariate, since these measures were not standardized by fish length, but distance traveled and velocity were. Time spent in the inner zone was square-root transformed to achieve normal distribution errors. For aerobic scope, maximum oxygen uptake, and resting oxygen uptake, CO_2 and temperature treatments and mean-centered mass were fixed effects. For all linear mixed effect models, tank was included as a random effect. Assumptions of normality and homogeneity of residuals were visually assessed with Q-Q plots and frequency distributions. When the variance of the model residuals increased as the fitted values increased, a power variance function was used to allow for heteroscedasticity. Parameters were estimated using restricted maximum-likelihood. Covariates and interactions between the fixed factor and covariates were dropped when not significant for model simplification and fit. Correlations between behavioral and physiological traits were calculated using linear models (LM), with mass as a covariate, and separate analyses were undertaken between ambient vs. elevated CO_2 treatments and ambient vs. elevated temperature treatments. The dataset generated and analyzed during the current study is available from the corresponding author on request or via the Tropical Research Data Hub (doi:10.4225/28/5ae15a2d946b4).

3. Results

3.1. Behavior

Elevated temperature significantly affected the distance traveled in body lengths (t_{103} = 6.08, $p < 0.0001$; Figure 2A). Fish maintained at 25 °C swam 138% further on average than fish maintained at 21 °C, whereas CO_2 treatment did not affect the distance traveled (t_{103} = 0.41, p = 0.68; Figure 2A), and there was no significant interaction between CO_2 and temperature treatments. Elevated temperature also significantly affected the average velocity of fish in body lengths per second (t_{103} = 4.47, $p < 0.0001$; Figure 2B), with fish maintained at 25 °C swimming 59% faster than fish maintained at 21 °C. Again, CO_2 treatment did not affect average velocity (t_{103} = −0.34, p = 0.74; Figure 2B), and there was no interaction between CO_2 and temperature. Elevated temperature also affected the time that fish spent active (t_{103} = 5.95, $p < 0.0001$; Figure 2C), but CO_2 had no effect (t_{103} = 0.59, p = 0.56; Figure 2C), and there was no interaction between CO_2 and temperature. Fish maintained at 25 °C were, on average, 55% more active than fish maintained at 21 °C. There were no significant main effects of either CO_2 (t_{103} = −1.13, p = 0.26; Figure 3) or temperature (t_{103} = −0.88, p = 0.38; Figure 3) on time spent in the inner zone.

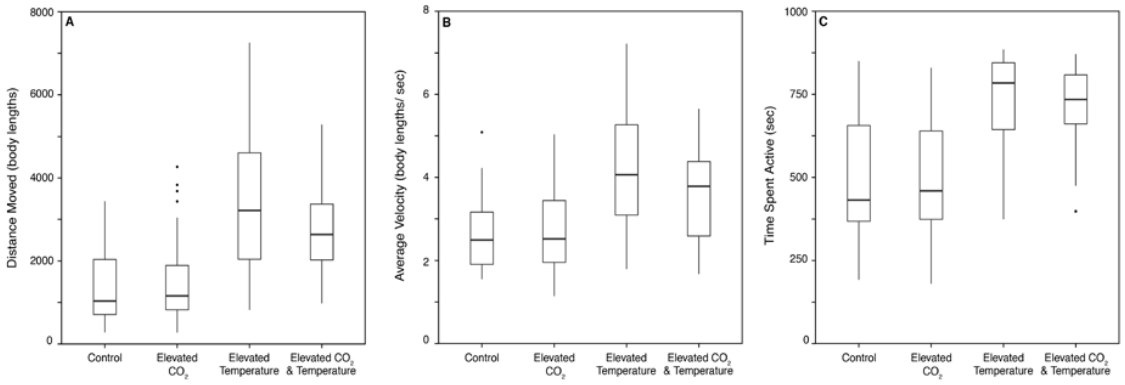

Figure 2. The effect of elevated CO_2 and temperature treatments on: (**A**) the total distance moved, standardized by body length; (**B**) average velocity, standardized by body lengths; and (**C**) the time spent active during a 15 min open field test of larval yellowtail kingfish. Boxplots show median and inter-quartile range. N = 29, 38, 29, and 34, respectively.

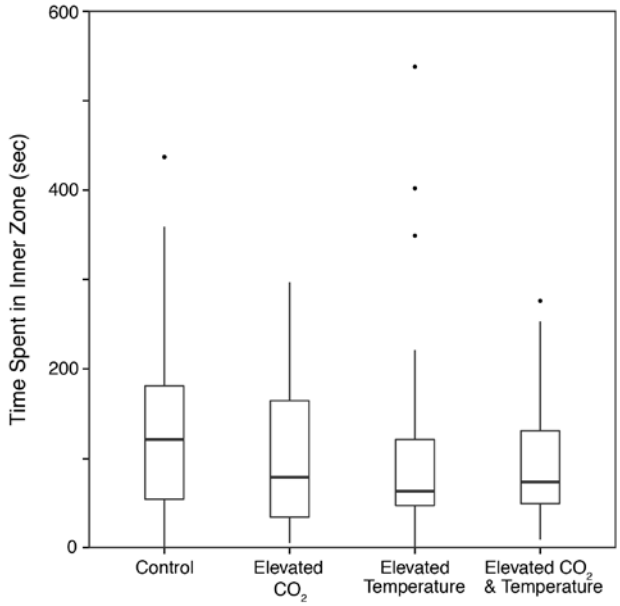

Figure 3. The effect of elevated CO_2 and temperature treatments on the time spent in the inner zone during a 15 min open field test in larval yellowtail kingfish. Boxplots show median and inter-quartile range. N = 29, 38, 29, and 34, respectively.

3.2. Physiology

Both elevated CO_2 (t_{18} = 2.59, p = 0.02; Figure 4A) and temperature (t_{18} = 2.15, p = 0.04; Figure 4A) significantly affected $\dot{M}O_{2Rest}$. Fish maintained under elevated CO_2 exhibited a 21% increase in $\dot{M}O_{2Rest}$ compared to fish maintained under ambient CO_2 levels, and those maintained at 25 °C showed a 20% increase in $\dot{M}O_{2Rest}$ compared to fish maintained at 21 °C. There was a trend toward a negative interaction between CO_2 and temperature which was marginally significant (t_{18} = −2.00, p = 0.06; Figure 4A).

Neither elevated CO_2 nor temperature significantly affected $\dot{M}O_{2Max}$ (t_{18} = 0.83, p = 0.42 and t_{18} = 0.15, p = 0.88, respectively; Figure 4A), and there was no interaction between treatments. Aerobic scope was significantly affected by fish mass (t_{24} = −3.46, p = 0.002; Figure 4B), but not elevated CO_2 or temperature (t_{18} = −0.48, p = 0.64 and t_{18} = −0.27, p = 0.79, respectively; Figure 4B), and there was no interaction between treatments.

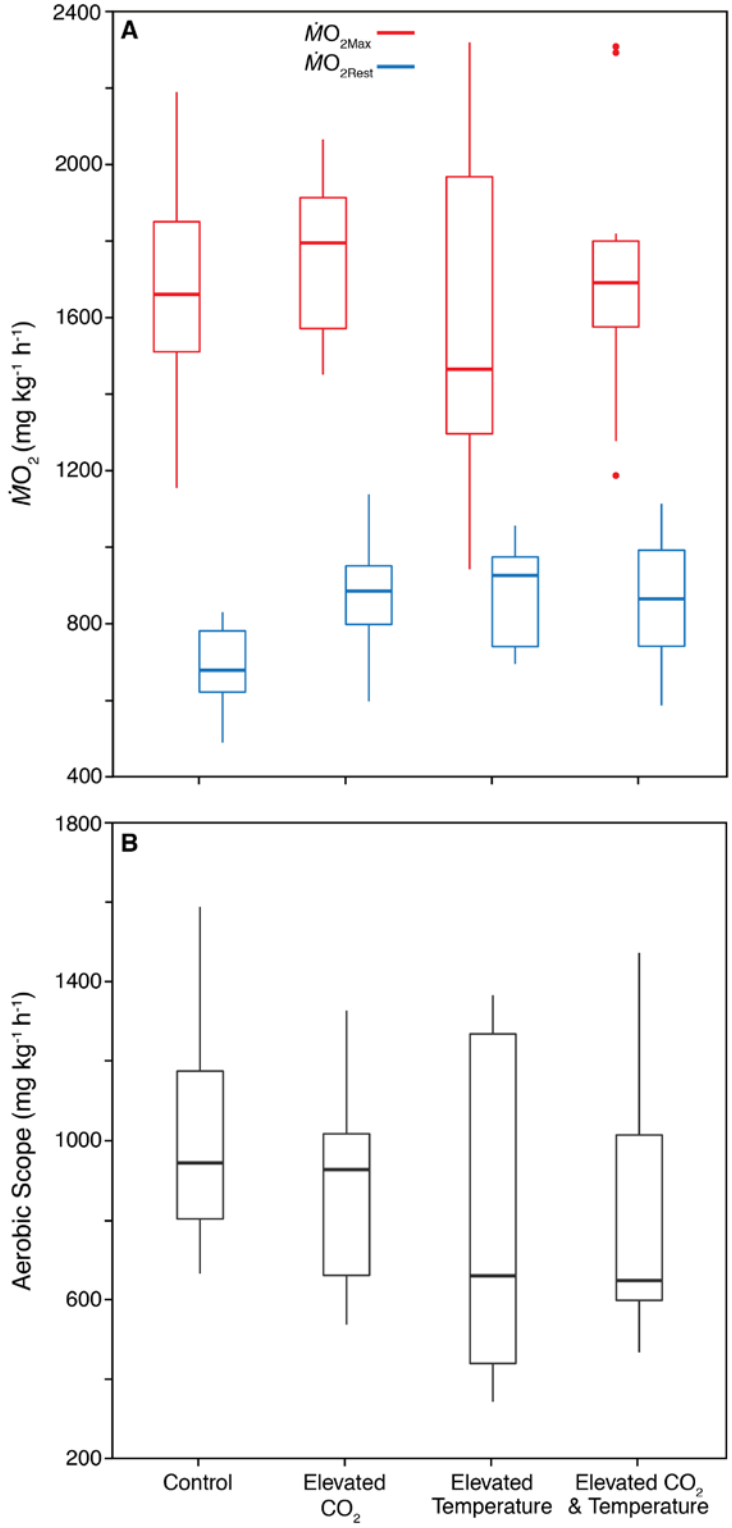

Figure 4. The effect of elevated CO_2 and temperature treatments on: (**A**) resting and maximal oxygen uptake rates ($\dot{M}O_{2Rest}$ and $\dot{M}O_{2Max}$), and (**B**) aerobic scope ($\dot{M}O_{2Max} - \dot{M}O_{2Rest}$) of larval yellowtail kingfish. Boxplots show median and inter-quartile range. N = 14, 13, 14, and 11, respectively.

3.3. Correlations

There were a number of correlations between behavioral and physiological traits. When comparing across temperature treatments, there was a significant positive relationship between $\dot{M}O_{2Rest}$ and time spent in the inner zone (i.e., boldness) in fish maintained at 25 °C (LM $t = 2.99$, slope estimate = 2.22, $p = 0.009$; Figure 5A). By contrast, there was a negative relationship between $\dot{M}O_{2Rest}$ and boldness in fish maintained at 21 °C (LM $t = -2.95$, slope estimate = −0.80, $p = 0.008$; Figure 5A). Within each temperature treatment, these trends were consistent across CO_2 treatments (ANOVAs of LMs, CO_2 treatment × Time spent in inner zone interaction, $p > 0.05$). Fish maintained at 21 °C also exhibited a significant negative relationship between boldness and $\dot{M}O_{2Max}$ (LM $t = -2.61$, slope estimate = −1.50, $p = 0.02$; Figure 5B), but this relationship was absent in fish maintained at 25 °C. These trends were also consistent across CO_2 treatments (ANOVAs of LMs, CO_2 treatment × Time spent in inner zone interaction, $p > 0.05$).

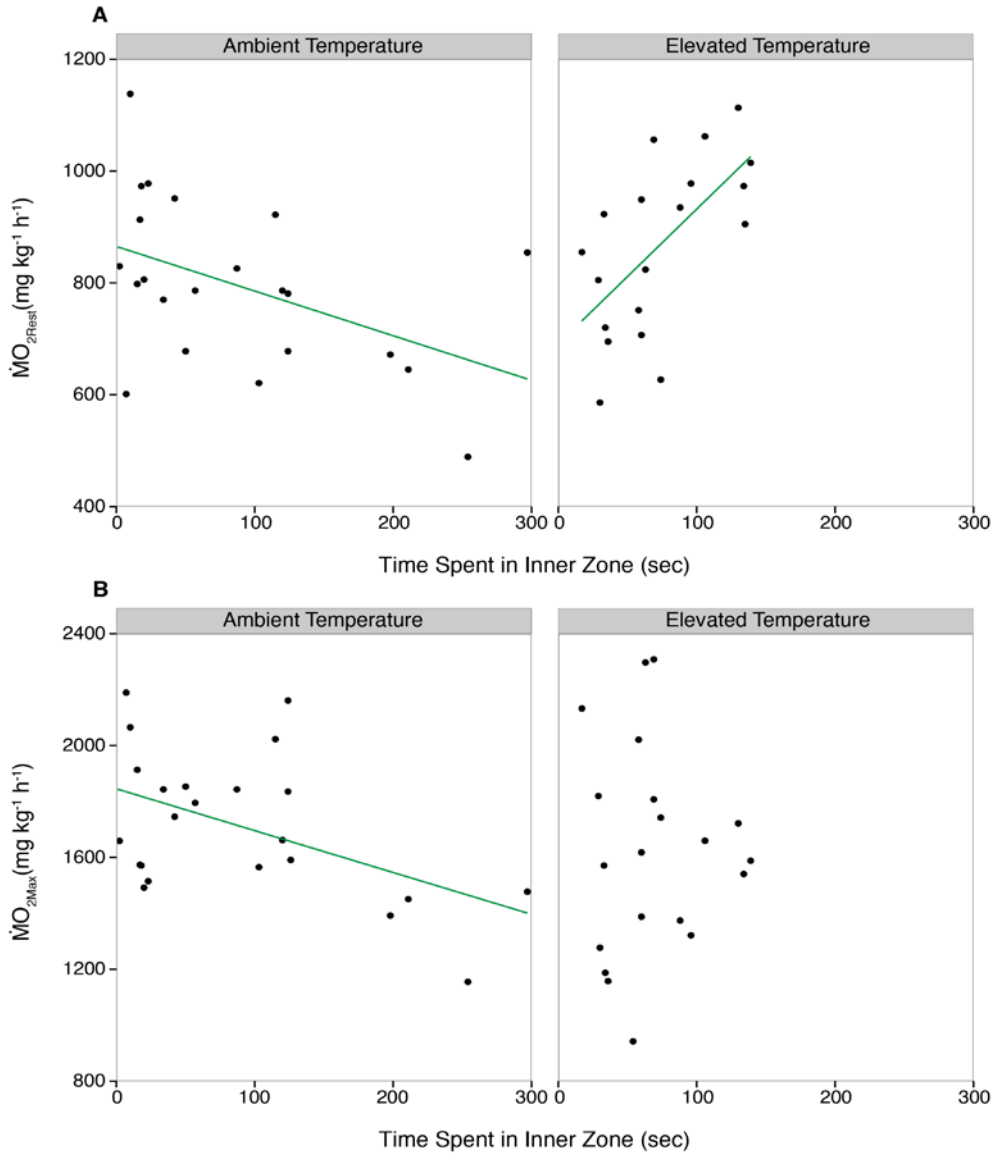

Figure 5. The relationship between (**A**) time spent in the inner zone of the arena and $\dot{M}O_{2Rest}$ and (**B**) time spent in the inner zone of the arena and $\dot{M}O_{2Max}$ in larval yellowtail kingfish. Panels represent ambient (21 °C) and elevated (25 °C) temperature treatments. Trend lines are shown as derived from linear models, and are only displayed for statistically significant relationships.

4. Discussion

Our results indicate that, while elevated temperature dominated effects on behavioral traits, elevated CO_2 and temperature had an equal effect on metabolic traits in larval yellowtail kingfish. Fish maintained under elevated temperature traveled further, had a higher mean velocity, and spent more time active than fish maintained under ambient temperature. By contrast, elevated CO_2 had no effect on these traits. Both elevated CO_2 and temperature increased resting oxygen consumption rates. There was also evidence of an antagonistic interaction, as the combined elevated CO_2 and temperature treatment did not have a significantly different $\dot{M}O_{2Rest}$ from the single-stressor treatments. Boldness, aerobic scope, and $\dot{M}O_{2Max}$ were not affected by elevated CO_2 or temperature. Finally, we found that temperature influenced the relationship between boldness and $\dot{M}O_{2Rest}/\dot{M}O_{2Max}$, which is important because it could influence natural selection and consequently adaptive potential to ocean acidification and warming.

Kingfish reared under elevated temperature exhibited higher activity levels than individuals reared under ambient temperature. This aligns well with other studies that have observed increased activity at higher temperature (e.g., [36,37]). It has been proposed that variation in activity level may be attributed to differences in $\dot{M}O_{2Rest}$, because fish with higher $\dot{M}O_{2Rest}$ have higher energetic demands, causing them to become more active and seek food [40]. Alternatively, fish with higher activity levels might develop a higher $\dot{M}O_{2Rest}$ to cope with the increased energetic demands of a highly active lifestyle [41]. Regardless of the mechanism, our results support a link between $\dot{M}O_{2Rest}$ and activity.

Increased activity under elevated temperature could have either positive or negative effects on larval kingfish. Higher activity rates are likely to increase foraging success [78], which could help fish to meet the high energetic demands of their elevated $\dot{M}O_{2Rest}$. Conversely, increased activity could also make fish more vulnerable to predation, particularly in the larval and early juvenile stages [30–32]. Mortality in the early life stages of pelagic fishes can have significant effects on recruitment patterns in adult populations [24]. The relative effects of increased foraging success versus increased mortality due to predation will thus largely depend on the abundance and distribution of both predators and prey, which are themselves subject to temperature-induced changes [79].

In contrast to temperature, elevated CO_2 levels did not affect any of the activity metrics. The effect of elevated CO_2 on activity of larval pelagic fishes appears to be highly variable, with some species showing decreased activity [34,37] and others experiencing no change in activity [33–36]. Coral reef fishes have shown similar variability, with both increases [45] and no changes [80,81] in activity under elevated CO_2. These results suggest some degree of inter-species variation in response to elevated CO_2, although the variation in results could also be attributed to different experimental methodologies and CO_2 levels. Nevertheless, our results indicate that larval kingfish will likely not experience changes in activity levels due to elevated CO_2 under relevant ocean acidification conditions.

Boldness of larval yellowtail kingfish, as measured by time spent in the inner zone of the test arena, did not vary regardless of CO_2 or temperature treatment. Boldness has not been previously assessed for the larval stages of large pelagic fishes, but studies on other fish species have found both an increase [45] and a decrease [47] in boldness under elevated CO_2. As with activity levels, it appears that larval kingfish are resilient to changes in boldness due to elevated CO_2. Boldness has been linked with elevated temperature previously [38,48], but notably, prior studies have predominantly examined temperature changes on time-scales of hours to days. We exposed kingfish to elevated temperature from the egg stage to 18–24 dph, which may have conferred some benefits in mitigating the effects of elevated temperature. Alternatively, we may not have found any differences in boldness between treatments because the kingfish were well fed. Previous work has shown that fish adopt more risky behaviors when food is scarce [30], and that elevated temperature can exacerbate risky behaviors under low food conditions [48]. It is likely that food will be less abundant in nature; therefore, future work could cross food availability with long-term temperature exposure to tease out the relationship between boldness and temperature.

A significant increase in $\dot{M}O_{2Rest}$ was observed in fish maintained at both elevated CO_2 and temperature. The positive correlation between temperature and $\dot{M}O_{2Rest}$ in marine fishes has been well established due to the influence of temperature on biochemical reactions, and our results are consistent with these findings [24,27]. The effects of CO_2 on $\dot{M}O_{2Rest}$ have been more varied; recent meta-analyses indicate that, on average, elevated CO_2 has no effect on $\dot{M}O_{2Rest}$ [11,54], although both increases [14,82] and decreases in $\dot{M}O_{2Rest}$ [37] under elevated CO_2 have been observed. This inter-species variability suggests that the effect of CO_2 on metabolism is species-specific, and underscores the importance of studying the responses of many species of different lifestyles to elevated CO_2 [54].

The observed increase in $\dot{M}O_{2Rest}$ at both elevated CO_2 and temperature is indicative of a higher cost of living under end-of-century climatic conditions. With respect to elevated CO_2, this increase in $\dot{M}O_{2Rest}$ suggests an increased metabolic cost of acid-base regulation. Importantly, oceanic pH is relatively uniform in comparison with the strong latitudinal gradient of temperature, indicating that a geographical shift in distribution will not ameliorate the costs of elevated CO_2. By comparison, adult kingfish have a peak distribution at 22.5 °C in Australian waters and display seasonal shifts away from warmer waters [83], suggesting that at present, they avoid warmer temperatures and the higher metabolic costs they incur. Nonetheless, an overall increase in average temperature is predicted for the end of the century, which would force even greater distribution shifts to avoid an increased metabolic cost.

In contrast to $\dot{M}O_{2Rest}$, neither $\dot{M}O_{2Max}$ nor aerobic scope of larval kingfish was significantly affected by elevated CO_2 or temperature. In other marine fishes, the effects of CO_2 on $\dot{M}O_{2Max}$ and aerobic scope have been diverse, with many species showing no effect [54], but some increases [53] and decreases [14] have been observed. Therefore, our results are consistent with most previous studies. By contrast, temperature tends to increase $\dot{M}O_{2Max}$ [52], although decreases in $\dot{M}O_{2Max}$ have been observed in some tropical fish species, likely because they live closer to their thermal limits than temperate species [84]. Aerobic scope has shown a strong species-dependence in response to elevated temperature as well [11,84]. It is possible that we did not detect an effect of elevated temperature on $\dot{M}O_{2Max}$ or aerobic scope because there was higher individual variation in $\dot{M}O_{2Max}$ in the elevated temperature treatment as compared with controls, which may have masked a significant effect. This high variability could represent true inter-individual differences in $\dot{M}O_{2Max}$, but could also be indicative of differential recovery times from the exhaustive chase. Still, our results suggest that elevated CO_2 and temperature are unlikely to have a meaningful impact on $\dot{M}O_{2Max}$ or aerobic scope in yellowtail kingfish, which is consistent with findings for many other species of marine fishes [11].

The increase in $\dot{M}O_{2Rest}$ in conjunction with the lack of change in $\dot{M}O_{2Max}$ and aerobic scope suggests that, while there are higher maintenance costs under elevated CO_2 and temperature conditions, this does not diminish the capacity of fish to perform aerobic activities. However, while the overall capacity for aerobic activity did not diminish, $\dot{M}O_{2Rest}$ comprises a larger proportion of the aerobic scope at higher CO_2 and temperature conditions than under ambient conditions. Thus, a smaller fraction of the metabolic scope is available for aerobic activities such as growth, development, and reproduction. This is relevant because fish in this experiment were fed ad libitum with highly nutritious food, meaning that they could easily meet the higher energy requirements of fast growth. In nature, food distribution is more patchy and unreliable, making it more difficult for fish to meet higher energetic demands. Therefore in food-scarce environments, we might expect to see a decline in aerobic scope at higher temperatures [76].

We observed opposing relationships between boldness and $\dot{M}O_{2Rest}$ under elevated versus ambient temperature treatments. Under elevated temperature, bolder individuals had a higher $\dot{M}O_{2Rest}$, while under ambient temperature, bolder individuals had a lower $\dot{M}O_{2Rest}$. Correlations between boldness and $\dot{M}O_{2Rest}$ have been observed previously [40], and two models have been proposed to explain these patterns. The "performance model" posits that bolder individuals will consume more energy, and thus require a higher resting oxygen uptake rate to support their higher

metabolic needs [56]. The performance model predicts a positive relationship between $\dot{M}O_{2Rest}$ and boldness. Conversely, the "allocation model" is based on the idea that organisms have a finite supply of energy and must balance their energy budget between $\dot{M}O_{2Rest}$ and boldness-related activities [56]. This model predicts a negative relationship between $\dot{M}O_{2Rest}$ and boldness. The performance model has broader support from experimental evidence than the allocation model [40], and our results support the performance model under elevated temperature. However, we observed a negative relationship between $\dot{M}O_{2Rest}$ and boldness under ambient temperature conditions. It is possible that the allocation model holds for this population under ambient temperature, and that the performance model applies under elevated temperature. Indeed, it has been suggested that environmental stressors such as temperature can alter the relationship between behavioral and physiological traits [58]. Still, the proximal cause for this shift from a positive to a negative relationship is not clear, and represents a fruitful avenue for future research.

Importantly, our results show that boldness and $\dot{M}O_{2Rest}$ have opposing relationships under different thermal, but not CO_2, regimes. The implication of this pattern is that different thermal regimes could have opposing influences on selection, driving changes in rates of adaptation to environmental change. We cannot predict with certainty the direction that selection will take, as the relative benefits of having a higher $\dot{M}O_{2Rest}$ or boldness will depend upon environmental factors such as food availability and predator density. Still, both boldness [85,86] and aerobic scope [87] have shown heritability in fishes, suggesting that their correlation could indeed influence the rate and direction of adaptation.

A similar relationship between $\dot{M}O_{2Max}$ and boldness was also detected, with bolder individuals having a lower $\dot{M}O_{2Max}$ at ambient temperature, but the relationship disappeared under elevated temperature. The links between $\dot{M}O_{2Max}$ and behavioral traits have been relatively understudied, and there are no models to explain the underlying causes of such patterns. However, $\dot{M}O_{2Rest}$ and $\dot{M}O_{2Max}$ have shown correlations in fishes [88], suggesting that the similar relationships that we saw between $\dot{M}O_{2Rest}$ and $\dot{M}O_{2Max}$ with boldness are plausible.

In conclusion, this study indicates that elevated temperature has a greater effect than elevated CO_2 levels on the behavior, physiology, and correlations between behavior and physiology of larval kingfish. At elevated temperature, larval kingfish displayed elevated activity levels and $\dot{M}O_{2Rest}$, indicating a higher cost of living. However, the overall effect of these traits will ultimately depend upon the distribution and abundance of predators and food sources for the larval fish. It is likely that as ocean warming progresses, kingfish will shift their distributions polewards to avoid incurring the metabolic and behavioral costs associated with warmer waters, as has been documented in a range of marine species [89]. Unlike temperature, pCO_2 does not have a strong latitudinal gradient, and thus rising CO_2 levels cannot be avoided through range shifts. This implies that kingfish may still experience increased metabolic costs in future climatic conditions, unless they can adapt to elevated CO_2. Further work is needed to determine whether variation in $\dot{M}O_{2Rest}$ to elevated CO_2 is heritable, and thus whether adaptation is possible. Our results also showed correlations between $\dot{M}O_{2Rest}$ and boldness that were temperature-dependent. These opposing correlations could influence the rate and direction of future adaptation, but their consequences will depend on additional factors such as predation risk and food availability. Future work could include additional stressors to determine the relative benefits of boldness and $\dot{M}O_{2Rest}$, revealing the evolutionary implications of climate change. Our findings provide novel insights into the behavioral and physiological impacts of future climate conditions on the early life stages of a large pelagic fish, a critical knowledge gap in climate change research.

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