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Ocean acidification and seasonal temperature extremes combine to impair the thermal physiology of a sub-Antarctic fish

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Abstract

To predict the potential impacts of climate change on marine organisms, it is critical to understand how multiple stressors constrain the physiology and distribution of species. We evaluated the effects of seasonal changes in seawater temperature and near-future ocean acidification (OA) on organismal and sub-organismal traits associated with the thermal performance of *Eleginops maclovinus*, a sub-Antarctic notothenioid species with economic importance to sport and artisanal fisheries in southern South America. Juveniles were exposed to mean winter and summer sea surface temperatures (4 and 10 ºC) at present-day and near-future pCO_2 levels (~500 and 1800 μatm). After a month, the Critical Thermal maximum and minimum (CTmax, CTmin) of fish were measured using the Critical Thermal Methodology and the aerobic scope of fish was measured based on the difference between their maximal and standard rates determined from intermittent flow respirometry. Lipid peroxidation and the antioxidant capacity were also quantified to estimate the oxidative damage potentially caused to gill and liver tissue. Although CTmax and CTmin were higher in individuals acclimated to summer versus winter temperatures, the increase in CTmax was minimal in juveniles exposed to the near-future compared to present-day pCO_2 levels (there was a significant interaction between temperature and pCO_2 on CTmax). The reduction in the thermal tolerance range under summer temperatures and near-future OA conditions was associated with a reduction in the aerobic scope observed at the elevated pCO_2 level. Moreover, an oxidative stress condition was detected in the gill and liver tissues. Thus, chronic exposure to OA and the current summer temperatures pose limits to the thermal performance of juvenile *E. maclovinus* at the organismal and sub-organismal levels, making this species vulnerable to projected climate-driven warming.

Keywords: *Eleginops maclovinus*, thermal tolerance, aerobic scope, oxidative metabolism, multiple stressors, climate change biology.

1. Introduction

The Beagle Channel is a unique subpolar marine ecosystem located in the southern extreme of South America, connecting the Pacific and the Atlantic Oceans (Flores Melo et al., 2020). Due to its location, this area hosts a mixture of marine ichthyofauna with different origins that have experienced distinct evolutionary histories (Fernández et al., 2019). Fishes of the suborder Notothenioidei are the most dominant component of the ichthyofauna in the Beagle Channel (Lloris and Rucabado, 1991; Vanella et al., 2007). The 'róbalo' or Patagonian blenny, *Eleginops maclovinus* (Cuvier, 1830) is an endemic notothenioidei species that has an extended bioceanic distribution in temperate and subantarctic waters from Valparaíso in the Pacific Ocean (33°S) to San Matías Gulf in the Atlantic Ocean (40°S) down to the Beagle Channel (55°S) and Malvinas/Falklands Islands (López, 1963; Guzmán and Campodonico, 1973; Pequeño, 1989; Eastman, 1993). This species inhabits shallow coastal waters, estuaries, and rivers throughout their geographic distribution and is a key component of many food webs, both as prey and predator (Riccialdelli et al., 2017; 2020). In the Atlantic Ocean and the Beagle Channel, *E. maclovinus* is also targeted by recreational (sport) and artisanal fisheries (Lattuca et al., 2018).

Climate change represents one of the main threats to biodiversity (Heller and Zavaleta, 2009; IPBES, 2019) and its effects are appearing more rapidly and with greater impacts at high latitudes (Fabry et al., 2009; IPCC, 2022). When studying the effects of climate change in marine ecosystems, the roles of temperatures and carbon dioxide (CO_2) are invariably linked (Enzor et al., 2013). The increase in atmospheric CO_2 observed since the beginning of the industrial revolution has caused not only ocean warming but also a decrease in seawater pH, known as ocean acidification (OA; Levitus et al., 2005; Caldeira and Wickett, 2003).

Temperature is the most pervasive abiotic factor governing the biology of organisms (Beitinger and Lutterrschmidt, 2011). Thus, the organism's thermal sensitivity is a fundamental factor in climate-induced changes in marine ecosystems (Pörtner and Farrell, 2008). It has been hypothesized that temperature sets important limitations for aquatic ectotherms mainly by reducing their aerobic scope, caused by the limited capacity of the circulatory and ventilatory systems to match oxygen demand [Oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis], in fully oxygenated waters (Pörtner, 2002; 2010; Pörtner and Farrell, 2008). Within their thermal tolerance limits or thermal windows, at the sub-organismal level, the organisms progressively enhance the exploitation of protective mechanisms, such as the capacity of anaerobic metabolism, antioxidant defenses, and the heat-shock response, as oxygen limitations are set during warming and cooling (Pörtner, 2002). The thermal tolerance limits or thermal windows of fishes are generally assessed by determining the

Critical Thermal Maximum (CTmax) and Minimum (CTmin) and represent a useful relative proxy for the temperatures at which fishes are unable to escape conditions that will ultimately lead to thermal death (Becker and Genoway, 1979; Beitinger et al., 2000; Beitinger and Lutterschmidt, 2011, Åsheim et al., 2020). Several works have highlighted the importance of determining physiological limits to different environmental drivers in order to develop predictions for the future geographical distribution or productivity of species based on future climate scenarios (Pörtner and Peck, 2010; Burrows et al., 2011; Deutsch et al., 2015; Marras et al., 2015). In this regard, Lattuca et al. (2018) showed that under laboratory conditions, juvenile *E. maclovinus* inhabiting coastal areas of the Beagle Channel is a eurythermic species that can acclimate well to different temperatures and has a broad thermal window and a positive relationship between preferred and acclimation temperatures. To understand the effects of global warming on the thermal tolerance of a given ectotherm organism, it is critical to understand how they respond to seasonal temperature extremes and identify the mechanisms involved in the responses. Since *E. maclovinus* lives in much cooler environments than their maximum thermal tolerance, Lattuca et al. (2018) proposed that *E. maclovinus* populations from Tierra del Fuego could experience enhanced performances in response to ocean warming.

OA has been recognized as a critical process impacting marine life (Hendriks et al., 2010; Kroeker et al., 2010), and its effects are fundamentally linked to the ecology and physiology of the organisms (Pörtner, 2008). In particular, fish were initially thought to be resilient to OA as they can maintain a constant internal pH through increased buffering capacity and net acid excretion (Claiborne et al., 2002; Melzner et al., 2009; Cattano et al., 2018). However, these compensation responses have additional energetic costs and, thus, potential consequences on other fitness-related traits (Ishimatsu et al., 2008; Heuer and Grosell, 2014). Accordingly, increased pCO_2 levels can affect fish metabolism, internal calcification, yolk consumption, and behavioral performance along with increased predation risk and decreased foraging efficiency, particularly for larvae (Cattano et al., 2018). Furthermore, several fitness-related traits in fish are suggested to be modulated by the combined effect of pCO_2 and temperature levels (Pörtner et al., 2005).

Predicting and understanding the potential impacts of interacting climate change stressors, such as changes in temperature and pCO_2 levels, on marine organisms is one of the most urgent challenges that environmental scientists face. Interacting stressors may cause either additive, synergistic, or antagonistic impacts on marine organisms (Vinebrooke et al., 2004). Several studies have reported on physiological responses of notothenioid species to increased temperature and elevated pCO_2 levels (Strobel et al., 2012; Strobel et al., 2013a,b; Enzor et al., 2013; Enzor and Place, 2014). For example, by measuring the routine metabolic rate, mitochondrial capacity, and the intra- and extracellular acid-

base status, Strobel et al. (2012) reported that *Notothenia rossii* could, in part, acclimate to ocean warming and OA. However, Strobel et al. (2013a) demonstrated the existence of different tissue capacities to compensate for such conditions in terms of energy metabolism and mitochondrial enzymes. Compared to *N. rossii*, the sub-Antarctic *Lepidonotothen squamifrons* exhibited higher plasticity in energy usage in response to changing temperature and hypercapnia (Strobel et al., 2013b). Moreover, Enzor et al. (2013) demonstrated that *Trematomus bernacchii*, *Trematomus hansoni*, *Trematomus newnesi* and *Pagothenia borchgrevinki* were capable of rapidly acclimating to increased pCO_2 levels but that warmer temperature continued to impact their routine metabolic rates for at least 28 days. Enzor and Place (2014) also documented that *T. bernacchii*, *P. borchgrevinki* and *T. newnesi* maintained an antioxidant capacity necessary to offset predicted warming and OA.

Most of the notothenioid fish in southern South America can be found in coastal waters. These shallow-water environments have little thermal inertia, so they are expected to be the first to reflect a rise in atmospheric temperature (Madeira et al., 2012). Moreover, these waters are subjected to a wide range of CO_2 concentrations across different time scales (Waldbusser and Salisbury, 2014). In particular, fish inhabiting the coasts of the Beagle Channel are naturally exposed to fluctuations in temperature and CO_2 due to the freshwater input from rivers, coastal runoff and the thawing of glaciers (Giesecke et al., 2021). Thus, fish in the Beagle Channel are exposed to higher pCO_2 levels than fish living in pelagic environments where CO_2 levels are more stable. Waldbusser and Salisbury (2014) noted that such varying conditions should not prevent scientists from studying how the processes modifying coastal conditions interact to affect organisms, nor does it mean that they are better adapted to higher CO_2 levels. Despite being the most prominent channel of the South American continent, to our knowledge, no studies have documented the impacts of climate change stressors on fish inhabiting these waters.

We measured the response of thermal tolerance, aerobic scope, and oxidative metabolism of juvenile *E. maclovinus* from coastal Channel waters exposed to two temperatures (4 and 10 ºC) at both present-day and near-future pCO_2 levels (~500 and ~1800 μatm). The temperatures used here match the mean winter and mean summer sea surface temperatures in the Beagle Channel (Lattuca et al., 2018). The pCO_2 levels correspond to present-day pCO_2 measured in coastal areas in this region and to future levels projected for the end of 2200, respectively (Caldeira and Wickett, 2003; IPCC, 2014). We hypothesized that the combined effect of chronic exposure to OA at the mean summer temperature would reduce the thermal performance of the fish at organismal and sub-organismal levels and that climate change will cause negative impacts on these fish. At the organismal level, we expected that the interaction between temperature and pCO_2 would lower the aerobic scope of juvenile *E. maclovinus*

and, hence, its thermal tolerance range. At the sub-organismal level, we expected an oxidative stress response. To our knowledge, this is the first study to investigate the interactive effects of OA levels and temperature on functional traits linked with the thermal performance of a notothenioid fish inhabiting coastal waters surrounding the tip of South America.

2. Materials and methods

Ethics statement

The methods described in this study were reviewed and approved by the Bioethics Institutional Committee of the Austral Centre for Scientific Research (CIB-CADIC), which assesses animal care in research activities.

2.1. Fish collection and habituation to captivity

Juvenile *E. maclovinus* were collected during the austral autumn 2019 at Golondrina Bay (54° 50'S, 68° 20'W), located on the Beagle Channel (Tierra del Fuego, Argentina) close to Ushuaia city, where all the laboratory experiments were conducted. Fish were captured using a seine net (25 m long, 1.5 m high, and 12 mm stretch mesh) and then transported to the laboratory in 50-L tanks equipped with aeration. The temperature at the capture site was ~5 °C. Once in the laboratory, fish were habituated to captivity for one month in 120-L aquaria containing seawater at 4 ± 0.5 °C, with a salinity of 24.6 ± 0.2 and dissolved oxygen concentrations of 9 ± 0.3 mg L^{-1}. During this period, fish were fed to satiation (~10% body mass) with chopped hake (*Merluccius hubbsi*) muscle every other day and held under a 12:12-h (light:dark) light regime with light-emitting diodes (LEDs) as the light source (250 lx). Water quality was maintained by daily partial water changes (20–25% of total volume).

2.2. CO_2 mixing system and carbonate system determination

A flow-through CO_2 mixing system similar in design to that described by Manríquez et al. (2019; 2020) was built to manipulate pCO_2 levels inside the rearing containers (experimental units). This system consisted of three mass flow controllers (model GdFC, Aalborg, New York, USA) used to blend pure CO_2 gas with the (ambient) air provided by an oil-free compressor (MSV 12/100, Schulz, Sao Paulo, Brazil) to obtain CO_2-enriched air (Torres et al., 2013). This procedure allowed to obtain CO_2-enriched air of ~1800 µatm for the future pCO_2 condition and CO_2-enriched air of ~500 µatm for the present-day condition. The enriched air was bubbled into four 230-L plastic reservoirs (mixing tanks, n = 2 for each pCO_2 condition) filled with 1 µm filtered seawater (Hidroquil, Buenos Aires,

Argentina). The filtered and pCO_2-treated seawater was delivered to the different rearing containers by electrical submersible pumps located inside the mixing tanks. The delivery of the treated seawater was controlled automatically by solenoid valves to achieve a flow rate of ~250 mL min^{-1}, which renewed one-third of the total volume of each rearing container (750 mL) six times a day. During the entire experimental period, all rearing containers were semi-immersed in four large fiberglass water baths maintained thermally stable at the two experimental temperatures (two at 4 ºC and two at 10 ºC). The two temperature treatments were achieved using two independent seawater recirculating systems that connected the water baths to aquarium chillers (C-2500, Pacific Coast Imports, Oregon, USA) set at the corresponding temperatures. The rearing container had two holes in the lid (for plastic water and air tubing) and a third hole located on the side and above the thermal bath's water level as a seawater overflow. Filtered and treated seawater flowed from the mixing tanks into each rearing container, as did a stream of air with the appropriate pCO_2 (~500 or 1800 μatm).

The carbonate system parameters, total alkalinity, temperature, pH, and salinity were quantified weekly in seawater samples taken from three randomly chosen rearing containers per treatment (Table 1). Total alkalinity was measured using an automated, open-cell titration system (Haraldsson et al., 1997), and its accuracy was verified using certified reference material (CRM) supplied by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA). The correction factor was approximately 1.002, corresponding to a difference of ~5 μmol kg^{-1}. The pH was measured in a closed 60 mL cell, at 25.0 °C, with a Hanna Edge pH meter provided with a HI11310 digital glass pH-electrode (Hanna Instruments, Inc., Rhode Island, USA) calibrated with standard Tris buffer in synthetic seawater. The buffer was prepared following the standard operating procedures outlined by Dickson and Goyet (1994), with a nominal pH value of 8.063 pH units at 25.0 °C and a salinity of 26 on the total hydrogen ion scale. The pH values were reported using the total hydrogen ion scale (Dickson and Goyet, 1994). Temperature and salinity were measured using a multiparameter meter HANNA HI9828 (Hanna Instruments, Inc., Rhode Island, USA). Finally, the temperature, pH, salinity, and total alkalinity data were used to calculate pCO_2 and CO_3^{2-} and seawater saturation stages for calcite and aragonite using the CO_2SYS program for Microsoft Excel (Lewis and Wallace, 1998) set with Mehrbach solubility constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987).

2.3. Experimental rearing

The experimental setup included two different exposure temperatures (4 and 10 °C) at each of two pCO_2 levels (P: ~500 and F: ~1800 μatm). At the end of the habituation phase, four groups of 40

similar-sized fish (total n = 160) were randomly assigned to one of the four treatments (4P, 4F, 10P and 10F) and were kept in pairs in each rearing container (n = 20 replicates per treatment, two fish per replicate). Pilot tests indicated that this density was not stressful for this shoaling species. The temperature for fish in the 10P and 10F treatments was increased from 4 to 10 °C at a rate of 1 °C day^{-1} as suggested in the literature (Beitinger and Lutterschmidt, 2011). As in most OA studies, the pCO_2 exposure occurred acutely, directly after the treatment temperatures were reached (Manríquez et al., 2019).

Fish were exposed to the different treatments (4P, 4F, 10P and 10F) for one month, experiencing the same light and feeding regime used during the habituation phase. Once a week, fish were transferred into a new and clean rearing container filled with fresh filtered and treated seawater at the corresponding temperature and pCO_2 level. At the end of the exposure period, the fish were anesthetized with 0.5 g L^{-1} tricaine methano-sulphonate (MS-222), and the total length (TL, ± 0.1 mm) and body mass (BM, ± 10^{-2} g) were measured using a digital caliper (Sylvac, S 235 PAT, Yverdon, Switzerland) and a digital balance (Ohaus, TA302, NJ, USA), respectively. After a recovery time of ~1 h, they were randomly assigned to thermal, metabolic or biochemical measurements.

2.4. Thermal tolerance

The Critical Thermal Maximum (CTmax) and the Critical Thermal Minimum (CTmin) were estimated using the Critical Thermal Methodology (CTM, Becker and Genoway, 1979; Paladino et al., 1980; Beitinger et al., 2000). Fish initially acclimated to a specific temperature were subjected to a constant temperature change until the temperature at which a predefined sub-lethal endpoint was reached (Lutterschmidt and Hutchison, 1997; Beitinger and Lutterschmidt, 2011).

After 24 h of fasting, 80 fish were chosen at random for CTmax (n = 10 per treatment, total n = 40, Table 2) or CTmin (n = 10 per treatment, total n = 40, Table S1) trials and placed individually into 300 mL plastic beakers filled with seawater at the corresponding temperature and pCO_2 levels. For CTmax trials, the beakers were suspended within a 50-L plastic test chamber, and the temperature inside them was increased at a constant rate of 3 °C h^{-1} by heating the water in the test chamber with a thermoregulator Techne TU-20D (Bibby Scientific Limited, Stone, Staffordshire, UK). For CTmin trials, a constant cooling rate of 3 °C h^{-1} was achieved inside the beakers by placing them inside a cooling chamber. The selected rate of temperature change was chosen to address relevant physiological responses to experimental temperatures and pCO_2 levels and can also be considered ecologically relevant, even when it is beyond rates predicted to occur in the context of climate change (Åsheim et

al., 2020). During the trials, water temperature change continued until fish reached a sub-lethal endpoint, the loss of equilibrium (LOE), defined as the inability of fish to maintain dorso-ventral orientation for at least 1 min (Beitinger et al., 2000). When LOE was observed, the corresponding water temperature was recorded with a digital thermometer Lutron TM-917 provided with a Pt100 thermoresistance Luftman 3636 (Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) and then fish were returned to the initial experimental conditions to allow recovery.

The CTmax and CTmin of fish at each experimental condition were calculated as the mean temperature at which LOE was observed (Becker and Genoway, 1979; Beitinger et al., 2000). The thermal tolerance window (TTW) at each pCO_2 level was calculated as the difference between CTmax and CTmin.

2.5. Aerobic scope

Forty-eight fish (n = 12 per treatment, Table S1) were tested to estimate the aerobic scope (AS) by measuring their standard (SMR) and maximal (MMR) metabolic rates. Before the measurements, fish were fasted for 72 h to ensure a post-absorptive state. Then, they were introduced individually into a circular 2-L tank and chased until exhaustion for MMR determinations (Clark et al., 2013, Roche et al., 2013). Fish were considered exhausted when they did not respond to mechanical stimulation (Roche et al., 2013). After the chasing protocol, each fish was immediately transferred to a 100-mL static respirometry chamber, provided with a Strathkelvin 1302 Clark-type polarographic O_2 electrode connected to a Strathkelvin 928 6-channel O_2 system (Strathkelvin Instruments Limited, North Lanarkshire, Scotland). Each respirometry chamber was immersed in a water bath, and temperature and pCO_2 levels inside the chamber and the water bath were kept constant at the respective treatment condition throughout the measurements.

The instantaneous O_2 uptake was measured by intermittent flow respirometry (Steffensen, 1989; Svendsen et al., 2016) for 24 cycles (120 min each) that lasted 48 h. During each cycle, chambers were sequentially closed (105 min) and flushed (15 min) with clean and aerated treated seawater to prevent O_2 saturation levels from falling below 70% and also to eliminate potential hypercapnia and nitrogenous waste buildup in the chamber (Steffensen, 1989). An electrical submersible pump achieved the flush of water from the bath through the respirometry chamber. After 48 h, fish were removed and the background microbial respiration was measured for 24 h. The fish O_2 consumption rate was then calculated using linear least-squares regressions, excluding the first and last 2 min of

each closed phase. The background (water only) O_2 consumption rates were then subtracted from fish O_2 consumption rates, following Svendsen et al. (2016).

The MMR, corresponding to the maximal rate of aerobic metabolism of the fish in non-limiting conditions, was calculated using only the first 15 min (excluding the first 2 min) of measurements immediately after closing each respirometry chamber (Marras et al., 2015). The SMR, corresponding to the minimal cost of living measured in an inactive, post-absorptive fish, was calculated using the 15th percentile method in the last 12 measurement cycles (Chabot et al., 2016). The AS, corresponding to the energy available for activities above maintenance, such as muscular exercise, growth, or reproduction (Pörtner and Peck, 2010), was calculated as the difference between MMR and SMR.

2.6. Oxidative metabolism

Fish were euthanized following deep anesthesia with 0.5 g L^{-1} MS-222, and the gills and liver of 24 individuals (n = 6 per treatment, Table S1) were dissected, weighed (± 10^{-5} g), and stored at -80 ºC for two weeks until biochemical analyses were performed (see below). All the measurements were quantified in the gill and liver tissues. Even when the gills are the first organ to contact the environment, becoming a target for a more significant oxidative disruption, the liver, characterized by high metabolic activity, is a primary site for lipid peroxidation (Pörtner et al., 2005).

2.6.1. Oxidative damage

The lipid peroxidation of fish was quantified as the content of 2-thiobarbituric acid reactive substances (TBARS). Gill and liver tissues were homogenated in 50 mM potassium phosphate buffer (pH 7.0) and 30% (w/v) trichloroacetic acid, in a 1:4 (w/v) ratio. After centrifugation (4000 rpm for 10 min at 4 °C), the content of TBARS was determined in the supernatant, according to Malanga et al. (2004).

2.6.2. Antioxidant capacity

The activity of catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) enzymes were measured to quantify the effects of temperature and pCO_2 on the antioxidant capacity of fish. Homogenates from gill and liver tissues were prepared in 50 mM potassium phosphate 120 mM KCl (pH = 7.4). After centrifugation (2500 rpm for 10 min at 4 °C), the supernatants were stored at -80 °C until enzymes activities were determined. CAT activity was evaluated spectrophotometrically at 25 °C by the decomposition rate of H_2O_2 at λ = 240 nm in a reaction mixture consisting of the supernatant, 50 mM potassium phosphate buffer (pH 7.0) containing 1% Triton-

X100, 1:9 (w/v) and 12.5 mM H_2O_2 (Aebi, 1984). One CAT unit was defined as the amount of enzyme catalyzing the elimination of 1 mmol of H_2O_2 per minute.

SOD activity was measured by the epinephrine method (Misra and Fridovich, 1972), based on the capability of SOD to inhibit the autooxidation of epinephrine to adrenochrome at 480 nm at 30 ºC. One SOD unit was defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50% under the assay conditions.

GST activity was determined by incubating reduced glutathione with 1-chloro-2,4-dinitrobenzene as a substrate at 25 °C and measuring the absorbance increase at 340 nm (Habig et al. 1974). One GST unit was defined as the amount of enzyme catalyzing the formation of 1 µmol of 2,4 dinitrophenyl-S-glutathione per min.

2.7. Statistics

A one-way ANOVA or Kruskal-Wallis was used to test for differences among treatments in the TL and BM of juvenile *E. maclovinus* selected for thermal tolerance (Ctmax/Ctmin), metabolic (AS) or oxidative metabolism (lipid damage and antioxidant capacity). Assumptions of normality and homoscedasticity of residuals were evaluated through Shapiro–Wilks and Levene tests, respectively. A two-way ANOVA followed by a pairwise multiple comparison procedure (Tukey test) was then used to evaluate the effect of temperature and pCO_2 on the measured traits. If normality and/or homoscedasticity were not met, the effect of temperature and pCO_2 was examined after an aligned rank transformation (ART Analysis, Wobbrock et al., 2011). Statistical determinations were performed at a significance level of 5% (Zar, 1984; Sokal and Rohlf, 2011). All the analyses were performed using R software (version 3.6.1, R Core Team, 2019).

3. Results

At the end of the exposure period, the different groups of juvenile *E. maclovinus* selected for thermal tolerance (CTmax and CTmin), metabolic (AS) or oxidative metabolism (lipid damage and antioxidant capacity) determinations were not significantly different in mean total length or mean body mass among treatments (Table S1).

3.1. Thermal tolerance

Across all treatments, CTmax values ranged between 24.61 and 28.52 °C (Fig. 1a). Temperature, pCO_2 and the interaction between these factors significantly affected CTmax (all $p < 0.001$, Table 2). Across all treatments, CTmin values ranged between -1.94 °C and -1.50 °C (Fig. 1b). CTmin was significantly affected by temperature ($p < 0.001$) and pCO_2 ($p < 0.001$) but not by their interaction ($p = 0.1338$) (Table 2). At the present-day pCO_2 level, the TTW increased on average from 26.87 to 30.15 ºC between 4 and 10 °C, respectively. In contrast, at the future pCO_2 level, the increase in TTW from 4 to 10 °C was much more modest (26.31 to 27.53 ºC).

3.2. Aerobic scope

The SMR of fish was significantly affected by temperature ($p < 0.001$) but not by pCO_2 level ($p = 0.958$) and there was a significant interaction between the two factors ($p = 0.023$) (Table 3). The SMR of fish exposed to 4 and 10 °C, regardless of pCO_2 level, was on average 0.04 and 0.11 mg O_2 g BM^{-1} h^{-1}, respectively (Fig. 2a). At both experimental temperatures, the MMR and the AS significantly decreased with increasing pCO_2 (MMR: $p = 0.035$, AS: $p = 0.039$; Table 3). Compared to the present-day level, MMR (Fig. 2b) and AS (Fig. 2c) were 34 and 49% lower, respectively at the near-future pCO2 level. MMR was not significantly affected by temperature ($p = 0.867$) or the interaction between temperature and pCO_2 ($p = 0.851$) and the same was found for AS (temperature: $p = 0.093$; temperature × pCO_2: $p = 0.750$) (Table 3).

3.3. Oxidative metabolism

Acclimation to different temperatures and pCO_2 levels revealed lipid damage in the liver and the gills of juvenile *E. maclovinus*. The TBARS content showed tissue-specific differences in magnitude, with the liver displaying values around 6-fold higher than in the gills (Fig. 3a,b). Neither the temperature ($p = 0.607$) nor the pCO_2 ($p = 0.741$) or their interaction ($p = 0.138$) affected the TBARS content in the liver (Table 4). On the other hand, a significant effect of temperature ($p = 0.027$) was detected in the gills, resulting in higher TBARS contents at 10 °C than at 4 °C (Fig. 3a,b; Table 4).

The CAT activity of the liver tissue was significantly affected by temperature ($p = 0.022$) and pCO_2 ($p < 0.001$) but not by their interaction ($p = 0.507$) (Fig. 4a; Table 4). It increased with summer temperatures, resulting in 1.56-fold higher at present-day pCO_2 levels and 2.19 higher at near-future pCO_2 levels. On the contrary, near-future pCO_2 levels reduced CAT activity to values ranging from 600.78 at 4ºC to 1313.27 U mg FW^{-1} at 10 ºC (Fig. 4a; Table 4). The CAT activity of the gill tissue

was affected by pCO_2 levels ($p = 0.044$), showing lower values at near-future pCO_2 levels, but not by temperature ($p = 0.640$) or the interaction between both factors ($p = 0.983$) (Fig. 4b; Table 4).

The SOD activity in the liver tissue was significantly affected by temperature ($p < 0.001$) but not by pCO_2 level ($p = 0.117$), and there was a significant interaction between the two factors ($p = 0.020$). Regardless of pCO_2 levels, activity levels ranged from 40.22 (4 °C) to 91.80 U mg FW^{-1} (10 °C) (Fig. 4c; Table 4). The SOD activity in the gill tissue was not affected by temperature ($p = 0.094$), but it was significantly affected by pCO_2 levels ($p < 0.001$) and by the interaction between both factors ($p = 0.005$). Activity levels in the gills ranged from 32.23 and 141.84 mg FW^{-1} in fish exposed to present-day and near-future pCO_2, respectively (Fig. 4d; Table 4).

The activity of GST in the liver differed significantly with temperature (1.15-fold increase between 4 and 10 °C, $p = 0.010$) and pCO_2 (1.26-fold increase from present-day to near-future pCO_2 levels, $p = 0.038$) but not by the interaction between them ($p = 0.348$) (Fig. 4e, Table 4). Conversely, in the gill tissue no effects of temperature ($p = 0.060$), pCO_2 ($p = 0.415$) or their interaction ($p = 0.424$) were detected in the GST activity (Fig. 4f; Table 4).

4. Discussion

The present study is, to our knowledge, the first report of the combined effects of seasonal seawater temperature extremes (summer and winter) and OA on functional traits linked with the thermal performance of a notothenioid fish inhabiting the Beagle Channel at the southern tip of South America. By examining both organismal and sub-organismal responses in juvenile *E. maclovinus*, we found a reduction in the thermal tolerance range under summer temperatures and near-future OA conditions associated with a reduction in the AS, registered at the elevated pCO_2 level. An oxidative stress condition was also detected in the gill and liver tissues. Such responses may have significant consequences under the present trajectories of climate change.

The critical thermal limits measured for juvenile *E. maclovinus* were typical of temperate species inhabiting a wide range of temperatures, both seasonally and spatially (Nati et al., 2021). Furthermore, the upper thermal tolerance limits (average CTmax: 26.73 ºC) of *E. maclovinus* were higher than that reported for other notothenioid fish from the high Antarctic at McMurdo Sound and the maritime Antarctic on the Antarctic Peninsula (Bilyk and DeVries, 2011). Under present-day pCO_2 levels, fish exhibited broad TTWs (26.87 to 30.15 °C, at 4 and 10 °C, respectively), with values for both CTmax and CTmin increasing with increasing temperature exposure, which is in accordance with Beitinger and Bennett (2000). However, such TTWs were narrower than those previously estimated for juvenile

E. maclovinus from the Beagle Channel (29.31 and 32ºC at 4 and 10 °C, respectively; Lattuca et al., 2018). The broader thermal tolerance limits previously reported for this species may be due to a methodological difference; CTM trials conducted by Lattuca et al. (2018) applied a different warming/cooling rate (18 °C h^{-1}) compared with the present study (3 °C h^{-1}). The rate of temperature change used here was chosen to be the same as other studies conducted in the "CLIMAR" research program examining the effects of warming and ocean acidification on the thermal tolerance of a range of species living in different marine habitats (e.g. Manríquez et al., 2020; Alter and Peck, 2021). Fast heating rates tend to produce higher CTmax values, though the same is not necessarily valid for cooling rates and CTmin (Kovacevic et al., 2019). Moreover, *E. maclovinus* had a wider thermal tolerance than the sub-Antarctic notothenioids *Harpagifer bispinis* and *Patagonotothen tessellata,* also inhabiting the coastal waters of the Beagle Channel. According to Giménez et al. (2021), TTWs of *H. bispinis* range between 25.57 and 27.02 ºC and those of *P. tessellata* range between 24.99 and 25.88 ºC in fish acclimated at 4 and 10 ºC, respectively.

The TTWs of juvenile *E. maclovinus* were reduced by future pCO_2 levels, with values ranging between ~26 and ~28 °C at 4 and 10 °C, respectively. Furthermore, a more significant influence of high pCO_2 was observed on the upper than on the lower thermal tolerance limit since CTmax values at summer temperatures were much lower (5.44%) than those registered under the present-day pCO_2 level. This significant difference suggests that, under near-future pCO_2 levels, juvenile fish may not fully acclimate to seasonal changes in temperature. Despite the reduction in TTW measured at near-future pCO_2 levels and summer temperatures, thermal tolerance could still be broad enough to allow this fish species to cope in near-future increases in temperatures at cold-temperate latitudes.

Quantifying the TTW of a species is central to understanding how present-day distributions can potentially change in response to variations in environmental conditions projected for specific ecosystems. In this regard, a strong surface warming has already been observed in the southwest South Atlantic Ocean over the last two decades due to a southward displacement of the Brazil Current (Goni et al., 2011; Yang et al., 2020). This warming allows marine fish and other species to colonize higher latitudes and/or for migratory species to display a more extended residency in regions sub-optimally cold in the winter (Franco et al., 2020). For example, an increase in fish richness was driven by an influx of species from warmer waters in Northern and Central Patagonia (Galván et al., 2021). The tropicalization of temperate waters is an ongoing global phenomenon that has impacted sea surface temperatures to 48ºS latitude (Galván et al., 2021). Furthermore, in the last five decades, increased anthropogenic CO_2 has altered the chemical conditions of the Argentine Basin, with all depths displaying ocean acidification (Fontela et al., 2021). According to Pörtner (2008), organisms exposed

to increased pCO_2 levels will have reduced tolerance to thermal extremes. In line with this idea, the narrowest TTW of juvenile *E. maclovinus* in the present study occurred under future pCO_2 levels at a high summer temperature.

Temperature-induced limitations on the capacity of the cardiorespiratory system to transport oxygen from the environment reduce AS and have been proposed as the main factor determining the critical thermal limits of fish and other water-breathing ectotherms (Pörtner, 2002; Pörtner and Knust, 2007). However, varying responses have been documented concerning the effect of OA on fish metabolism, depending on their life stages, physiological types (i.e., stenohaline or euryhaline), climatic zones or habitats. Furthermore, the directionality of each metabolic response (SMR, MMR or AS) of fish may differ under the same OA conditions (Cattano et al., 2018). In the present study, juvenile *E. maclovinus* showed an AS decrease following a one-month acclimation to near-future pCO_2 levels. Reductions in AS are expected when increasing temperatures raise the standard metabolism in ectothermic animals, while maximum oxygen supply fails to increase correspondingly (Melzner et al., 2009; Pörtner and Farrell, 2008). Accordingly, and in good agreement with expected responses for ectotherm organisms with broad latitudinal distributions (Markle and Kozak, 2018), the SMR of juvenile *E. maclovinus* was significantly affected by increasing exposure temperature and by the interactive effect of temperature and pCO_2. The SMR at 10 °C and present-day pCO_2 levels in this study were slightly higher than rates previously measured in *E. maclovinus* by Vanella et al. (2012, 2017). Such differences could result from different fish body masses between studies. On the other hand, the MMR significantly decreased with increasing pCO_2 levels, with a consequent reduction of the AS. Therefore, within the narrow temperature range explored in this study (4-10 °C), this reduction could explain the reduction of the TTW of juvenile *E. maclovinus* at austral summer temperatures and near-future pCO_2 levels.

The effects of elevated pCO_2 levels on MMR, and consequently the AS, could have been due to either direct disturbances or costs associated with compensatory mechanisms. Physiological effects of CO_2 are mediated through low pH in acidified water and diffusive CO_2 entry into the organism. Elevated CO_2 elicits an acidosis in tissues and body fluids; acute effects may occur when plasma pH is rapidly lowered, and oxygen transport by pH-sensitive blood pigments is disrupted (Pörtner et al. 2005). Even under normal (present-day) pCO_2 levels, work by Brauner et al. (2000) on rainbow trout (*Oncorhynchus mykiss*) indicated that arterial CO_2 may build-up during exercize due to diffusion limitations of CO2 causing the onset of respiratory acidosis. Bicarbonate accumulation and active ion transport are used by fish to compensate for increasing acidosis and to regulate their acid-base balance (Claiborne et al. 2002). Such ATP-demanding compensation activities for acid-base balance and

enhanced transport of ions may incur elevated energetic costs (Heuer and Grosell 2014). Pörtner (2004) indicated that temperature effects lead to higher costs for pH regulation in cold-adapted eurytherms (as *E. maclovinus*) compared to polar stenotherms; however, knowledge of high pCO_2 levels' effects on the metabolism of cold environment fish is scarce (Cattano et al., 2018).

According to the OCLTT hypothesis, the physiological performance of ectothermic animals should decline with the decline of the AS (Pörtner 2008, 2010). However, Gräns et al. (2014) reported that an increased AS of Atlantic halibut (*Hippoglossus hippoglossus)* did not translate into improved growth when exposed to elevated temperatures and pCO_2 levels, suggesting that oxygen uptake was not the factor limiting growth performance. Therefore, future studies should consider moving beyond seasonal temperature changes and include the effect of a broader range of temperatures and pCO_2 levels to test whether the response of *E. maclovinus* agrees with the OCLTT hypothesis. This will allow more robust predictions of how this species will respond under near-future climate change scenarios to ocean warming and acidification.

As seen with ecologically relevant increases in temperatures (Abele and Puntarulo, 2004; Lesser, 2006), increases in pCO_2 levels can also disrupt the oxidative metabolism of marine organisms (Pimentel et al., 2015; Carney Almroth et al., 2019). Therefore, the combined effect of these two environmental drivers may exacerbate decrements in cellular homeostasis (Pörtner, 2008). After a one-month exposure period to different pCO_2 levels coupled with seasonal temperature extremes, juvenile *E. maclovinus* showed different levels of oxidative damage (i.e., lipid peroxidation) in the liver and gill tissues. These results could be grounded in the different functional capacities of both organs and susceptibility to ROS (Oliveira et al., 2008; Nahrgang et al., 2010). Similarly, Enzor and Place (2014) found higher levels of oxidative damage in the liver than in the gills of the Antarctic notothenioid *Trematomus bernacchi*, *Pagothenia borchgrevinski* and *Trematomus newnesi* exposed to elevated temperature and pCO_2 levels in a 56-day exposure period. Additionally, summer temperatures in the present study caused an increase in gills' lipid peroxidation that might be influenced by the increase in the SMR of juvenile *E. maclovinus* under similar acclimation conditions. Different works have shown that an increase in the metabolic rate causes an increase in radical production and, consequently, in oxidative damage (Sohal et al., 1989; Zielinski and Pörtner, 2000). Thus, present data could indicate that lipid peroxidation is possibly linked to the metabolic production of ROS in *E. maclovinus* and that metabolic capacity could play a significant role in adapting to elevated temperatures in this species.

Oxidative damage is counteracted by the antioxidant defence systems and repair mechanisms (Lushchak, 2011). Particularly SOD and CAT, catalyzing the breakdown of oxygen radicals and H_2O_2, respectively, serve as primary antioxidants (Lushchak, 2011), and GST is responsible for the metabolic

inactivation of electrophilic compounds and toxic substrates (Habig et al., 1974). In juvenile *E. maclovinus*, the activity of these enzymatic antioxidants varied between tissues. While changes in the antioxidant enzyme defenses in response to different temperatures were restricted to the liver tissue, changes in response to different pCO_2 levels were detected in both organs. Specifically, the increase in SOD, CAT and GST activities in the liver tissue translated into an absence of significant changes in lipid peroxidation. However, the increase of SOD and the decrease of CAT activities in the gills were insufficient to neutralize the effects of potentially higher ROS generated under changing environmental conditions. Overall, these results indicate the generation of an oxidative stress condition (Lushchak, 2014) in the liver and gills of the sub-Antarctic *E. maclovinus* exposed to OA and warmer temperatures. These findings also suggest that this species does not maintain a constant high antioxidant defence level, as the Antarctic *T. bernacchi*, *P. borchgrevinski* and *T. newnesi* do to compensate for predicted temperature and pCO_2 increases in the Southern Ocean (Enzor and Place, 2014).

Taken together, the results of the present study suggest that the combined effect of chronic exposure to OA and the current summer temperatures pose limits to the thermal performance of juvenile *E. maclovinus* at the organismal (thermal tolerance and aerobic scope) and sub-organismal (oxidative metabolism) levels, making this species vulnerable to projected climate-driven warming. Following the general expectations for biological and ecological responses to warming (Poloczanka et al., 2016), *E. maclovinus* is expected to shift its distribution poleward under projected near-future warming. Distribution shifts often track the same trajectories as the species' optimal climates (Flanagan et al., 2019). However, in the southern hemisphere, there is a limit to how far poleward temperate species will be able to shift before they are blocked by the Southern Ocean (Fraser et al., 2012).

Future studies are needed to evaluate the potential consequences of the combined direct effect of OA and projected warming (+1 to 3 °C) on the same and other important fitness-related traits of *E. maclovinus*. We suggest using physiological data, such as those obtained here, to forecast shifts in habitat suitability across the distribution range of *E. maclovinus* in the context of climate change, which could be a valuable tool for management and conservation.

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847 **Captions**

848 Fig. 1. (a) Critical thermal maximum (CTmax) and (b) minimum (CTmin) of juvenile *Eleginops*
849 *maclovinus* exposed to a combination of temperatures (4 and 10 °C) and pCO_2 levels (500, open box,
850 and 1800 µatm, filled box) for one month. Box plots display the 25th and 75th percentiles, the median
851 (solid lines), the mean (dotted lines), the 10th and 90th percentiles (whiskers) and outliers (dots). n =
852 10 is the sample size for each box plot. Different letters above the box plots represent significant
853 differences ($P \leq 0.05$) between temperatures (uppercase) and pCO_2 levels (lowercase) in Tukey *post*
854 *hoc* tests.

855

856 Fig. 2. (a) Standard metabolic rates (SMR), (b) maximal metabolic rates and (c) aerobic scope (AS) of
857 juvenile *Eleginops maclovinus* exposed to a combination of temperatures (4 and 10 °C) and pCO_2
858 levels (500, open box, and 1800 µatm, filled box) for one month. Box plots display the 25th and 75th
859 percentiles, the median (solid lines), the mean (dotted lines), the 10th and 90th percentiles (whiskers)
860 and outliers (dots). n = 11 (present-day pCO_2 at 4 °C), n = 8 (future pCO_2 at 4 °C), n= 10 (present-day
861 pCO_2 at 10 °C) and n = 10 (future pCO_2 at 10 °C) are the sample sizes for corresponding box plots.
862 Different letters above the box plots represent significant differences ($P \leq 0.05$) between temperatures
863 (uppercase) and pCO_2 levels (lowercase) in Tukey *post hoc* tests.

864

865 Fig. 3. Lipid damage, measured as TBARS contents in (a) the liver and (b) gills of juvenile *Eleginops*
866 *maclovinus* from the Beagle Channel exposed to a combination of temperatures (4 and 10 °C) and
867 pCO_2 levels (500, open box, and 1800 µatm, filled box) for one month. Box plots display the 25th and
868 75th percentiles, the median (solid lines), the mean (dotted lines). n = 6 is the sample size for each box
869 plot. Different letters above the box plots represent significant differences ($P \leq 0.05$) between
870 temperatures (uppercase) and pCO_2 levels (lowercase) in Tukey *post hoc* tests. Note the difference in
871 the y-axis scale between results for liver and gills.

872

873 Fig. 4. Antioxidant capacity, quantified as (a, b) catalase (CAT), (c, d) superoxide dismutase (SOD)
874 and (e, f) glutathione S-transferase (GST) enzymes activities in the liver and gills of juvenile *Eleginops*
875 *maclovinus* exposed to a combination of temperatures (4 and 10 ºC) and pCO_2 levels (500, open box,
876 and 1800 µatm, filled box) for one month. Box plots display the 25th and 75th percentiles, the median
877 (solid lines), the mean (dotted lines). n = 6 is the sample size for each box plot. Different letters above
878 the box plots represent significant differences ($P \leq 0.05$) between temperatures (uppercase) and pCO_2
879 levels (lowercase) in Tukey *post hoc* tests. Note the difference in the y-axis scale between results for
880 liver and gills.

881 Table 1. Seawater parameters (mean ± SE) throughout the experiment with juvenile *Eleginops maclovinus*. 4P: present-day pCO_2 at 4 °C, 4F: future pCO_2
882 at 4 °C, 10P: present-day pCO_2 at 10 °C, 10F: future pCO_2 at 10 °C.

883

Treatments	pH at 25ºC (pH units)	Temperature (º C)	Total alkalinity (µmol kg^{-1} SW)	pCO_2 in situ (µatm)	$[CO_3^{2-}]$ in situ (µmol kg SW^{-1})	Salinity	Ω calcite	Ω aragonite
Natural seawater	8.03 ± 0.01	6.98 ± 1.02	2056 ± 6	536 ± 9	65 ± 3	24.63 ± 0.06	1.64 ± 0.08	1.01 ± 0.05
4P	8.04 ± 0.02	4.51 ± 0.04	1960 ± 18	502 ± 32	58 ± 2	24.58 ± 0.07	1.45 ± 0.06	0.88 ± 0.04
4F	7.47 ± 0.01	4.47 ± 0.03	1934 ± 16	1887 ± 28	16 ± 1	24.62 ± 0.06	0.41 ± 0.01	0.25 ± 0.01
10P	8.02 ± 0.01	10.56 ± 0.13	1929 ± 25	536 ± 11	67 ± 2	24.74 ± 0.07	1.69 ± 0.04	1.04 ± 0.01
10F	7.48 ± 0.01	10.56 ± 0.13	1871 ± 32	1886 ± 40	20 ± 1	24.78 ± 0.06	0.51 ± 0.01	0.31 ± 0.01

884

Table 2. Two-way ANOVA or ART Analysis followed by two-way ANOVA for the critical thermal maximum/minimum (CTmax/CTmin) of juvenile *Eleginops maclovinus* after a one-month exposure to a combination of temperatures (4 and 10 °C) and pCO_2 levels (~500 and 1800 µatm). Asterisks indicate statistically significant differences ($P \leq 0.05$).

Source of variation	Degrees of freedom	F values	P	Comparisons
CTmax (Two-way ANOVA)				
Temperature	1	675.93	< 0.001*	
pCO_2	1	213.72	< 0.001*	
Temperature × pCO_2	1	127.61	< 0.001*	
Residual	36			
CTmin (ART + Two-way ANOVA)				
Temperature	1	32.77	1.623×10^{-6}*	4 < 10
pCO_2	1	16.86	0.0002*	P < F
Temperature × pCO_2	1	2.35	0.1338	
Residual	36			

892 Table 3. Summary of two-way ANOVA for the standard (SMR) and maximum metabolic rates (MMR)
893 and aerobic scope (AS) of juvenile *Eleginops maclovinus* after a one-month exposure to a combination
894 of temperatures (4 and 10 °C) and pCO_2 levels (~500 and 1800 µatm). Asterisks indicate statistically
895 significant differences ($P \leq 0.05$).
896

Source of variation	Degrees of freedom	F values	P	Comparisons
	SMR (Two-way ANOVA)			
Temperature	1	76.45	< 0.001*	
pCO_2	1	0.01	0.958	
Temperature × pCO_2	1	5.67	0.023*	
Residual	34			
	MMR (Two-way ANOVA)			
Temperature	1	0.03	0.867	
pCO_2	1	4.83	0.035*	P > F
Temperature × pCO_2	1	0.04	0.851	
Residual	33			
	AS (Two-way ANOVA)			
Temperature	1	2.99	0.093	
pCO_2	1	4.63	0.039*	P > F
Temperature × pCO_2	1	0.10	0.750	
Residual	33			

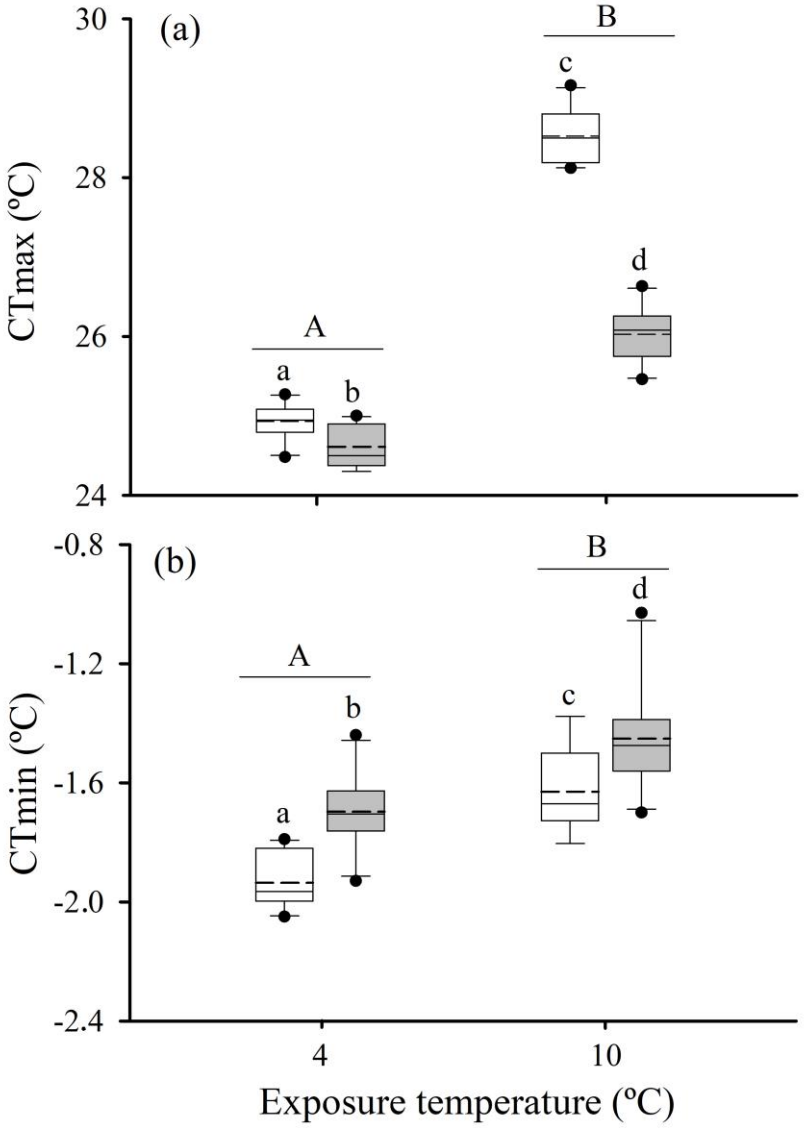
897

898

899 Table 4. Two-way ANOVA or ART Analysis followed by two-way ANOVA for the lipid damage and
900 the antioxidant capacity in the liver and gills of juvenile *Eleginops maclovinus* after a one-month
901 exposure to a combination of temperatures (4 and 10 °C) and pCO_2 levels (~500 and 1800 µatm).
902 Asterisks indicate statistically significant differences ($P \leq 0.05$).

Source of variation	Degrees of freedom	F values	P	Comparisons
Lipid damage				
TBARS liver (Two-way ANOVA)				
Temperature	1	0.273	0.607	
pCO_2	1	0.112	0.741	
Temperature × pCO_2	1	2.392	0.138	
Residual	20			
TBARS gills (ART + Two-way ANOVA)				
Temperature	1	5.950	0.027*	4 < 10
pCO_2	1	1.497	0.239	
Temperature × pCO_2	1	0.049	0.828	
Residual	16			
Antioxidant capacity				
CAT liver (ART + Two-way ANOVA)				
Temperature	1	6.14	0.022*	4 < 10
pCO_2	1	21.264	1.68×10^{-4}*	P > F
Temperature × pCO_2	1	0.456	0.507	
Residual	20			
CAT gills (Two-way ANOVA)				
Temperature	1	0.225	0.64	
pCO_2	1	4.644	0.044*	P > F
Temperature × pCO_2	1	0.000486	0.983	
Residual	20			
SOD liver (ART + Two-way ANOVA)				
Temperature	1	48.544	3.17×10^{-6}*	
pCO_2	1	2.742	0.117	
Temperature × pCO_2	1	6.616	0.020*	
Residual	16			
SOD gills (ART + Two-way ANOVA)				
Temperature	1	3.082	0.094	
pCO_2	1	37.691	5.34×10^{-6}*	
Temperature × pCO_2	1	9.981	0.005*	
Residual	20			
GST liver (ART + Two-way ANOVA)				
Temperature	1	7.993	0.010*	4 < 10
pCO_2	1	4.942	0.038*	P < F
Temperature × pCO_2	1	0.922	0.348	
Residual	20			
GST gills (Two-way ANOVA)				
Temperature	1	3.982	0.060	
pCO_2	1	0.694	0.415	
Temperature × pCO_2	1	0.667	0.424	
Residual	20			

903 Figure 1.



904

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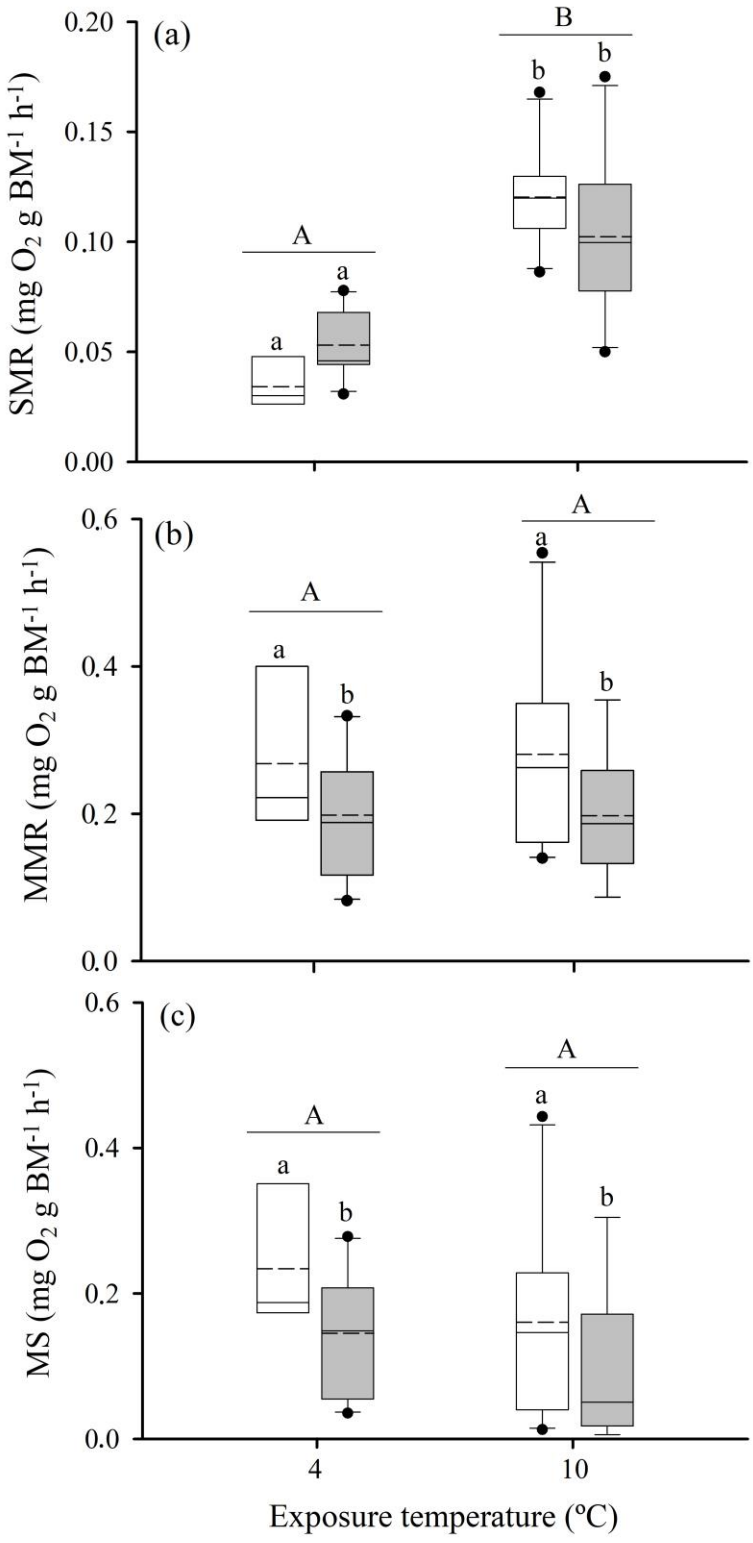
906 Figure 2.



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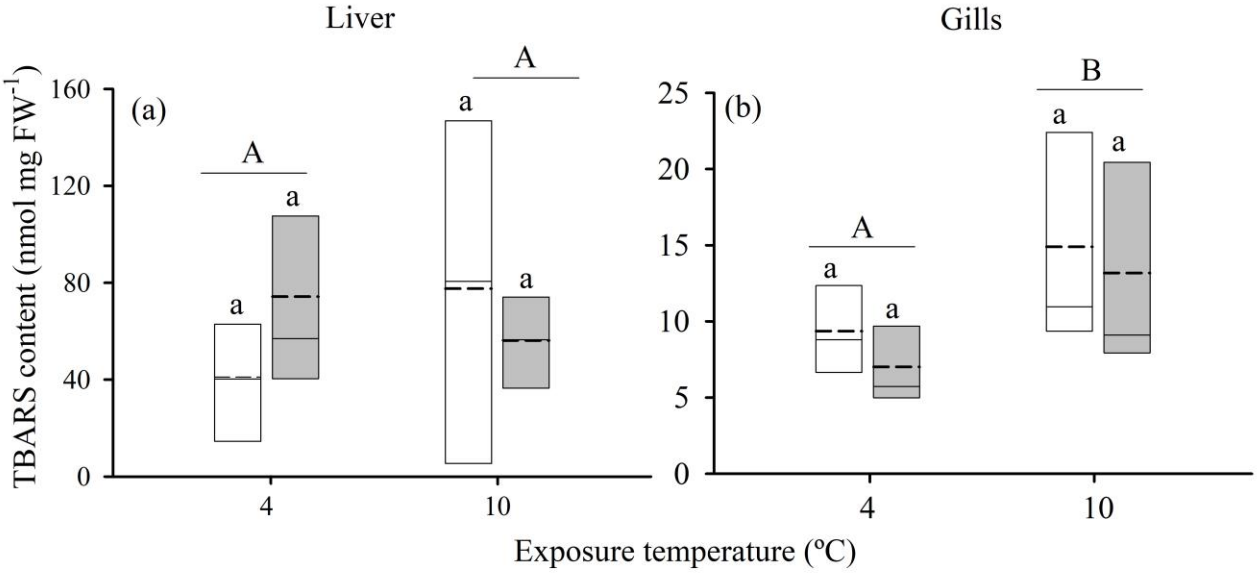
909 Figure 3.



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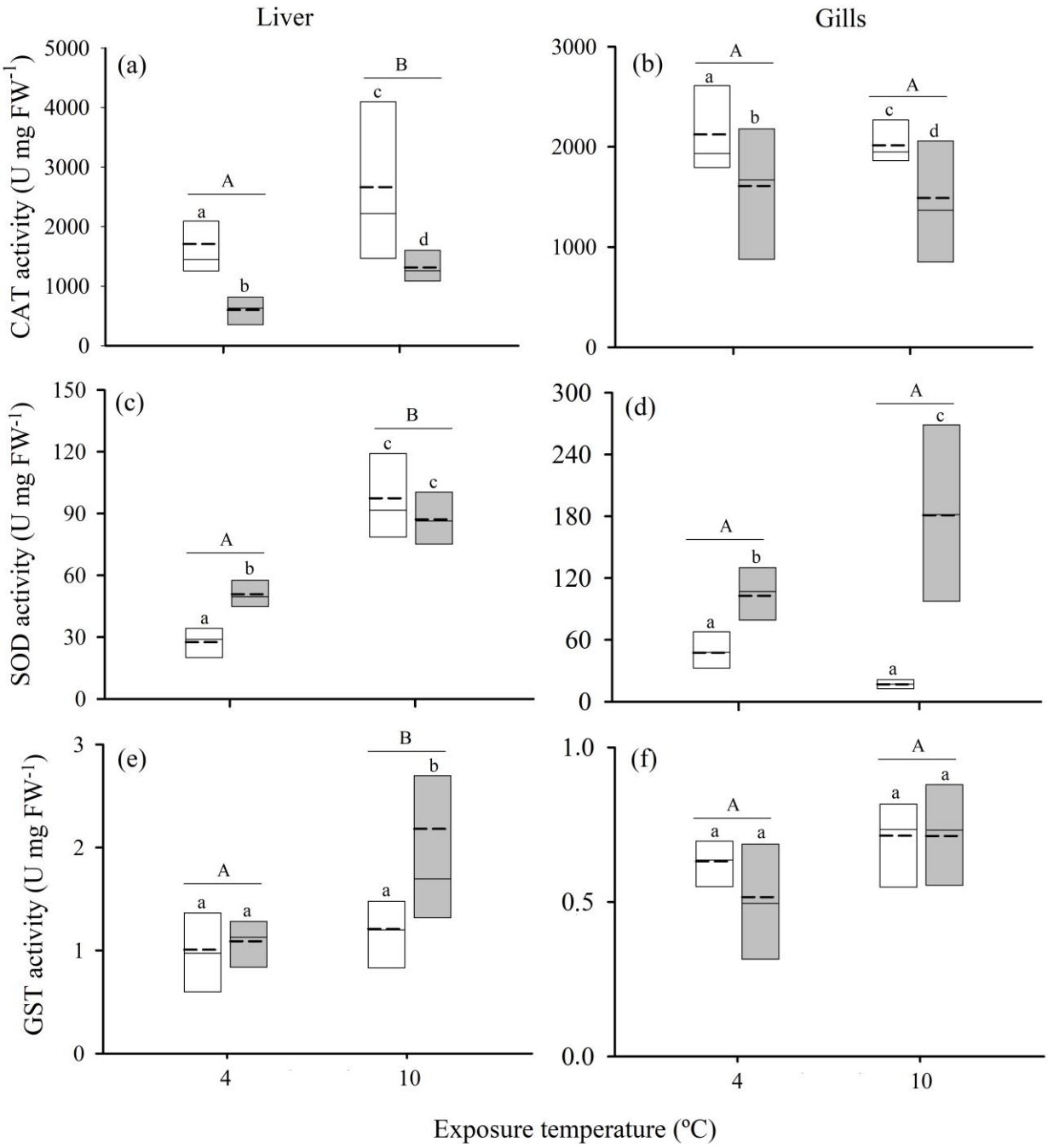
913 Figure 4.



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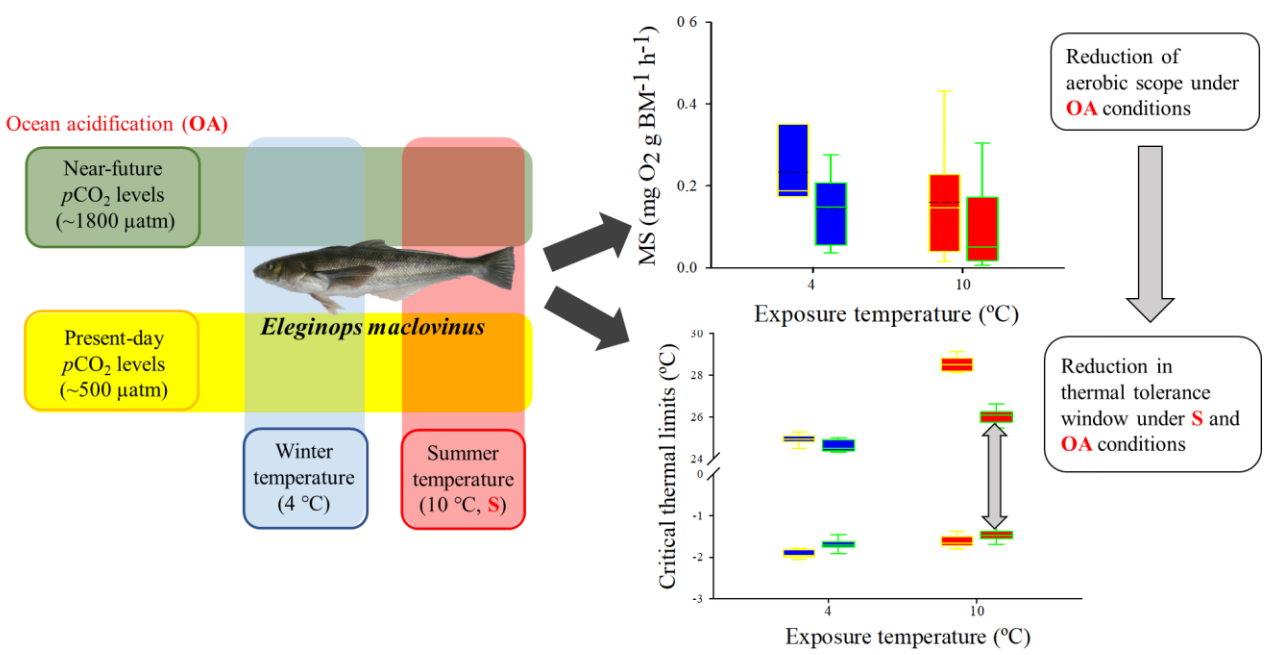
916 Graphical abstract



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Highlights

1. Climate change stressors impaired the thermal physiology of *Eleginops maclovinus*
2. Summer temperature and near-future pCO_2 levels reduced its thermal tolerance
3. Concomitant reductions occurred in fish aerobic scope at near-future pCO_2
4. An oxidative stress condition was detected in the gills and liver tissues

Table S1. One-way ANOVA or Kruskal-Wallis testing for differences among treatments in the total length (mm) and body mass (g) of juvenile *Eleginops maclovinus* selected for the critical thermal maximum/minimum (Ctmax/Ctmin), aerobic scope (AS) or oxidative metabolism (lipid damage and antioxidant capacity) determinations after a one-month exposure to a combination of temperatures (4 and 10 °C) and pCO_2 levels (500 and 1800 µatm).

Source	Body parameter	Average ± SE	n	Test	Degrees of freedom	F/H values	P
CTmax	Total length	55.87 ± 0.76	40	One-way ANOVA	3	0.327	0.806
	Body mass	1.01 ± 0.05	40	Kruskal-Wallis	3	3.854	0.278
CTmin	Total length	54.98 ±0.86	40	Kruskal-Wallis	3	4.302	0.231
	Body mass	1.11 ± 0.05	40	Kruskal-Wallis	3	6.667	0.083
AS	Total length	66.26 ± 1	48	One-way ANOVA	3	2.194	0.106
	Body mass	1.80 ± 0.07	48	Kruskal-Wallis	3	2.868	0.412
Oxidative metabolism	Total length	67.42 ± 0.89	24	One-way ANOVA	3	1.633	0.213
	Body mass	2.07 ± 0.08	24	One-way ANOVA	3	0.978	0.423