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Ocean acidification and elevated temperatures alter the behavior of a sub-Antarctic fish

María E. Lattuca [a,*], María E. Barrantes [a], Eloísa M. Giménez [a], Patricio H. Manríquez [b,c], Rodrigo Torres [d,e], Paolo Domenici [f,g], Myron A. Peck [h,i], Daniel A. Fernández [a,j]

[a] Laboratorio de Ecología, Fisiología y Evolución de Organismos Acuáticos, Centro Austral de Investigaciones Científicas (CADIC-CONICET), Bernardo Houssay 200, V9410BFD Ushuaia, Argentina
[b] Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Av. Bernardo Ossandón 877, 1781681 Coquimbo, Chile
[c] Laboratorio de Ecología y Conducta de la Ontogenia Temprana (LECOT), Larrondo 1281, 1781421 Coquimbo, Chile
[d] Centro de Investigación en Ecosistemas de la Patagonia (CIEP), José de Moraleda 16, 5951369 Coyhaique, Chile
[e] Centro de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Av. El Bosque 01789, 6200000 Punta Arenas, Chile
[f] CNR-IAS, Località Sa Mardini, 09070 Torregrande, Oristano, Italy
[g] CNR-IBF, Area di Ricerca San Cataldo, Via G. Moruzzi N°1, 56124 Pisa, Italy
[h] Royal Netherlands Institute for Sea Research (NIOZ), Department of Coastal Systems (COS), P.O. Box 59, 1790 AB Den Burg, Texel, the Netherlands
[i] Marine Animal Ecology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands
[j] Universidad Nacional de Tierra del Fuego, Instituto de Ciencias Polares, Ambiente y Recursos Naturales (UNTDF - ICPA), Fuegia Basket 251, V9410BXE Ushuaia, Argentina

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ABSTRACT

The interaction of multiple climate change stressors can affect the behavior of marine fish. While these effects have been reported in tropical and temperate species, much less is known for fish inhabiting high latitudes. We analyzed the combined effects of ocean acidification and the highest and lowest seasonal temperatures on the activity level and boldness of *Eleginops maclovinus*, an ecologically and commercially important notothenioid fish from the subantarctic area. Juveniles were acclimated for one month to two temperatures (T = 4 and 10 °C) and two pCO_2 levels (~500 and ~1800 μatm) in a full factorial design. In an open field test, the time spent active was significantly affected by temperature, with fish at 10 °C 1.63 times more active than those at 4 °C, but not by pCO_2 or the interaction (T × pCO_2). No differences were observed in the average swimming velocity measured when active, nor in the time spent in the inner zone of the tank. A refuge emergence test indicated increased boldness under near-future pCO_2 levels with fish emerging 2.06 (4 °C) and 1.23 (10 °C) times faster than those acclimated to present-day pCO_2 levels. The disruptions of these fundamental behaviors by these climate-driven stressors could have consequences for foraging and predator-prey interactions, with likely detrimental effects on the interactions among sympatric subantarctic fishes under projected climate change scenarios.

1. Introduction

Since the beginning of the Industrial Revolution, the carbon dioxide (CO_2) released from anthropogenic activities has increased atmospheric CO_2 concentrations (Raven et al., 2005), resulting in a greenhouse effect that has increased the temperature of ocean surface waters (ocean warming, OW) (Levitus et al., 2005). The increased concentrations of CO_2 in seawater (pCO_2) have changed the seawater carbonate system causing a decrease in the pH (ocean acidification, OA) (Doney et al., 2009). Consequently, the average pH of ocean surface waters has already decreased by approximately 0.1 units and is expected to decrease an additional 0.3–0.4 pH units by 2100 (Caldeira and Wickett, 2003; IPCC, 2021). Due to these unprecedented changes, studies reporting the effects of OA and its interaction with OW on marine fish inhabiting open ocean and coastal ecosystems have markedly increased in the last decade. These studies include impacts on vital rates such as survival (Stiasny et al., 2016; Dahlke et al., 2017), growth (Gräns et al., 2014) and reproduction (Miller et al., 2015; Welch and Munday, 2016), as well as aspects of growth physiology such as morphology (Pimentel et al., 2014; Yona et al., 2016; Muller et al., 2021), metabolism (Strobel

* Corresponding author.
E-mail address: elattuca@gmail.com (M.E. Lattuca).

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et al., 2012, 2013), thermal tolerance (Alter and Peck, 2021; Lattuca et al., 2023), otolith formation (Bignami et al., 2013) and yolk provisioning (Murray and Klinger, 2022). In their meta-analysis, Cattano et al. (2018) reported that differences in fish sensitivity to OA depended on the magnitude of climate change (CO_2 scenarios), fish stage, and species-specific traits and ecological characteristics. Furthermore, the interaction between OA and OW may mitigate, reverse, or enhance the effects of elevated pCO_2 on marine fishes (Harvey et al., 2013; Kroeker et al., 2010; Alter et al., 2024).

The effects of stressors associated with climate change on fish behavior, such as activity, boldness, predator avoidance and lateralization among others, have garnered considerable attention as these and other behaviors mediate biological interactions that, in turn, can impact the structure and functioning of ecosystems (Clements and Hunt, 2015). Particularly, routine activity is a behavioral trait that has been studied extensively because it affects the acquisition of food, daily energy expenditure, and, ultimately, the fitness of individuals (Careau et al., 2008). Fish often face a trade-off where increased activity levels lead to both greater feeding opportunities, and hence growth rates, and a higher risk of mortality due to higher incidences of predation (Biro et al., 2003; Werner and Anholt, 1993). Temperature is known to increase activity in marine fishes (Peck et al., 2006; Biro et al., 2010) and it has been discussed that this effect is mediated indirectly through its influence on metabolic rates (Laubenstein et al., 2018).

Contrasting results on the effect of OA on activity have been reported for marine fishes. For instance, activity increased in juvenile coral trout, *Plectropomus leopardus*, tested under experimental conditions in the laboratory (Munday et al., 2013) and two cardinalfish, *Apogon cyanosoma* and *Cheilodipterus quinquelineatus*, living at natural volcanic CO_2 seeps (Munday et al., 2014). On the other hand, the activity of the larvae of *Rachycentron canadum*, a large pelagic fish, was mainly unaffected after exposure to 800 and 2100 μatm pCO_2 (Bignami et al., 2013). However, the larvae of *Coryphaena hippurus* exhibited a decrease in swimming duration and maximum swimming velocity at similarly high pCO_2 levels (Bignami et al., 2014).

Boldness, considered here as the propensity to take risks, is another commonly measured behavioral trait with ecological and evolutionary relevance in fishes (Brown et al., 2007). Different levels of boldness could impact how individuals handle everyday ecological challenges, such as competing for mates or food resources, foraging under the threat of predators, and selecting habitats. As a result, boldness can affect reproductive success, survival, and overall fitness of individuals (White et al., 2013). Moreover, boldness may have physiological underpinnings and could be inherited, making it a trait that might evolve through natural selection over generations (Brown et al., 2007). As with activity, changes in boldness have been linked to higher predation rates (Biro et al., 2003; Smith and Blumstein, 2008). Boldness has been reported to either increase, decrease, or be unaffected by OA. For instance, exposure to high pCO_2 was reported to increase boldness in the common coral trout, *Plectropomus leopardus* (Munday et al., 2010), decrease boldness in the stickleback *Gasterosteus aculeatus* (Jutfelt et al., 2013) and have not effects on boldness in juvenile Atlantic cod, *Gadus morhua*, (Jutfelt and Hedgärde, 2015). Regarding temperature, its relationship with activity and boldness was suggested to be driven by the direct impact of temperature on metabolic rates and appetite (Biro et al., 2010).

Given the large differences reported in the literature, it is not surprising that the effect of OA on fish behavior is a source of debate (Clark et al., 2020; Munday et al., 2020), with initial studies reporting large effects, but subsequent studies reporting fewer effects (Clements et al., 2022; Munday, 2022; Esbaugh, 2023). Initial research suggested that OA impaired the functioning of neurotransmitters (Heuer and Grosell, 2014), with several studies proposing disruption in the $GABA_A$ receptor as the primary mechanism of altering behavior (Nilsson et al., 2012; Hamilton et al., 2014). However, other physiological functions were also suggested to act independently or synergistically with $GABA_A$ to alleviate or amplify OA effects on some behaviors (Briffa et al., 2012). The most recent review on the subject suggested that the behavioral effects of OA on fish may result from either neurophysiological or sensory mechanisms (Esbaugh, 2023). Unfortunately, OA-induced behavioral changes have been investigated mainly on tropical and temperate fishes (see review by Clements and Hunt, 2015), while far fewer studies have examined the impact of OA on the behavior of fishes from other latitudes (Sundin and Jutfelt, 2016; Schmidt et al., 2017, among others). To our knowledge, no studies have examined the effect of OA on the behavior of fishes from the sub-Antarctic or Antarctic areas. The lack of information on high-latitude fishes represents a critical knowledge gap since high latitudes are hotspots of climate change (IPCC, 2021). Moreover, it is necessary to consider the interacting effect of different environmental stressors, such as increased temperature, for a more comprehensive understanding of the effects of OA on fish behavior.

The 'róbalo' *Eleginops maclovinus* (Cuvier, 1830), a notothenioid fish from the subantarctic area, is a species with ecological relevance that is targeted by sport and artisanal fisheries in Patagonia. The species can be found in a wide range of habitats, from shallow coastal waters to estuaries and rivers (Vanella et al., 2017) of temperate and subantarctic areas in southern South America (López, 1963; Guzmán and Campodónico, 1973; Pequeño, 1989; Eastman, 1993). The broad distribution range and the ability of *E. maclovinus* to acclimatize to wide environmental conditions (Vanella et al., 2017; Lattuca et al., 2018) suggest that it may be unaffected by the increases in pCO_2 projected at the end of the century. A recent study by Lattuca et al. (2023), however, demonstrated that the thermal tolerance range of juvenile *E. maclovinus* was reduced under OA conditions and summer temperatures and that such reduction was related to a decrease in aerobic scope at near-future pCO_2 levels.

In this study, we analyzed the combined effect of OA and the highest and lowest seasonal temperatures on the activity level and boldness of juvenile *E. maclovinus*. We exposed the fish to present-day and near-future pCO_2 levels (~500 and ~1800 μatm) at two temperatures (4 and 10 °C). The pCO_2 levels correspond to present-day pCO_2 measured in coastal areas in this region and levels projected at 2100, respectively (Caldeira and Wickett, 2003; IPCC, 2021), and the temperatures used match the mean winter and mean summer sea surface temperatures in the Beagle Channel (Lattuca et al., 2018). To date, this is the first study investigating the interactive effects of OA levels and temperature on the behavioral responses of fish inhabiting coastal waters surrounding Tierra del Fuego, the tip of South America. Located about 1500 km from the most stenothermic region on the planet (Antarctica), understanding climate change impacts on ichthyofauna in Tierra del Fuego is potentially a bellwether for future impacts of environmental changes on the fish communities inhabiting subpolar regions in the southern hemisphere.

2. Materials and methods

2.1. Ethics statement

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

2.2. Animal collection and maintenance

Juvenile *E. maclovinus* were caught with a seine net (25 m long, 1.5 m high, and 12 mm stretch mesh) in coastal waters of the Beagle Channel (Tierra del Fuego – Argentina) in Golondrina Bay (54° 50′S, 68° 20′W) during fall 2019. Fish were transported in 50-L tanks containing seawater with constant aeration to CADIC facilities and habituated to captivity for 30 days in 120-L aquaria with daily renewal of seawater (20–25 % of total volume) at controlled temperature (4 ± 0.5 °C), salinity (24.6 ± 0.2) and dissolved oxygen concentrations (9 ± 0.3 mg L^{-1}) under a 12:12-h (light:dark) light regime. Feeding consisted of chopped hake (*Merluccius hubbsi*) filets offered ad libitum every other

day.

2.3. Experimental acclimation

At the end of the habituation phase, juvenile *E. maclovinus* were acclimated in individual rearing containers for one month to one of two temperatures (4 and 10 °C) and pCO_2 levels (Present, P: ~500 and μatm, Future, F: ~1800 μatm) in a full factorial design (4P, 4F, 10P and 10F) with 20 fish per treatment (total n = 80, 5.8 ± 0.1 cm body length, BL). Temperature for fish in the 10P and 10F treatments was adjusted by 1 °C day^{-1} (Beitinger and Lutterschmidt, 2011), and pCO_2 in 4F and 10F treatments was increased acutely after the desired temperature was reached (Manríquez et al., 2019). During the acclimation period to the four treatments, fish experienced the same light and feeding regime used in the habituation phase.

2.4. Rearing system and CO_2 chemistry

Constant pCO_2 levels (~500 and 1800 μatm) in the rearing containers were generated through a flow-through CO_2 mixing system used in a previous study on *E. maclovinus* (Lattuca et al., 2023). The system consisted of three mass flow controllers (model GdFC, Aalborg, New York, USA) and an oil-free compressor (MSV 12/100, Schulz, Sao Paulo, Brazil) that mixed pure CO_2 gas with ambient air. Filtered seawater (1 μm, Hidroquil, Buenos Aires, Argentina) was then aerated with air or CO_2-enriched air in 230-L mixing tanks, two for each condition, and delivered to each rearing container by submersible electrical pumps located inside the tanks. Treated seawater flowed into each rearing container by plastic tubing entering the lid and overflowing through a hole on the side of the container. The selected treated seawater flow rate (~250 mL min^{-1}) renewed one-third of the total volume of each rearing container six times a day. The desired pCO_2 level was also maintained by bubbling the appropriate mix of gases in each rearing container. Rearing containers were semi-immersed in thermally stable water baths at 4 and 10 °C with temperatures maintained by aquarium chillers (C-2500, Pacific Coast Imports, Oregon, USA).

Temperature, salinity, and pH were measured at least once each week in seawater samples taken from three randomly chosen rearing containers per treatment. Temperature and salinity were measured using a HANNA HI9828 meter and pH, reported using the total hydrogen ion scale (Dickson and Goyet, 1994), was measured with a Hanna Edge pH meter provided with a HI11310 digital glass pH-electrode (Hanna Instruments, Inc., Rhode Island, USA). The pH meter was calibrated with standard Tris buffer in synthetic seawater. 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). 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 (Table 1) 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).

2.5. Behavioral assays

After the acclimation phase to treatment conditions, fish were assayed for routine activity levels and boldness in an open field test and boldness traits were also examined by conducting a refuge emergence test. Open-field tests measure different components of animal temperament, generally boldness and exploratory behavior (i.e., activity), by placing an animal in a novel open environment and registering ambulation, freezing and use of outer versus inner zones of the open field, among other behaviors (Burns, 2008). Emergence tests measure the propensity of an animal to leave a safe area (i.e., refuge) with the latency (time) to leave a refuge considered a proxy of boldness (Brown and Braithwaite, 2005; Brown et al., 2007).

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 at 4 °C, 10P: present-day pCO_2 at 10 °C, 10F: future pCO_2 at 10 °C.

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

2.5.1. Open field test

The open field arena consisted of a circular plastic opaque aquarium (58 cm total diameter × 15 cm height) placed within a square glass aquarium (60 cm length × 60 cm width × 30 cm height) to minimize disturbance. An inner zone (IZ) was defined at 44 cm in diameter from the central point of the opaque aquarium. The aquarium was filled up to 10 cm with seawater from the corresponding pCO_2 and temperature combination treatment. A GoPro Hero 2 camera was positioned ~90 cm above the tank.

Fish (total n = 80, with n = 20 per treatment) were randomly selected and gently placed individually inside an opaque PVC tube (10 cm diameter × 15 cm height) in the center of the IZ. The fish was allowed to habituate to the experimental condition for 5 min. The tube did not allow the fish to swim freely. The tube was removed and the fish was filmed without external stimulation for 20 min. The total duration of each trial was 25 min.

The recordings of the fish movement were then analyzed blind to treatment using Kinovea 0.8.15 (Kinovea.org). The following parameters were measured: 1) the time spent active, 2) the average velocity, and 3) the time spent in the IZ. Since juvenile *E. maclovinus* showed low mobility and a discontinuous swimming pattern, the time spent active was considered as the time fish swam and did not stop for more than two consecutive seconds. Considering this pattern, the swimming velocity was only calculated in those activity bouts that lasted 1 min or more (mean duration ± SE in sec: 4P: 132 ± 25; 4F: 95 ± 15; 10P: 107 ± 20; 10F: 179 ± 41). To make average velocity values comparable, fish were tracked for the first minute of each bout. The average velocity was then expressed in BL per second (BL s^{-1}) to compensate for any possible effect of small differences in body size among individuals. Note, the BL of fish was not significantly different among treatments (see below). The time spent active and the swimming velocity were used to estimate the routine activity level. The time spent in the IZ, considering the whole fish inside this zone, was used to quantify boldness based on the idea that the inner area of an open field is considered dangerous and that venturing into this zone represents boldness or the willingness to undertake risk (Burns, 2008; Ariyomo and Watt, 2012; Laubenstein et al., 2018).

2.5.2. Refuge emergence test

The boldness behavior related to willingness to leave a refuge (total n = 80, with n = 20 per treatment) was measured in square opaque

plastic aquaria (45 × 25 × 25 cm, length × height × width) filled to a depth of 7 cm with treatment-specific water. As a refuge, an opaque flat-bottomed PVC tube (12 × 4 cm, length × diameter) was located with one open end facing the middle of an aquarium side. Fish were filmed throughout the trial with a Go Pro Hero2 camera positioned ~110 cm above the tank. Each trial started when the fish from a selected treatment were placed in the aquarium. After 10 min. of habituation, the fish were gently chased into the refuges with a hand net. Each trial lasted 1 h, and the area was free from external stimulation during the trial. Videos of the fish movement were analyzed with the observer blind to treatment, and the latency (in seconds) for the whole fish to emerge from the refuge was quantified as a proxy of boldness (Brown et al., 2007).

2.6. Statistics

A one-way ANOVA was used to test for differences in the BL of juvenile *E. maclovinus* assigned to each treatment. 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 behavioral traits. If the conditions of 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

3.1. Juvenile Eleginops maclovinus

The mean BL of fish acclimated to the four treatment groups (4P, 4F, 10P, 10F) was not significantly different (One-way ANOVA, $F = 1.391$, $p = 0.252$). No mortalities were observed across the acclimation and behavioral testing.

3.2. Open field test

The time spent active by *E. maclovinus* was significantly greater in the summer (10 °C) as opposed to the winter (4 °C) temperature ($p = 0.048$). Regardless of pCO_2 level, fish acclimated to 10 °C were 1.63 times more active than those at 4 °C. On the other hand, the time spent active was not significantly affected by pCO_2 level ($p = 0.132$) or the interaction between temperature and pCO_2 ($p = 0.065$) (Fig. 1a, Table 2). The mean ± SE swimming velocity was between 0.96 ± 0.28 and 1.23 ± 0.27 BL s^{-1} for fish acclimated to 4 °C and present-day pCO_2 and 10 °C and near-future pCO_2 levels, respectively. Swimming velocity was not significantly influenced by temperature ($p = 0.464$), pCO_2 level ($p = 0.249$) or their interaction ($p = 0.791$) (Fig. 1b, Table 2). Moreover, no significant differences were detected among treatments in the amount of time fish spent in the IZ of the open field arena (temperature: $p = 0.265$; pCO_2 level: $p = 0.807$; temperature × pCO_2 level: $p = 0.234$) (Fig. 1c, Table 2).

3.3. Refuge emergence test

Across the four treatments, the mean ± SE time to emerge from the refuge ranged from 634 ± 0.08 s in fish acclimated to 4 °C and near-future pCO_2 levels to 1445 ± 0.15 s in fish acclimated to 4 °C and present-day pCO_2 levels. The time to emerge was significantly affected by pCO_2 level ($p = 0.004$) but not by temperature ($p = 0.153$) or the interaction between these factors ($p = 0.170$) (Fig. 2, Table 2). Fish exposed to the winter temperature and near-future pCO_2 level were bolder and took significantly less time to leave the refuge than fish in the other treatments (Tukey Test, $p < 0.05$).

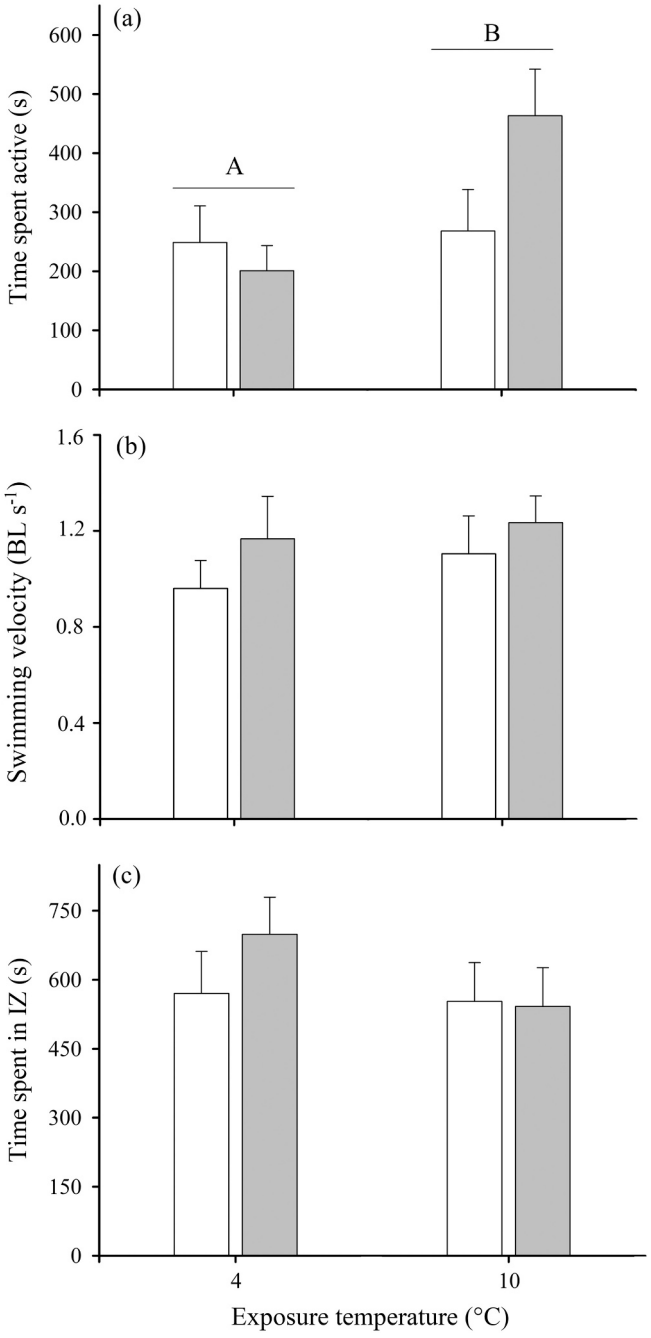


Fig. 1. (a) Time spent active, (b) length-specific swimming velocity, and (c) time spent in the IZ of juvenile *Eleginops maclovinus* from the Beagle Channel after a one-month exposure to one of two temperatures (4 and 10 °C) and pCO_2 levels (~500 and 1800 µatm, white and grey bars, respectively). Error bars represent the standard error (SE). Different letters indicate significant differences ($p < 0.05$) between temperatures.

4. Discussion

This is the first study investigating the combined effects of OA and temperature on the behavioral responses of a fish inhabiting coastal waters surrounding the tip of South America and adds important information on the debated impacts of OA on fish behavior (Clark et al., 2020; Munday et al., 2020; Clements et al., 2022; Esbaugh, 2023). Our experiments revealed that the activity level of juvenile *E. maclovinus* increased with summer temperatures in an open field test. The results also revealed that fish were bolder with near-future pCO_2 levels in a

Table 2
Two-way ANOVA on row or aligned rank transformed data (‡) for activity level and boldness assessments in 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 ($p < 0.05$).

Source	DF	F	p	Comparison
Open field test				
Time spent active (‡)				
Temperature	1	4.047	0.048*	10 > 4
pCO_2	1	2.314	0.132	
Temperature × pCO_2	1	3.495	0.065	
Residual	78			
Swimming velocity (‡)				
Temperature	1	0.560	0.464	
pCO_2	1	1.419	0.249	
Temperature × pCO_2	1	0.072	0.791	
Residual	18			
Time spent in the IZ zone (‡)				
Temperature	1	1.262	0.265	
pCO_2	1	0.060	0.807	
Temperature × pCO_2	1	1.442	0.234	
Residual	78			
Refuge emergence test				
Latency to emerge				
Temperature	1	2.081	0.153	
pCO_2	1	8.808	0.004*	F < P
Temperature × pCO_2	1	1.915	0.170	
Residual	82			

refuge emergence test but not when boldness was tested using the open field arena. The differences in results between these two commonly used methods may explain, in part, the challenges of detecting significant impacts of OA on fish behavior.

Fish exposed to summer temperatures spent more time active than those exposed to winter temperatures. However, no effect of such elevated temperature was observed in the fish swimming velocity, which remained constant across treatments. Different studies reported increased activity of different fish species at higher temperatures. Schmidt et al. (2017) analyzed the combined effect of ocean warming and acidification on the behavior of the polar cod *Boreogadus saida* and *G. morhua* and observed that the temperature influenced the activity level of *B. saida* but not in *G. morhua*. However, the authors suggested that a possible temperature effect in the latter species may have been masked by high inter-individual variability. Peck et al. (2006) demonstrated exponential increases in swimming speed with increasing acclimation temperature of larval and juvenile *G. morhua*. Laubenstein et al. (2018) reported that elevated temperatures increased the time spent active by kingfish *Seriola lalandi*. They also reported a similar relationship between the temperature and the swimming velocity of the species, with fish maintained at 25 °C swimming 59 % faster than fish maintained at 21 °C. Unlike Laubenstein et al. (2018), we did not obtain an analogous pattern between the time spent active and velocity with temperature. This could be explained, in part, by differences in the way velocity was calculated. While Laubenstein et al. (2018) quantified swimming velocity throughout the whole trial, making this trait comparable to activity level, we measured speed only when *E. maclovinus* was active. Therefore, in our case, the time spent active is a more indicative trait of activity level than speed which was merely an indicator of swimming performance during the first minute of their occasional bouts of activity.

Differences in the activity level have been related to differences in metabolic rates (Laubenstein et al., 2018). Our results indirectly support a link between metabolic rates and activity, as the standard metabolic rate was also higher in this species at higher temperatures (Lattuca et al., 2023). Such an increase in activity may have positive or negative effects. Increased activity can improve foraging success (Metcalfe et al., 2016) but also increase the vulnerability of fish to predation, particularly in larval and juvenile stages (Biro et al., 2003). The outcome will largely depend on the abundance, distribution and temperature-induced changes in predators and prey (Llopiz et al., 2014; Akimova et al., 2019). Moreover, activity has also been linked to boldness, as a high rate of movement can be taken as an indication of increased boldness because it reflects an increased ability for the fish to explore its environment (Ariyomo and Watt, 2012).

Our results indicate that there was no significant effect of elevated

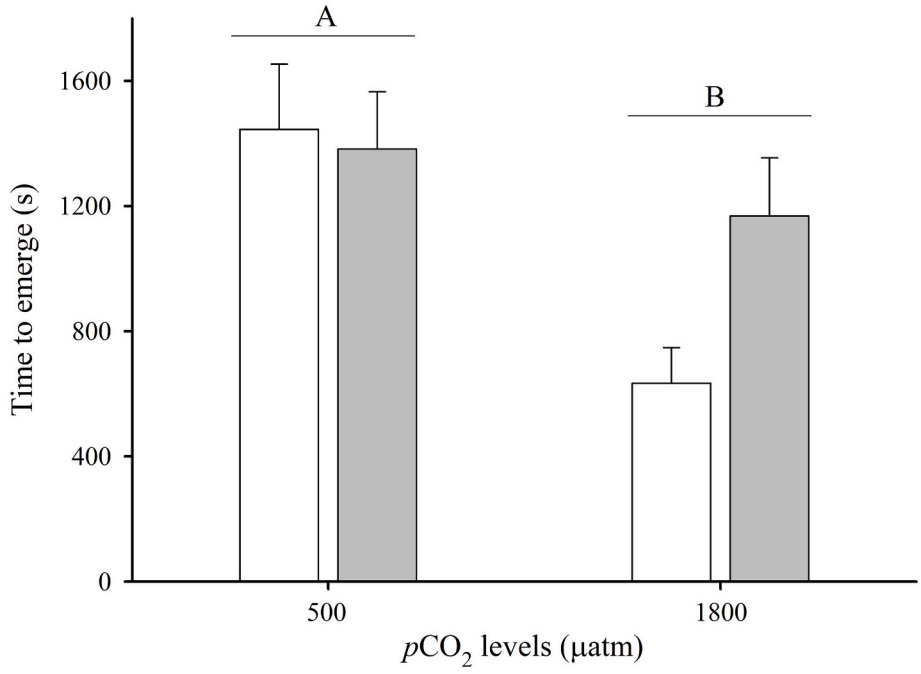


Fig. 2. Time to emerge from a refuge for juvenile *Eleginops maclovinus* from the Beagle Channel after a one-month exposure to one of two temperatures (4 and 10 °C, white and grey bars, respectively) and pCO_2 levels (~500 and 1800 μatm). Error bars represent the standard error (SE). Different letters indicate significant differences ($p < 0.05$) between pCO_2 levels.

pCO_2 on the time spent active nor the swimming velocity of *E. maclovinus* (Table 2). The results of studies examining the effect of pCO_2 on fish activity are equivocal. Some studies have reported significant effects of pCO_2 exposure (clownfish *Amphiprion percula* and damselfish *Pomacentrus wardi*: Munday et al., 2010; brown dottyback *Pseudochromis fuscus*: Cripps et al., 2011) while others reported no significant effect of pCO_2 exposure (anemonefish *Amphiprion melanopus*: Nowicki et al., 2012; mahi-mahi *Coryphaena hippurus*: Bignami et al., 2014; kingfish *S. lalandi*: Laubenstein et al., 2018) on fish activity. Our finding that activity levels appear to be robust to near-future pCO_2 levels in subantarctic *E. maclovinus*, agrees with those reported for other high-latitude fish species (*G. morhua*: Jutfelt and Hedgärde, 2015; goldsinny wrasse *Ctenolabrus rupestris*: Sundin and Jutfelt, 2016; *B. saida* and *G. morhua*: Schmidt et al., 2017). Finally, it must be pointed out that, even when we did not observe a significant interaction between temperature and pCO_2 levels, there was a clear trend in the current data for fish to be more active in high pCO_2 at elevated temperatures. Future experiments with an increased number of individuals could help validate such a trend.

Juvenile *E. maclovinus* were also screened for boldness, by measuring the time fish spent in the IZ of the open field test and the time they took to emerge from a refuge (Sneddon, 2003; Burns, 2008). Our results indicate that the effect of temperature and pCO_2 on boldness depended on the test employed; while the time spent by fish in the IZ of the open field arena did not change with temperatures and/or pCO_2 levels, near-future pCO_2 levels caused a reduction in the time to emerge from a refuge. Boldness can be defined and measured in different ways which makes it a challenging trait to assess (Yuen et al., 2017). It can be defined as [1] risk taken in the presence of a threat (Réale et al., 2007) or [2] risk taken in a novel environment (Ward et al., 2004) and ecologists select different indices of boldness according to the definition followed (e.g., [1] novel object, predator inspection or [2] open field, emergence from a refuge, among others) or the conditions (laboratory or natural settings) under which they are assessed (Burns, 2008; White et al., 2013; Mitchell et al., 2023). In this line, Stamps (2007) also mentioned that scores on different indices of boldness are not necessarily correlated with one another across individuals in different contexts. Arguably, the effect of temperature and pCO_2 on boldness can be considered context-specific (Brown et al., 2005; Burns, 2008), and therefore, its relevance for fish behavior in natural settings needs further attention.

In general, boldness has been shown to increase with temperature in marine fishes (Biro et al., 2010; Laubenstein et al., 2018). Similar to activity, this increase has been suggested to be driven by the direct impact of temperature on metabolic rates (Biro et al., 2010; Metcalfe et al., 2016). For instance, Biro et al. (2010) demonstrated that the boldness behavior of the coral reef fish *Pomacentrus bankanensis*, measured as the latency to emerge from a refuge following a simulated animal threat, increased significantly as temperature increased from 26.2 to 29.0 °C. Similarly, Forsatkar et al. (2016) found a positive effect of elevated temperatures on the boldness of the male Siamese fighting fish *Betta splendens* tested across different and complementary experimental settings. Moreover, Moffett et al. (2022) expected that long-term exposure of the mosquito fish *Gambusia affinis* to elevated temperatures would moderate metabolic rate, reducing the temperature sensitivity of metabolism, with concomitant reductions in boldness and activity. In contrast to expectations, however, those authors found relative decreases in metabolism and increases in boldness, producing a novel combination of physiological and behavioral traits that would impact the performance of that species in warming conditions. On the contrary, our results did not show any significant effect of temperature on boldness, measured both as the time spent in the IZ of the open field arena or the time to emerge from the refuge. In line with our findings, Laubenstein et al. (2018) observed that increasing temperatures did not alter the boldness of larval *S. lalandi*, suggesting this was likely because they exposed kingfish to elevated temperatures from the egg stage and thus conferring them some benefits in mitigating the effects of warming. Alternatively, considering that previous works have shown that risky behaviors (increased boldness) under low food conditions could be exacerbated by elevated temperatures (Lienart et al., 2014), they proposed that larvae were well-fed, and thus the effects of elevated temperatures were not detected. This latter explanation may also apply to our study, in which *E. maclovinus* was fed ad libitum. While this feeding regime allowed us to detect differences in the activity levels, it may have masked the effects of temperature on fish boldness. Nonetheless, it remains possible that boldness in this species is inherently less sensitive to thermal variation, highlighting the need for further studies across a broader range of environmental and developmental conditions.

On the other hand, the boldness of *E. maclovinus*, when measured as the time to emerge from a refuge, consistently increased under near-future pCO_2 levels. Previous studies examined the effects of OA on fish boldness and reported a wide range of responses, including increases (Munday et al., 2014), decreases (Jutfelt et al., 2013) or no effects (Jutfelt and Hedgärde, 2015). In this regard, Munday et al. (2014) found that OA at marine CO_2 seeps promoted bolder behavior in four juvenile reef fish species - two damselfishes, *Dascyllus aruanus* and *Pomacentrus moluccensis*, and two cardinalfishes, *A. cyanosoma* and *C. quinquelineatus*. In aquarium tests with a coralline refuge, control reef fish spent over 80 % of their time in the shelter, whereas CO_2 seep-exposed damselfish used it <12 % of the time and cardinalfish avoided it entirely. Conversely, Jutfelt et al. (2013) reported that CO_2-exposed adult *G. aculeatus* was less bold than control fish when they were presented with a novel object; after 20 days, the average time spent investigating the object was five times longer for control fish than CO_2-exposed fish. Additionally, Jutfelt and Hedgärde (2015) indicated that the boldness behavior of juvenile *G. morhua* was not affected by exposure to elevated CO_2 in a refuge emergence test. These contrasting results, along with those obtained in our study suggest that the effects of CO_2 on boldness behavior are species-specific across teleosts. This highlights the need to better understand the full geographical and phylogenetic variability in behavioral responses to CO_2 exposure.

The impacts of changes in boldness are also debated. It might be assumed that bold fish are more inclined to forage despite predation risk, inspect potential predators, and explore new habitats. However, these tendencies can have varying impacts on growth and mortality. Foraging under predation risk is thought to supply the resources needed for growth but simultaneously raises the immediate risk of mortality (Biro and Post, 2008). On the other hand, taking time to observe and avoid predators is believed to consume energy that, in turn, is no longer available for growth-promoting activities, but that does provide information that reduces risks of mortality (Walling et al., 2004; Stamps, 2007; Ólafsdóttir and Magellan, 2016). Furthermore, it is suggested that bold fish will have increased fitness via increased reproductive success (Ariyomo and Watt, 2012). While boldness may have positive or negative effects at the individual level, changes in any direction in this trait will likely cause disruptions at the ecosystem level (Bolnick et al., 2011; Sih et al., 2012; Alter et al., 2024). In this regard, Wang et al. (2021) offered insights into changes that occurred in a three-trophic level population model (perch–roach–zooplankton) due to changes in fish boldness, reporting that their altered foraging behavior caused skewed size distributions, reduced biomass and reduced reproduction success and thus, ecosystem state shifts.

It must be also pointed out that the relevance of modified behaviors in response to elevated temperatures and near-future pCO_2 levels to the fitness of *E. maclovinus* remains unknown. Although no impacts on survival or growth over the exposure period were found in the laboratory, we cannot rule out that exposure to elevated temperatures and near-future pCO_2 levels may influence more complex behaviors such as foraging on benthic resources (Riccialdelli et al., 2017) or competitive interactions between *E. maclovinus* and other fishes such as the pejerrey *Odontesthes nigricans*, the puyen *Galaxias maculatus* (Lattuca et al., 2018) and other notothenioid species (Giménez et al., 2021). Notothenioid fishes, such as *E. maclovinus*, are the most dominant component of the

ichthyofauna and climate-driven changes in species interactions and Patagonian food-web dynamics need to be studied.

To better assess the ecological consequences of these behavioral modifications, it is crucial to explore how they interact with other biotic and abiotic factors in more complex and realistic settings. Future studies that incorporate additional environmental and biological factors, likely to interact with the variables manipulated in the present study, will help to expand our current understanding. Moreover, since activity and boldness can vary substantially among individuals from different populations (Heckley et al., 2025), investigating how these behavioral traits are influenced by factors that differ within and among populations may also provide insight into the ecologically and evolutionarily drivers of behaviors.

5. Conclusions

This study provides the first experimental evidence of how elevated temperature and pCO_2 levels affect the behavior of *E. maclovinus*, a notothenioid fish native to Tierra del Fuego. Although conducted under laboratory conditions, our findings offer valuable baseline data for assessing how climate change-related drivers may influence the behavioral ecology of subpolar ichthyofauna. Located about 1500 km from Antarctica, Tierra del Fuego represents a transitional zone where early behavioral shifts could foreshadow broader ecological impacts on southern hemisphere fish communities. Future studies incorporating a wider range of biotic and abiotic factors, as well as inter- and intra-population variability, will be crucial to improving our understanding of species resilience and informing conservation strategies under climate change scenarios. In particular, understanding how boldness is modulated by temperature in *E. maclovinus* is relevant, given its potential influence on predator-prey interactions, dispersal, and habitat use. Comparative studies across notothenioid species from different thermal environments will further clarify the adaptive significance and plasticity of behavioral traits, reinforcing the importance of continued research into this field.

CRediT authorship contribution statement

María E. Lattuca: Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Conceptualization. **María E. Barrantes:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Eloísa M. Giménez:** Investigation. **Patricio H. Manríquez:** Writing – original draft, Funding acquisition, Conceptualization. **Rodrigo Torres:** Investigation. **Paolo Domenici:** Writing – review & editing, Funding acquisition, Conceptualization. **Myron A. Peck:** Writing – review & editing, Funding acquisition, Conceptualization. **Daniel A. Fernández:** Resources, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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