



The effects of CO₂ level and temperature on embryos and free embryos of the Patagonian pejerrey *Odontesthes hatcheri* (Actinopterygii, Atherinopsidae)

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Abstract Climate Change includes an increase of both the concentration of atmospheric CO₂ and of global temperature. Embryos (=eggs) of *Odontesthes hatcheri* were collected in spring–summer from the littoral of Lake Morenito (Andes of Argentina). Embryos and free embryos were exposed to different temperatures (within a currently natural thermal range) and CO₂ levels (obtained by bubbling lake water with gaseous CO₂). High temperature and high CO₂ led to low embryonic survival. Embryonic abnormalities were observed. Incidence of colorless blood cells and delayed dorsal pigmentation increased significantly at high CO₂ level. Free embryo survival decreased with time, being particularly low at high CO₂ level. Free embryo body shape also signaled the effects of high CO₂. The combined effect of temperature and CO₂ was additive. As temperature and

atmospheric pCO₂ increasing continues, the best survival at hatching observed in our experiments (50–60%, pH 7.5, 14 °C) will deteriorate (<20% at 18 °C and pH 7.0). These Climate Change-related results and the fact that the species is already threatened by translocated species suggest a fragile situation. Spatial variation of temperature and pCO₂ led us to reconsider the suitability of shallow littoral vegetated areas of the lakes as anti-predatory refuges for fish early life periods.

Keywords Acidification · Climate Change · Early life · Global warming · Silverside

Introduction

The principal causes and effects of Climate Change include an increase in the concentration of atmospheric CO₂ and in temperature, respectively (<https://climate.nasa.gov/>). The atmospheric abundance of CO₂ was 390.5 ppm in 2011, 40% greater than in 1750 (Hartmann et al., 2013) and the United Nations Intergovernmental Panel on climate change (IPCC) predicts that the atmospheric concentration of CO₂ will reach 794–1150 ppm by 2100 (Jansen et al., 2007; Ciais et al., 2014).

Information regarding the potential consequences of elevated atmospheric partial pressure of CO₂ (pCO₂) on freshwater ecosystems, which have poorly buffered pH levels, is scarce. When Phillips et al.

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(2015) evaluated the 1980–2100 effect of the increase of the atmospheric concentration of CO_2 on the water pH in the Great Lakes (ca. 0.3–0.4 pH units), they used only carbon chemistry and the assumption that Lake pCO_2 follows atmospheric pCO_2 . In the same way, mean pCO_2 values of four reservoirs in western Germany increased significantly with time (1980–2015) (Weiss et al., 2018). However, the total amount of free CO_2 in freshwater systems is affected by many factors (Hasler et al., 2016). Due to the complex interactions of these factors, freshwater was determined to be on average, CO_2 supersaturated (Cole et al., 1994). Moreover, freshwater experiences a wide range of CO_2 concentrations spatially temporally (Ma & Wang, 2021) and, in many lakes, CO_2 concentrations are far less than the air equilibrium, due to strong photosynthetic activity or high primary production (Balmer and Downing, 2011).

Freshwater fish have been relatively little studied with respect to their response to environmentally induced changes in the pCO_2 . Comparisons have been made with the effect of acid rain, which also results in a decrease in pH, and studies of different freshwater species revealed that no species would survive in highly acidic waters (pH=3–3.5) (Jellyman & Harding, 2014). However, comparisons between the effect of acid rain and elevated pCO_2 are limited because acid rain results in the addition of strong acids (e.g., sulfuric acid) and reduces pH much more than the weaker carbonic acid (Hasler et al., 2016). Dissolved CO_2 is controlled by the acid–base equilibrium within the total carbonate carbon system; i.e., carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) ion concentrations. Even though the physiological effects of high CO_2 in water can largely be ascribed to a decrease in body fluid pH, exposure to high CO_2 resulted in far higher mortality than acid exposure at the same pH levels, because the cell membrane is much more permeable to CO_2 than to the hydrogen ion (Ishimatsu et al., 2005). Studies conducted on the consequences of increased CO_2 for freshwater fish suggest that weak acidification may limit community diversity, specifically of invertebrates and fish (Hasler et al., 2018). High tolerance of increased CO_2 levels has been identified in juvenile and adult fish, with no effect on growth and survival even at high exposures; however, CO_2 sensitivity is higher during the early life of most aquatic organisms (Sayer et al., 1993; Dupont et al., 2008; Ishimatsu

et al., 2008; Talmage & Gobler, 2010; Baumann et al., 2012; Weiss et al., 2018), with documented effects at the level of fish nervous system (Nilsson et al., 2012). In South America, studies about Patagonian fishes were performed with only mineral acids (West et al., 1997; Gómez, 1998; Gómez et al., 2007; Barile et al., 2016).

Warming provokes changes in climatic variables that are ecologically important for freshwater fishes. From 1961 to 2019, the Mean Annual Air Temperature (MAAT), an estimator of water temperature at the lake bottom (Quirós & Drago, 1985; Quirós, 1991; Livingstone & Lotter, 1998), increased in northern Patagonia between 0.5 °C and 1.5 °C (<https://www.smn.gov.ar/clima/tendencias>). Water temperature varies in space and time and constitutes a key factor in determining the geographical and seasonal distribution of fish, since they are ectothermic poikilotherms (Cussac et al., 2009; Magnuson, 2010). However, fish have evolved behavioral, physiological, and biochemical responses to face the challenges of living in habitats with variable temperatures (Beitinger & McCauley, 1990; Beitinger & Lutterschmidt, 2011; Dabruzzi et al., 2012). In the Andean Region warming favored the northeast to southwest movement of Neotropical native fishes and the exotic common carp *Cyprinus carpio* Linnaeus, 1758 and also the subsequent decline or loss of populations of Andean species and exotic salmonids (Aigo et al., 2008; Cussac et al., 2009; Crichigno et al., 2016a; Becker et al., 2018). Several studies have been conducted on the thermal performance of Patagonian freshwater fish: the preferred temperatures of *Aplochiton zebra* Jenyns, 1842, *Galaxias maculatus* (Jenyns, 1842), *Galaxias platei* Steindachner, 1898, *Odontesthes hatcheri* (Eigenmann, 1909) (17.7 °C with an acclimation temperature of 15.4 °C), *Percichthys trucha* (Valenciennes, 1833), and the non-native *Oncorhynchus mykiss* (Walbaum, 1792) and tolerance of high temperatures in *Gymnocharacinus bergii* Steindachner, 1903, *Hatcheria macraei* (Girard, 1855), *P. trucha*, and *O. hatcheri* (30.8 °C with an acclimation temperature of 15.4 °C) (Gómez, 1990; Ortubay et al., 1997; Milano, 2003; Lattuca, 2006; Aigo, 2010; Aigo et al., 2014; Crichigno et al., 2018). Moreover, the combined effect of temperature and CO_2 may be additive, more than additive (synergistic), or less than additive (antagonistic), warming often significantly altering the effects of elevated CO_2 (Munday et al., 2019).

The aim of this work was to experimentally study how the combined effect of manipulated CO₂ level and temperature affects the embryos (eggs) and free embryos of *O. hatcheri*, analyzing survival, induced malformations, and body shape differences under CO₂ concentrations ranging between those of lake water (pH=7.5) and expected sub-lethal levels (pH=5.5) and within an usual thermal range (12.2–20.0 °C). The hypothesis is that the exposition to high CO₂ concentration, pending on water temperature, will provoke sub-lethal or lethal effects in *O. hatcheri* embryos and free embryos. These results will be important regarding the expected changes of freshwater fish populations in north Patagonia.

Materials and methods

Environmental characteristics

Lake Morenito (Argentina, 41°05" S, 71°30" W, 758 m a.s.l.) is a small shallow Andean Patagonian lake upstream of Lake Moreno and Lake Nahuel Huapi. The entire system, of glacial origin, has an Atlantic drainage. Lake Morenito has an area of 0.82 km² and a maximum depth of 15 m (Buria et al., 2007). The temperature is homogeneous throughout the water column most of the year, varying between 10 °C and 15 °C in spring and fall and reaching 22 °C in late summer (Online Resource 1, Modenutti et al., 2000). Andean Patagonian lakes have very low total alkalinity (0.51 meq l⁻¹, Quirós & Drago, 1999). Particularly, lake Morenito presented very low conductivity values (60 µS cm⁻¹), neutral pH (6.9–7.2) (Balseiro et al. 2014), and dissolved oxygen concentration at saturation levels throughout the water column (Modenutti & Pérez, 2001).

Water temperature was measured on the bottom, at 20 cm depth. The pH levels was measured on the bottom, at 5 cm depth. Both variables were recorded in the littoral zone over 24 h in early summer, in December 2018 and January 2019, with HOBO data loggers. Diel variation in pH was lower in December (range 7.78–8.01) than in January (range 7.49–8.08) (Online Resource 2), in line with increasing temperatures over the summer (Diaz et al., 2007) and higher photosynthetic and respiratory levels.

Species and sampling

The genus *Odontesthes* (family Atherinopsidae), which can be found in southern South America, includes freshwater silversides, such as *O. hatcheri* and *Odontesthes bonariensis* (Valenciennes, 1835), the most ancient records of the genus reaching the early Miocene (Dyer, 2006; Cione & Báez, 2007). These species have allopatric original distributions: *O. hatcheri* in the southwest (the Andean Region) and *O. bonariensis* in the northeast (the Neotropical Region) (López et al., 2008; Conte-Grand et al., 2015). Stocking practices of *O. bonariensis* within the original *O. hatcheri* distribution area, and their ability to hybridize, contributed to the extirpation of native populations of *O. hatcheri* and led to the establishment of a hybrid zone across northern Patagonia (Crichigno et al., 2013, 2016b; Conte-Grand et al., 2015; Rueda et al., 2017; Hughes et al., 2020). Two consecutive early life periods were considered here for *O. hatcheri*: embryo (=egg, from fertilization to hatching) and free embryo (from hatching to beginning of exogenous feeding) (Balon, 1990, 1999).

Lake Morenito lies within the original distribution of *O. hatcheri*. The population was previously identified at species level by key characters, mtDNA, and geometric morphometrics (Ringuelet et al., 1967; Crichigno et al., 2013; Conte-Grand et al., 2015; Rueda et al., 2017). Previous studies identified free embryos and larvae in the littoral zone of neighboring lakes during the summer (Cussac et al., 1992; Cervellini et al., 1993; Battini et al., 1995).

Four egg samplings were performed in November and December, 2018. Groups of eggs were found attached to macrophytes in the shallow littoral zone between 2 and 60 cm depth. The olive green eggs, with a smooth vitelline envelope and three or four chorionic filaments (Kunz, 2004), were collected in opaque plastic flasks with lake water and taken to the laboratory in the Centro de Salmonicultura Bariloche (CENSALBA) of Universidad Nacional del Comahue.

Treatments (CO₂ concentration and temperature) and effect on embryos

Levels of pH were used as an indicator of CO₂ concentrations. Dissolved CO₂ concentration can be calculated as a function of water pH, alkalinity, and

temperature. If the alkalinity and temperature of the water are relatively constant, pH can be used as an accurate measure of dissolved CO₂ concentration (Clingerman et al., 2007; Cupp et al., 2017). To reach the different CO₂ concentrations, we kept 200 l of lake water (pH 7.5) aerated ([O₂] = 7.03–7.36 ppm). One liter of this lake water was bubbled with gaseous CO₂ and the desired pH was adjusted by adding aerated lake water. The pH levels obtained and the calculated CO₂ concentrations, according to the equations of Clingerman et al. (2007) and Cupp et al. (2017), were as follows:

- (a) High CO₂, 520–1914 mg. l⁻¹ CO₂ (pH 5.5), a sub-lethal concentration, considering that Sayer et al. (1993) referred to a pH of 5.4–5.6 for uncontaminated rain water in equilibrium with atmospheric CO₂ in northern Europe and North America and that round goby *Neogobius melanostomus* (Pallas, 1814) loses equilibrium at 197–280 mg. l⁻¹ CO₂ (Cupp et al., 2017);
- (b) Medium CO₂, 57–120 mg. l⁻¹ CO₂ (pH 6.5), with expected negative effects, considering that a CO₂ concentration of 60 mg. l⁻¹ is avoided experimentally by rainbow trout (Clingerman et al., 2007) and a threshold of 99–169 mg. l⁻¹ CO₂ is avoided experimentally by round goby *Neogobius melanostomus* (Cupp et al., 2017); and
- (c) Low CO₂, 6–11 mg. l⁻¹ CO₂ (pH 7.5), close to the normal level of northern Patagonian lakes (Diaz et al., 2007) and close to the minimum value recorded in Morenito Lake, at sunrise (Online Resource 2).

The embryos involved in the experiment ranged from recognizable axial organization up to the presence of pigmented eyes. Embryos ($N=1340$) were exposed to controlled CO₂ and temperature in 15-ml transparent tubes (two to three eggs per tube) sealed without a gas chamber to prevent evaporation and gas exchange. A group of 10 tubes were considered an experimental unit (EU, Table 1).

Temperatures were selected according to the mean summer air temperature (1981–2010: 14–16 °C, <https://www.smn.gov.ar/clima/atlasclimatico>) and 2 °C and 4 °C above these values, considering the increase expected due to climate change (<https://www.ipcc.ch/assessment-report/ar6/>), within the expected range for Morenito lake water during spring summer (10–22 °C). Experimental temperatures were recorded with HOBO® data loggers (14.2 ± 2.0, 16.2 ± 2.3 and 18.1 ± 1.9 °C, mean and extremes, Fig. 1) in three incubators (Collection) with internal light.

A photoperiod of 10-h light and 14-h dark was applied. Water was replaced every 48 h to maintain the CO₂ and dissolved O₂ conditions. Minimum oxygen concentrations were always greater than 4.09 ppm. As the fertilization date of the embryos was unknown, time was considered in two ways: as days before hatching, to consider degree of development and as days after the beginning of the experiment (DAB), to consider the cumulative effect of the treatment. Both variables were highly correlated (Pearson Coefficient = 0.548, $N=552$, $P < 0.0001$). Thus, time was considered as DAB.

Table 1 Embryos and free embryos

Temperature (°C)		Embryos (1340 individuals)			Free embryos (273 individuals)		
		14.2	16.2	18.1	14.2	16.2	18.1
CO ₂ level							
Low (pH 7.5)	EU	5	5	5	7	7	7
Medium (pH 6.5)	EU	5	5	5	7	7	7
High (pH 5.5)	EU	5	5	5	7	7	7

Number of experimental units (EU = 10 tubes for embryos, 2–3 eggs per tube; EU = 1 flask for free embryos, 4–5 free embryos per flask) regarding Temperature and CO₂ level

Initial number of individuals are indicated in parenthesis

Survival records were taken daily during 20 days for embryos and daily during 8 days for free embryos

Respiratory frequency and incidence of five malformations were recorded every day in a sub-sample of the EUs

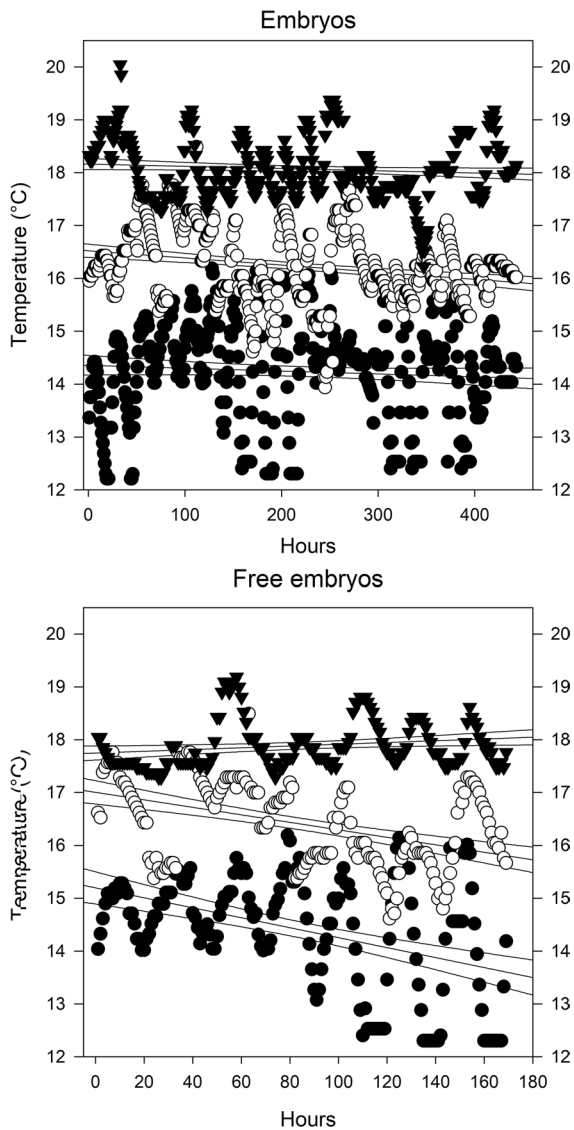


Fig. 1 Experimental temperatures for embryos (top panel) and free embryos (bottom panel). Data, linear regressions and 95% confidence intervals, are indicated

Dead individuals were removed from each EU and counted, every day, during 20 days. Each date, survival (S) was considered as

$S = 100 \cdot \text{Number of live embryos} / \text{Initial number of embryos}^{-1}$.

Videos of live embryos were recorded every day in a sub-sample of the experimental units (Table 1) with a camera (Nikon D5300) attached to a stereo microscope (Leica M3C), to record the respiratory frequency (RF),

$RF = \text{number of opercular cycles} \cdot \text{Second}^{-1}$,

as the mean value of the experimental unit (EU, Table 1) and observe the incidence (%) of possible abnormalities in the EU; cardiac arrhythmia, colorless blood cells, delayed dorsal pigmentation, pericardial edema, and weak heart contraction that could be indicators of an environmental perturbation of development (Incardona et al., 2014).

To compare effects of CO_2 levels and temperature on body shape, all newly hatched free embryos (NHFE) were euthanized with an excess ($0.1 \text{ g} \cdot \text{l}^{-1}$) of Benzocaine, fixed in 4% Formaldehyde, buffered (pH 7) with Sodium phosphate, monobasic and dibasic, and photographed (64x) under stereo microscope. The time of maintenance of the NHFE in formalin was recorded. Individuals were measured (Standard length, Area of the yolk sac, and Area of the sagitta otolith) with ImageJ.

Effect on free embryos

Embryos with eye pigmentation, without previous treatment, were incubated at room temperature ($15\text{--}21 \text{ }^\circ\text{C}$) until hatching, with aeration and the addition of NaCl ($5 \text{ g} \cdot \text{l}^{-1}$). NHFE ($N=273$) were placed in 63 glass flasks (350 ml each one, four to five free embryos per flask) and subjected to the same CO_2 and temperature treatment as the embryos (Fig. 1). One flask was considered an EU (Table 1). Survival was recorded every 24 h up to 8 days, in order not to reach the beginning of exogenous feeding (Battini et al., 1995).

In order to study the effect of CO_2 concentration and temperature on body shape along time, 4, 5, 6, and 7 days after hatching (DAH), 30, 42, 15, and 52 NHFE (pH = 6.5 and 7.5), respectively, were euthanized with 0.01% Benzocaine, fixed in buffered 4% Formaldehyde, and photographed (64x) in lateral view under stereo microscope. The time of maintenance of the NHFE in formalin was recorded. Individuals were measured (standard length, area of the yolk sac, and area of the eye) with ImageJ. In addition, to obtain high resolution regarding shape differences, twelve landmarks were placed on these images with TpsDig2v2.31 (Rohlf, 2013) according to Gobbi (1986) and Crichigno (2012) (Online Resources 3).

Data analysis

Embryos (and free embryos), factors, and levels were arranged in a two-way factorial design. Data (survival, incidence (%) of malformations, respiratory frequency, body measures) were transformed in order to obtain normality and homoscedasticity for Regression analysis, ANOVA, and pairwise multiple comparison procedures (Holm–Sidak method). When assumptions failed, Kruskal–Wallis One-Way Analysis of Variance on Ranks (K–W) was used, and pairwise multiple comparison procedures (Dunn’s method) were performed.

All morphometric analyses were conducted using MorphoJ ® V1.07a (Klingenberg, 2011). Images were first scaled and rotated to a common size and orientation using a generalized Procrustes superimposition approach (Rohlf and Slice, 1990). To characterize variation in shape dimensions, a principal component analysis was carried out based on the covariance matrix of shape. Canonical variate analysis (CVA) was used to identify the differences between temperatures, CO₂ levels, and days after hatching (Campbell & Atchley, 1981; Jolliffe, 2002). Mahalanobis distances and their *P*-values were extracted after a permutation test (10,000 runs). Wire-frame representation of the superimposition between the average lateral view for each CO₂ level, temperature, and days of treatment was performed to visualize variation in shape.

Results

Effect on embryos

Embryo survival could be predicted (Multiple Linear Regression, $N=552$, $R^2=0.619$, $F=445.795$, $P=0.001$) from a linear combination of CO₂ level ($P=0.001$) and DAB ($P=0.001$). High CO₂ concentration led to low survival. Survival also decreased over time (DAB) (Fig. 2).

The combined effect of temperature and CO₂ concentration was studied considering only survival at hatching: significant differences were observed between temperatures (ANOVA, $N=45$, $F=4.992$, $P=0.012$) and CO₂ levels (ANOVA, $N=45$, $F=20.910$, $P=0.001$), survival at 14.2 and 16.2 °C being higher than at 18.1 °C (Holm–Sidak method,

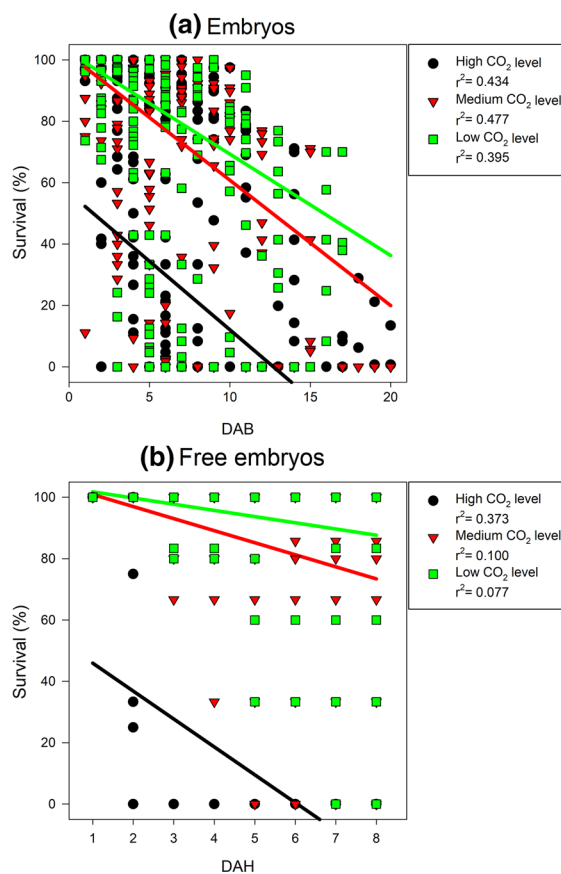


Fig. 2 The effect of CO₂ level on Survival (%) during a) embryo and b) free embryo development showing the decrease in survival with DAB (days after the beginning of the experiment) and DAH (days after hatching), with regard to CO₂ levels. Fitted regression lines and r^2 are indicated. High CO₂ level: black circles and line, Medium CO₂ level: red circles and line, Low CO₂ level: green circles and line

$P < 0.05$), and survival at each CO₂ levels being significantly different from each other (Holm–Sidak method, $P < 0.05$), being the lowest at high CO₂ concentration (pH 5.5). The interaction between temperature and CO₂ concentration was not significant ($P=0.323$) (Fig. 3).

The respiratory frequency of embryos could be predicted (Multiple Linear Regression, $N=177$, $R^2=0.105$, $F=6.789$, $P=0.001$) from a linear combination of CO₂ concentration ($P=0.045$) and DAB ($P=0.001$). No significant effects of temperature over the respiratory frequency were found ($P=0.138$). Respiratory frequency was lower at high CO₂ concentration and higher at low CO₂ concentration.

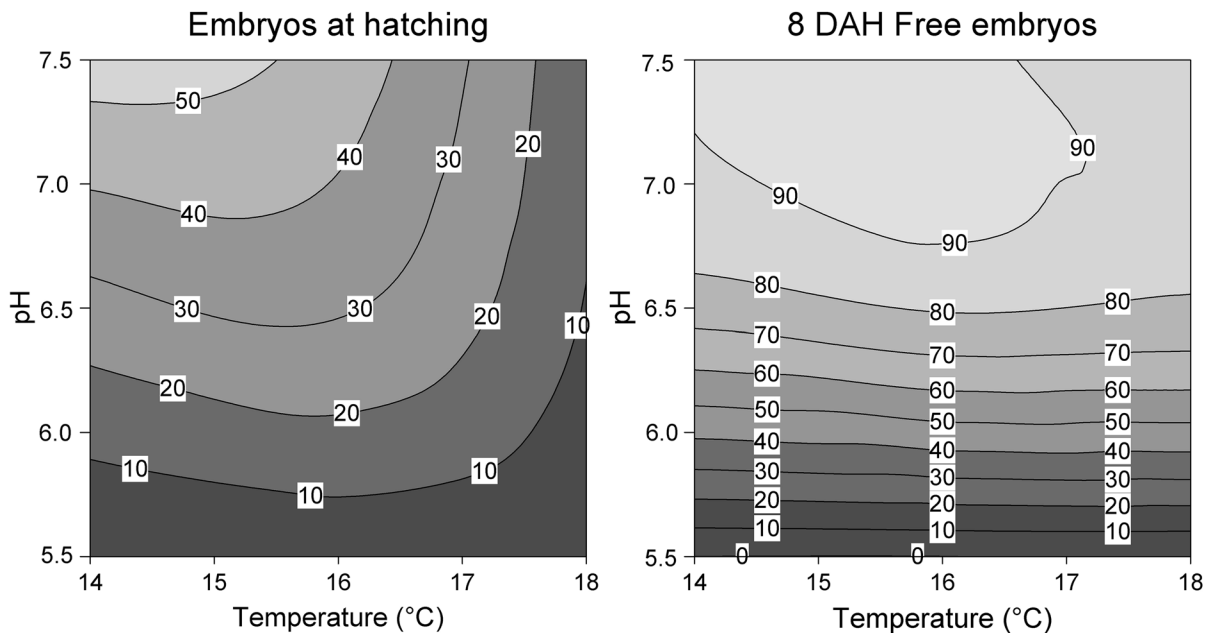


Fig. 3 Survival (%) of embryos at hatching (left) and of 8 DAH free embryos (right) versus temperature (°C) and pH levels. Contour plots and survival percentages (in box) are indicated

In the same way, the incidence of five malformations (cardiac arrhythmia, colorless blood cells, delayed dorsal pigmentation, pericardial edema, weak heart contraction) showed significant differences between CO_2 levels (K–W, 2 degrees of freedom, $N=57$, $P<0.05$), but only the incidence of colorless blood cells (K–W, $H=15.738$ with 2 degrees of freedom, $N=57$, $P=0.001$) and delayed dorsal pigmentation (K–W, $H=26.022$ with 2 degrees of freedom, $N=57$, $P=0.001$) was significantly higher at high (pH 5.5) than at low CO_2 levels (pH=7.5) (Dunn's method, $P<0.05$, Fig. 4).

Considering CO_2 concentration, temperature, DAB, and time of maintenance of the free embryo in formalin as independent variables, no significant effect was observed on standard length, area of the yolk sac, or area of the sagitta otolith of the NHFE (Multiple Linear Regression, $N=151$, $P>0.05$).

Effect on free embryos

Free embryo survival showed significant differences between CO_2 levels (K–W, $N=504$, $H=16.346$ with 2 degrees of freedom, $P=0.001$) and survival at high CO_2 level (pH 5.5) being lower than at medium (pH 6.5) and low values (pH 7.5) (Dunn's Method,

$P<0.05$). In addition, free embryo survival decreased with DAH (K–W, $N=504$, $H=55.431$ with 7 degrees of freedom, $P=0.001$), this being particularly marked for high CO_2 level (pH 5.5) (Fig. 2). No significant difference in free embryo survival was observed between incubation temperatures (K–W, $N=504$, $H=0.0684$ with 2 degrees of freedom, $P=0.966$).

The combined effect of Temperature and CO_2 concentration was studied with regard to the survival of free embryos only at 8 DAH. Significant differences were observed between CO_2 levels (ANOVA, $N=63$, $F=63.019$, $P<0.001$) but not between temperatures (ANOVA, $N=63$, $F=0.172$, $P=0.842$), survival at high CO_2 level (pH 5.5) being significantly lower than at medium (pH 6.5) and low CO_2 level (pH 7.5) (Holm–Sidak method, $P<0.05$). Interaction between these two factors was not significant ($P=0.957$) (Fig. 3).

With regard to CO_2 , temperature, DAH, and the time of maintenance of the free embryo in formalin, the standard length of free embryos increased significantly with DAH (Regression, $N=139$, $R^2=0.235$, $F=13.819$, $P=0.001$) and the lateral area of the yolk sac decreased significantly with DAH (Regression, $N=139$, $R^2=0.279$, $F=17.426$, $P=0.001$), and with high temperature (Regression, $N=139$, $R^2=0.279$,

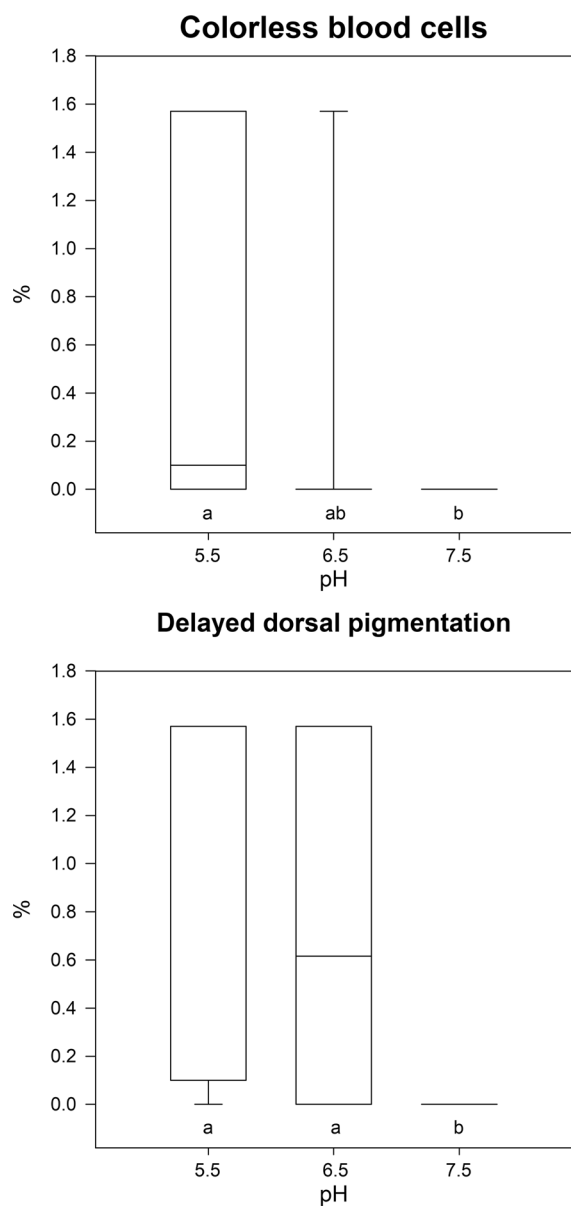


Fig. 4 Percentage of malformed embryos (Colorless blood cells, Delayed dorsal pigmentation) versus pH levels. Median, quartiles, and 10th and 90th percentiles are indicated. Different letters indicate significant differences

$P=0.001$, Fig. 5), whereas the area of the eye did not vary with any of these four variables (Regression, $N=139$, $R^2=0.0273$, $F=1.263$, $P=0.29$).

Geometric morphometrics provided the first three principal components (PC), explaining 71.1% of the variance (PC1: 37.6%, PC2: 19.9%, and PC3: 13.6%). CVA for CO_2 concentrations showed a significant

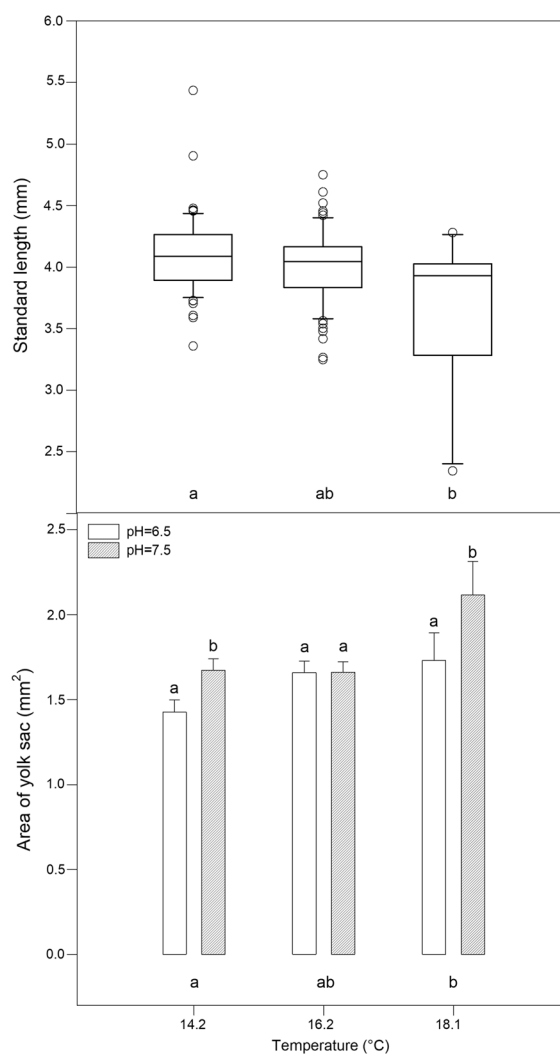


Fig. 5 Standard length (mm) of free embryos versus temperature ($^{\circ}\text{C}$). Median, quartiles, and data outside 10th and 90th percentiles are indicated (Top panel). Lateral area of the yolk sac (mm^2) versus pH and temperature ($^{\circ}\text{C}$). Means and standard deviation are indicated. (Bottom panel). Different letters indicate significant differences

Mahalanobis distance between medium (pH 6.5) and low CO_2 level (pH 7.5). The average shape for the different temperatures, DAH, and CO_2 levels differed mainly in the location of landmarks 5, 6, and 7 (anus, posterior ventral tip of operculum, and posterior tip of neurocranium) (Table 2, Online Resources 3, and Fig. 6). CVA among the three experimental temperatures showed two CVs, the first explaining 66.1% of the variation. The Mahalanobis distances between temperature groups were significantly different

Table 2 Geometric morphometrics of *O. hatcheri* free embryos

Temperature (°C)	14.2 (55)	16.2 (51)		
16.2 (51)	1.6806/0.0001			
18.1 (33)	1.4073/0.0022	1.6584/0.0001		
pH	6.5 (62)			
7.5 (77)	1.0439/0.0047			
DAH	4 (30)	5 (42)	6 (15)	
6 (15)	1.8714/0.0278	2.0373/0.0005		
7 (52)	2.5125/0.0001	2.4088/0.0001	2.7190/0.0001	

CVA according to temperature, pH, and DAH

The number of individuals (total=139) in each treatment is shown in parentheses

Only significant Mahalanobis distances between groups/p-values for permutation (10,000) tests are indicated

between all pairs (Table 2, Fig. 6). The CVA for DAH showed 3 CVs, the first two explaining 70.9% (CV1) and 18.4% (CV2) of variation. The Mahalanobis distances between age groups were significant between all groups except the distance 4–5 DAH (Table 2, Fig. 6).

Discussion

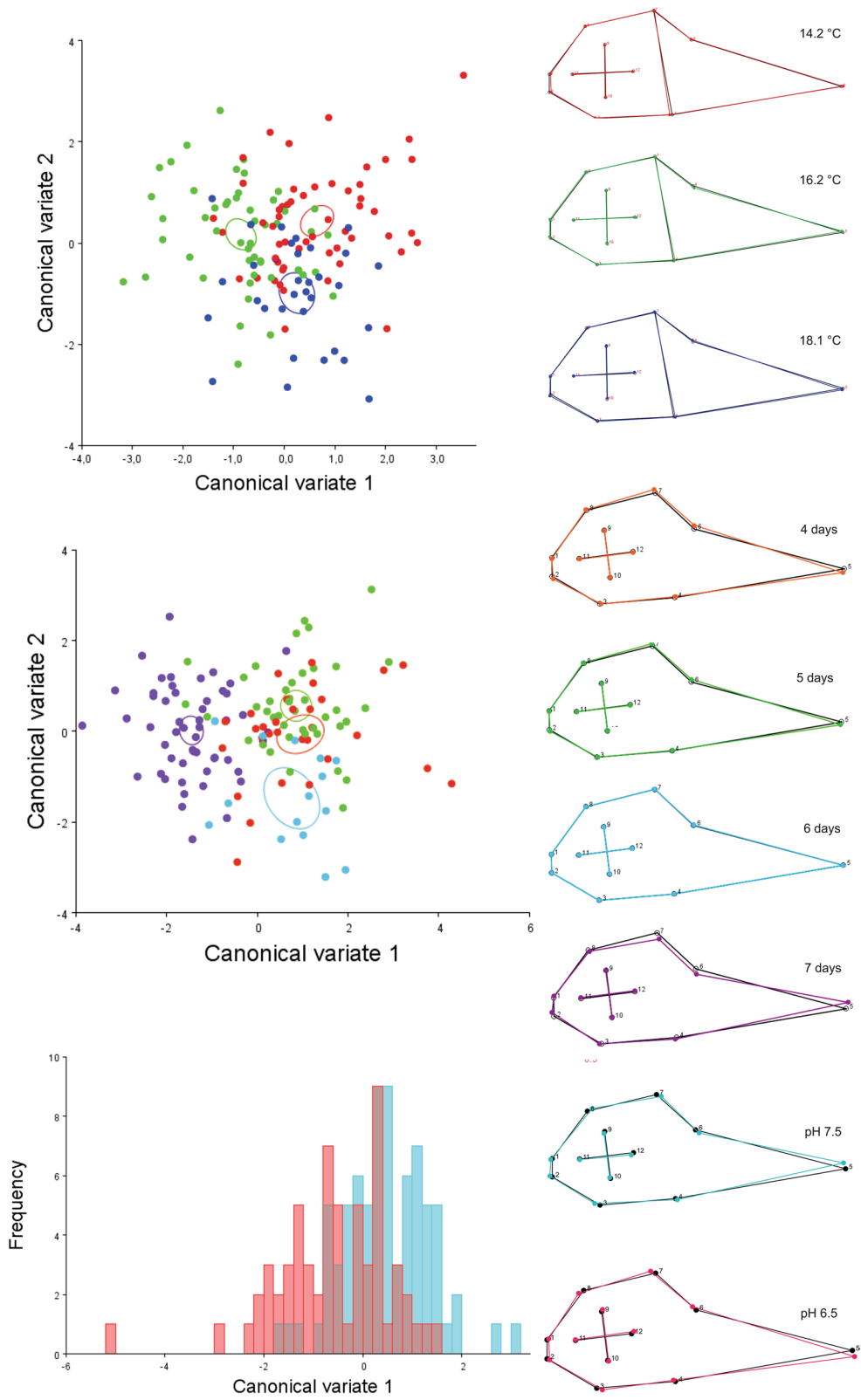
Our results show that as the temperature and CO₂ concentration increased, survival of *O. hatcheri* embryos decreased. Low survival in high CO₂ treatments was also observed in embryos of marine silver-sides (Baumann et al., 2012; Gobler et al., 2018; Murray & Baumann, 2018) and by Chambers et al. (2014) in embryos of the summer flounder *Paralichthys dentatus* (Linnaeus, 1766). Dead *O. hatcheri* eggs were opaque, so it was not possible to determine whether the cause of death was related to development or to hatching. Kwain & Rose (1985) assume that the first cause of mortality of the embryos of the brook trout *Salvelinus fontinalis* (Mitchill, 1814) is the impossibility of breaking the chorion, as observed for pH 4.5 and 5.5. According to Abernathy (2004), low pH levels inhibit the hatching enzyme (Kunz, 2004). Also the survival of the free embryo of *O. hatcheri* showed differences between CO₂ levels, the lowest survival being at high CO₂ level (pH 5.5). Free embryo survival decreased with DAH, and this was particularly marked for high CO₂ level (pH 5.5).

The incidence of cardiac arrhythmia, pericardial edema, and weak heart contraction showed small but significant differences between CO₂ levels in *O. hatcheri* embryos. The reduction in contractility of fish myocardium in vitro due to hypercapnia is well established. The high solubility of CO₂ lowers the intra-cellular pH of the myocardium, reducing contractility through antagonism between hydrogen ions and intra-cellular calcium ions. Moreover, in vivo cardiac responses to hypercapnia probably vary with the experimental temperature (Ishimatsu et al., 2005; Lo et al., 2021). The pericardial edema observed was similar to that described by Incardona et al. (2014) as a consequence of exposure of fish embryos to crude oil-derived polycyclic aromatic hydrocarbons (PAHs). Cardiac arrhythmia and weak heart contraction are also indicated as effects of exposition to PAHs. The underlying mechanism was shown to be a blockade of key potassium and calcium ion channels involved in cardiac excitation–contraction coupling (Incardona et al., 2004, 2014).

The incidence of colorless blood cells increased at high CO₂ level in *O. hatcheri* embryos. The higher incidence of colorless blood cells at higher CO₂ levels in *O. hatcheri* embryos could be the consequence of an affected primitive phase of erythropoiesis occurring in the mesenchyme of the yolk sac walls and in the embryonic mesenchyme, until resorption of the yolk sac (Kondera, 2019).

The incidence of delayed dorsal pigmentation in *O. hatcheri* embryos was higher at high CO₂ level. Chromatophores and pigmentation are useful indicators of degree of development in *O. bonariensis* (Chalde et al., 2011) and the structure and number of chromatophores change significantly in the catfish *Heteropneustes fossilis* (Bloch, 1794) on exposure to different levels of cadmium (Ahmad et al., 2018). However, the delayed dorsal pigmentation observed in *O. hatcheri* is probably related to a pH-mediated feed-forward mechanism of epigenetic regulation that enables selective amplification of the melanocyte maturation program observed in zebrafish (Raja et al., 2020).

Increases in environmental temperature may alter morphology (Lema et al., 2019). However, no significant effect of CO₂ concentration, temperature, and DAB was observed on standard length, area of the yolk sac, or area of the sagitta otolith of the *O. hatcheri* embryos at hatching. However, the standard



◀**Fig. 6** Geometric morphometrics. Canonical variate scores and associated morphospaces (colors maintain correspondence) are indicated, showing significant differences in overall body shape between temperatures: 14.2, 16.2, and 18.1 °C (top); DAH: 4, 5, 6, and 7 (medium); and pH: 6.5 and 7.5 (bottom)

length of free embryos did increase by growth with DAH, while the lateral area of the yolk sac decreased with DAH and with high temperature, probably due to yolk consumption. In the same way, in terms of geometric morphometrics, body shape showed significant differences between temperatures and between DAH, but it must be noted that significant differences in body shape were observed when free embryos were exposed to medium (pH 6.5) and low CO₂ levels (pH 7.5).

In brief, experimental exposure to an usual thermal range (12.2–20.0 °C), including the preferred temperature (17.7 °C) of juveniles (Aigo, 2010), lower than juvenile thermal tolerance (30.8 °C, Aigo, 2010), and lower than surface lake water temperature during summer (Online Resources 1 and 2), led to a decrease in embryonic survival at hatching, at high temperatures. The fact that eggs were obtained in the shallow littoral of the lake, under unfavorable thermal conditions raises the question of whether more eggs were present at greater depth and lower temperature or whether this low quality spatial location is the consequence of an anti-predatory trade-off. At the same time, the exposure of *O. hatcheri* embryos to high CO₂ concentrations, ranging between those of lake water (pH = 7.5 was recorded in midsummer at sunrise in Morenito lake, the maximum CO₂ concentration level attained before photosynthesis begins) and expected sub-lethal levels, led to increased incidence of embryo malformations and decreased survival in embryos and free embryos. The combined effect of these two factors was additive in embryos at hatching (no significant interaction). Thus, an additive effect, neither synergistic nor antagonistic, of temperature and CO₂ concentration on embryo survival at hatching can be observed.

Temperature and atmospheric pCO₂ increase will be continued (IPCC, 2014). In consequence, we can expect that the best survival at hatching observed for *O. hatcheri* in our experimental conditions (50–60%, pH 7.5, 14 °C) will deteriorate. Phillips et al. (2015) evaluated the 1980–2100 effect of the increase of the

atmospheric concentration of CO₂ on the water pH in the Great Lakes in ca. 0.3–0.4 pH units and Weiss et al. (2018) in ca. 0.3 pH units in four reservoirs in western Germany (1980–2015). In Fig. 3 we can note that *O. hatcheri* embryonic survival at 18 °C and pH 7.0 is less than 20%. Our results agree with those reviewed by Hasler et al. (2018), including mortality and sub-lethal injuries. Moreover, the effects of high water pCO₂ on fish can also include behavioral responses with unknown ecological consequences (Regan et al., 2016; Schunter et al., 2019).

Across its distribution range (Conte-Grand et al., 2015), the conservation situation of the Patagonian pejerrey *O. hatcheri* is already fragile due to salmonid and carp invasion and the genetic introgression of the co-generic *O. bonariensis* (Becker et al., 2018). Morenito Lake is a valued place where carp have not yet arrived (Crichigno et al., 2016a) and where salmonids are mostly excluded by high temperature (Aigo, 2010). It seems that the rule in the relationships between both air and water temperatures, atmospheric, and water pCO₂ is the spatial variation, due to water circulation, nutrient load, photosynthesis, and buffer capacity. As water temperature and pCO₂ increasing continues, we will need to revise the status of shallow littoral vegetated areas of the lakes as suitable anti-predatory refuges for fish eggs, larvae, and juveniles.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest To the best of our knowledge, none of the authors have any conflict of interest that might influence their objectivity on the topic of this manuscript.

Ethical approval All animal experiments complied with the U.K. Animals (Scientific Procedures) Act, 1986, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) guidelines.

Consent to participate Publication is approved by all authors and the responsible authorities.

Consent for publication The work described has not been published previously, is not under consideration for publication elsewhere, and will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright holder.

References

- Abernathy, M. A., 2004. Effect of water hardness on the survival of rainbow shark minnow. (*Epalzeorhynchus frenatum*) eggs and larvae. PhD Thesis, University of Florida. Available in http://etd.fcla.edu/UF/UFE0004915/abernathy_m
- Ahmad, S., S. Shukla, A. Mishra, D. Kasherwani, V. P. Swami & S. Shukla, 2018. Effect of cadmium chloride on general body colouration and chromatophores of stinging cat fish, *Heteropneustes fossilis* (Bloch). *Journal of Applied and Natural Science* 10: 655–660.
- Aigo, J., V. Cussac, S. Peris, S. Ortubay, S. Gómez, H. López, M. Gross, J. Barriga & M. Battini, 2008. Distribution of introduced and native fish in Patagonia (Argentina): patterns and changes in fish assemblages. *Reviews in Fish Biology and Fisheries* 14: 387–408.
- Aigo, J., 2010. Interacción entre peces nativos y salmónidos en Patagonia: su vulnerabilidad al cambio climático. PhD Thesis, Universidad Nacional del Comahue.
- Aigo, J., M. Lattuca & V. Cussac, 2014. Susceptibility of native perca (*Percichthys trucha*) and exotic rainbow trout (*Oncorhynchus mykiss*) to high temperature in Patagonia: different physiological traits and distinctive responses. *Hydrobiologia* 736: 73–82.
- Balmer, M. B. & J. A. Downing, 2011. Carbon dioxide concentrations in eutrophic lakes: undersaturation implies atmospheric uptake. *Inland Waters* 1(2): 125–132.
- Balon, E. K., 1990. Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. *Guelph Ichthyology Reviews* 1: 1–42.
- Balon, E. K., 1999. Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). *Environmental Biology of Fishes* 56: 17–38.
- Balseiro, E., T. Kitzberger, B. Modenutti, M. Bastidas Navarro, V. Ojeda, C. Úbeda, S. Ippi, P. Macchi, M. Alonso & P. Temporetti, 2014. RNU Morenito-Laguna Ezquerria, Universidad Nacional del Comahue, Centro Regional Universitario Bariloche, Informe Final., 67.
- Barile, J., M. Escudero & L. Jara, 2016. Efecto del pH sobre la supervivencia embrionaria, periodo embrionario y de eclosión de *Galaxias maculatus*. *Revista De Biología Marina y Oceanografía* 51: 181–185.
- Barriga, J.P., 2006. Estudio comparado de la distribución espacio temporal, el crecimiento y la alimentación de larvas y juveniles de *Galaxias* (Pisces, Galaxiidae) en lagos y ríos Patagónicos. PhD Thesis, Universidad Nacional del Comahue.
- Battini, M. A., M. F. Alonso & V. E. Cussac, 1995. Growth and nutritional condition of the larvae of *Odontesthes microlepidotus* (Atherinidae): An experimental approach. *Environmental Biology of Fishes* 42: 391–399.
- Baumann, H., 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Canadian Journal of Zoology* 97: 399–408.
- Baumann, H., S. C. Talmage & C. J. Gobler, 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change* 2: 38–41.
- Becker, L. A., S. A. Crichigno & V. E. Cussac, 2018. Climate change impacts on freshwater fishes: a Patagonian perspective. *Hydrobiologia* 816: 21–38.
- Beitinger, T. L. & R. W. McCauley, 1990. Whole-animal physiological processes for the assessment of stress in fishes. *Journal of Great Lakes Research* 16: 542–575.
- Beitinger, T. L. & W. I. Lutterschmidt, 2011. Measures of thermal tolerance. In Farrell, A. P. (ed), *Encyclopedia of fish physiology: from genome to environment* Elsevier Inc, San Diego: 1695–1702.
- Buria, L., S. J. Walde, M. Battini, P. J. Macchi, M. Alonso, D. E. Ruzzante & V. E. Cussac, 2007. Movement of a South American perch *Percichthys trucha* in a mountain Patagonian lake during spawning and prespawning periods. *Journal of Fish Biology* 70: 215–230.
- Campbell, N. A. & W. R. Atchley, 1981. The geometry of canonical variate analysis. *Systematic Zoology* 30: 268–280.
- Cervellini, P. M., M. A. Battini & V. E. Cussac, 1993. Ontogenetic shifts in the feeding of *Galaxias maculatus* (Galaxiidae) and *Odontesthes microlepidotus* (Atherinidae). *Environmental Biology of Fishes* 36: 283–290.
- Chalde, T., D. A. Fernández, V. E. Cussac & G. M. Somoza, 2011. The effect of rearing temperature in larval development of pejerrey, *Odontesthes bonariensis* – Morphological indicators of development. *Neotropical Ichthyology* 9: 747–756.
- Chambers, R. C., A. C. Candelmo, E. A. Habeck, M. E. Poach, D. Wiczorek, K. R. Cooper, C. E. Greenfield & B. A. Phelan, 2014. Effects of elevated CO₂ in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. *Biogeosciences* 11: 1613–1626.
- Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. Defries, J. Galloway & M. Heimann, 2014. IPCC AR5 WG1, Chapter 6: carbon and other biogeochemical cycles, Cambridge University Press., 465–570.
- Cione, A. L. & A. M. Báez, 2007. Peces continentales y anfibios cenozoicos de Argentina: los últimos cincuenta años. *Ameghiniana* 11: 195–220.
- Clingerman, J., J. Bebak, P. M. Mazik & S. T. Summerfelt, 2007. Use of avoidance response by rainbow trout to carbon dioxide for fish self-transfer between tanks. *Aquacultural Engineering* 37: 234–251.
- Cole, J. J., N. F. Caraco, G. W. Kling & T. K. Kratz, 1994. Carbon dioxide supersaturation in the surface waters of lakes. *Science* 265: 1568–1570.
- Conte-Grand, C., J. Sommer, G. Ortí & V. Cussac, 2015. Populations of *Odontesthes* (Teleostei: Atheriniformes) in the Andean region of Southern South America: body

- shape and hybrid individuals. *Neotropical Ichthyology* 13: 137–150.
- Crichigno, S., C. Conte-Grand, M. Battini & V. Cussac, 2013. Cephalic morphological variation in freshwater silversides, *Odontesthes hatcheri* and *Odontesthes bonariensis* in Patagonia: introgression and ecological relationships. *Journal of Fish Biology* 83: 542–559.
- Crichigno, S., P. Cordero, G. Blasetti & V. Cussac, 2016a. Dispersion of the invasive common carp *Cyprinus carpio* (Linnaeus 1758) in Southern South America: changes and expectations, westward and southward. *Journal of Fish Biology* 89: 403–416.
- Crichigno, S., R. S. Hattori, C. A. Strüssmann & V. Cussac, 2016b. Morphological comparison of wild, farmed and hybrids of two South American silversides, *Odontesthes bonariensis* and *Odontesthes hatcheri*. *Aquaculture Research* 47: 1797–1808.
- Crichigno, S., L. Becker, M. Orellana, R. Larraza, G. Mirena, M. A. Battini & V. Cussac, 2018. Rainbow trout adaptation to a warmer Patagonia and its potential to increase temperature tolerance in cultured stocks. *Aquaculture Reports* 9: 82–88.
- Crichigno, S., 2012. Variación morfológica y plasticidad fenotípica del aparato bucofaringeo de *Odontesthes hatcheri* (Eigenmann, 1909) y *Percichthys trucha* (Cuvier y Valenciennes, 1840). PhD Thesis. Universidad Nacional del Comahue, 224 pp. Available in: [http://crubweb.uncoma.edu.ar/docbiologia/Descargas/TESIS%20APR OBADAS/CRICHIGNO.pdf](http://crubweb.uncoma.edu.ar/docbiologia/Descargas/TESIS%20APR%20OBADAS/CRICHIGNO.pdf)
- Cupp, A. R., J. A. Tix, J. R. Smerud, R. A. Erickson, K. T. Fredricks, J. J. Amberg, C. D. Suski & R. Wakeman, 2017. Using dissolved carbon dioxide to alter the behavior of invasive round goby. *Management of Biological Invasions* 8: 567–574.
- Cussac, V. E., P. M. Cervellini & M. A. Battini, 1992. Intralacustrine movements of *Galaxias maculatus* (Galaxiidae) and *Odontesthes microlepidotus* (Atherinidae) during their early life history. *Environmental Biology of Fishes* 35: 141–148.
- Cussac, V. E., D. A. Fernández, S. E. Gómez & H. L. López, 2009. Fishes of southern South America: a story driven by temperature. *Fish Physiology and Biochemistry* 35: 29–42.
- Dabruzzi, T., W. A. Bennett, J. L. Rummer & N. A. Fangue, 2012. Thermal ecology of juvenile ribbontail stingray, *Taeniura lymma* (Forsska I, 1775), from a Mangal Nursery in the Banda Sea. *Hydrobiologia* 701: 37–49.
- Diaz, M., F. Pedrozo, C. Reynolds & P. Temporetti, 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. *Limnologia* 37: 17–27.
- Dupont, S., J. Havenhand, W. Thorndyke, L. Peck & M. Thorndyke, 2008. Near-future level of CO₂-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Marine Ecology Progress Series* 373: 285–294.
- Dyer, B. S., 2006. Systematic revision of the South American silversides (Teleostei, Atheriniformes). *Biocell* 30: 69–88.
- Gjedrem, T. & B. O. Rosseland, 2012. Genetic variation for tolerance to acidic water in salmonids. *Journal of Fish Biology* 80: 1–14.
- Gobbi, M., 1986. Osteología del pejerrey patagónico, *Patagonina hatcheri*. Licenciata Thesis. Universidad Nacional del Comahue. Argentina. 129 p.
- Gobler, C. J., L. R. Merlo, B. K. Morell & A. W. Griffith, 2018. Temperature, acidification, and food supply interact to negatively affect the growth and survival of the forage fish, *Menidia beryllina* (inland silverside) and *Cyprinodon variegatus* (sheepshead minnow). *Frontiers in Marine Science* 5: 86.
- Gómez, S. E., 1990. Some thermal ecophysiological observations on the catfish *Hatcheria macraei* (Girard, 1855) (Pisces, Trichomycteridae). *Biota* 6: 89–95.
- Gómez, S. E., 1998. Niveles letales de pH en *Odontesthes bonariensis* (Atheriniformes, Atherinidae). *Iheringia* 85: 101–108.
- Gómez, S. E., R. C. Menni, J. Gonzalez Naya & L. Ramirez, 2007. The physical–chemical habitat of the Buenos Aires pejerrey, *Odontesthes bonariensis* (Teleostei, Atherinopsidae), with a proposal of a water quality index. *Environmental Biology of Fishes* 78: 161–171.
- Greig, H. S., D. K. Niyogi, K. L. Hogsden, P. G. Jellyman & J. S. Harding, 2010. Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity. *Science of the Total Environment* 408: 3240–3250.
- Hartmann, D.L., A.M.G. Klein Tank, M. Rusticucci, L.V. Alexander, S. Brönnimann, Y. Charabi, F.J. Dentener, E.J. Dlugokencky, D.R. Easterling, A. Kaplan, B.J. Soden, P.W. Thorne, M. Wild, & P.M. Zhai, 2013. Observations: Atmosphere and Surface. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds)]. Cambridge University Press, Cambridge & New York.
- Hasler, C. T., D. Butman, J. D. Jeffrey & C. D. Suski, 2016. Freshwater biota and rising pCO₂. *Ecology Letters* 19: 98–108.
- Hasler, C. T., J. D. Jeffrey, E. V. C. Schneider, K. D. Hannan, J. A. Tix & C. D. Suski, 2018. Biological consequences of weak acidification caused by elevated carbon dioxide in freshwater ecosystems. *Hydrobiologia* 806: 1–12.
- Hughes, L. C., Y. P. Cardoso, J. A. Sommer, R. Cifuentes, M. Cuello, G. M. Somoza, M. González-Castro, L. R. Malabarba, V. Cussac, E. M. Habit, R. Betancur-R & G. Ortí, 2020. Biogeography, habitat transitions and hybridization in a radiation of South American silverside fishes revealed by mitochondrial and genomic RAD data. *Molecular Ecology* 29: 738–751.
- Incardona, J. P., T. K. Collier & N. L. Scholz, 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicology and Applied Pharmacology* 196: 191–205.
- Incardona, J. P., L. D. Gardner, T. L. Linbo, T. L. Brown, A. J. Esbaugh, E. M. Mager, J. D. Stieglitz, B. L. French, J. S. Labenia, C. A. Laetz, M. Tagal, C. A. Sloan, A. Elizur, D. D. Benetti, M. Grosell, B. A. Block & N. L. Scholz, 2014. Deepwater Horizon impacts on pelagic fish embryos.

- Proceedings of the National Academy of Sciences 111: E1510–E1518.
- IPCC, 2014. Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds)]. IPCC, Geneva, Switzerland, 151 pp.
- Ishimatsu, A., M. Hayashi & K. S. Lee, 2005. Physiological effects on fishes in a high-CO₂ world. *Journal of Geophysical Research* 110: C09S09.
- Ishimatsu, A., M. Hayashi & T. Kikkawa, 2008. Fishes in high-CO₂, acidified oceans. *Marine Ecology Progress Series* 373: 295–302.
- Jansen, E., J. Overpeck, K. Briffa, J. Duplessy, F. Joos, V. Mas-sondelmotte, D. Olago, B. Ottobliesner, W. Peltier & S. Rahmstorf, 2007. The physical science basis: contribution of working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge:
- Jellyman, P. G. & J. S. Harding, 2014. Variable survival across low pH gradients in freshwater fish species. *Journal of Fish Biology* 85: 1746–1752.
- Jolliffe, I. T., 2002. Principal component analysis, 2nd ed. Springer, New York:
- Klingenberg, C. P., 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11: 353–357.
- Kondera, E., 2019. Haematopoiesis and haematopoietic organs in fish. *Scientific Annals of Polish Society of Animal Production* 15: 9–16.
- Kunz, Y. W., 2004. Developmental biology of teleost fishes, Springer, Netherlands:
- Kwain, W. & G. A. Rose, 1985. Growth of brook trout *Salvelinus fontinalis* subject to sudden reductions of pH during their early life history. *Transactions of the American Fisheries Society* 114: 564–570.
- Lattuca, M.E., 2006. Ecología e historia de vida de *Aplochiton* (Pisces, Galaxiidae). PhD Thesis. Universidad Nacional del Comahue.
- Lema, S. C., S. L. Bock, M. M. Malley & E. A. Elkins, 2019. Warming waters beget smaller fish: evidence for reduced size and altered morphology in a desert fish following anthropogenic temperature change. *Biology Letters* 15: 20190518.
- Livingstone, D. M. & A. F. Lotter, 1998. The relationship between air and water temperatures in lakes of the Swiss Plateau: a case study with palaeolimnological implications. *Journal of Paleolimnology* 19: 181–198.
- Lo, M., A. Shahriari, J. N. Roa, M. Tresguerres & A. P. Farrell, 2021. Differential effects of bicarbonate on severe hypoxia- and hypercapnia-induced cardiac malfunctions in diverse fish species. *Journal of Comparative Physiology B* 191: 113–125.
- López, H. L., R. C. Menni, M. Donato & A. M. Miquelarena, 2008. Biogeographical revision of Argentina (Andean and Neotropical Regions): an analysis using freshwater fishes. *Journal of Biogeography* 35: 1564–1579.
- Ma, J. & P. Wang, 2021. Effects of rising atmospheric CO₂ levels on physiological response of cyanobacteria and cyanobacterial bloom development: a review. *Science of the Total Environment* 754: 141889.
- Magnuson, J. J., 2010. History and heroes: the thermal niche of fishes and long-term lake ice dynamics. *Journal of Fish Biology* 77: 1731–1744.
- Milano, D., 2003. Biología de *Galaxias platei* (Pisces, Galaxiidae): Especializaciones relativas a su distribución. PhD Thesis, Universidad Nacional del Comahue.
- Modenutti, B. E. & G. L. Pérez, 2001. Planktonic ciliates from an oligotrophic South Andean Lake, Morenito Lake (Patagonia, Argentina). *Brazilian Journal of Biology* 61: 389–395.
- Modenutti, B. E., E. G. Balseiro & C. P. Queimaliños, 2000. Ciliate community structure in two South Andean lakes: the effect of lake water on *Ophrydium naumanni* distribution. *Aquatic Microbial Ecology* 21: 299–307.
- Munday, P. L., M. D. Jarrold & I. Nagelkerken, 2019. Ecological effects of elevated CO₂ on marine and freshwater fishes: from individual to community effects. In Grosell, M., P. L. Munday, A. P. Farrell & C. J. Brauner (eds), Carbon dioxide: fish physiology, Vol. 37. Academic Press: 323–368.
- Murray, C. S. & H. Baumann, 2018. You better repeat it: complex CO₂ × temperature effects in Atlantic silver-side offspring revealed by serial experimentation. *Diversity* 10: 69.
- Nilsson, G., D. Dixon, P. Domenici, M. McCormick, C. Sørensen, S. Watson & P. Munday, 2012. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change* 2: 201–204.
- Ortubay, S. G., S. E. Gómez & V. E. Cussac, 1997. Lethal temperatures of a Neotropical fish relic in Patagonia, the scale-less characinid *Gymnocharacinus bergi* Steindachner 1903. *Environmental Biology of Fishes* 49: 341–350.
- Phillips, J. C., G. A. McKinley, V. Bennington, H. A. Bootsma, D. J. Pilcher, R. W. Sterner & N. R. Urban, 2015. The potential for CO₂-induced acidification in freshwater: a Great Lakes case study. *Oceanography* 28: 136–145.
- Quirós, R., 1991. Factores que afectan la distribución de salmónidos en Argentina. COPESCAL, FAO, Documento Técnico 9: 163–173.
- Quirós, R. & E. Drago, 1985. Relaciones entre variables físicas, morfológicas y climáticas en lagos patagónicos. *Revista De La Asociación De Ciencias Naturales Del Litoral* 16: 181–199.
- Quirós, R. & E. Drago, 1999. The environmental state of Argentinean lakes: an overview. *Lakes & Reservoirs: Research and Management* 4: 55–64.
- Raja, D. A., V. Gotherwal, S. A. Burse, Y. J. Subramaniam, F. Sultan, A. Vats, H. Gautam, B. Sharma, S. Sharma, A. Singh, S. Sivasubbu, R. S. Gokhale & V. T. Natarajan, 2020. pH-controlled histone acetylation amplifies melanocyte differentiation downstream of MITF. *EMBO Reports* 21: e48333.
- Regan, M. D., A. J. Turko, J. Heras, M. K. Andersen, S. Lefevre, T. Wang, M. Bayley, C. J. Brauner & T. T. Huong do, N. T. Phuong & G. E. Nilsson, 2016. Ambient CO₂, fish behaviour and altered GABAergic neurotransmission: exploring the mechanism of CO₂-altered behaviour by taking a hypercapnia dweller down to low CO₂ levels. *Journal of Experimental Biology* 219: 109–118.

- Ringuelet, R.A., Aramburu, R.H., Alonso, A., 1967. Los peces argentinos de agua dulce. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Argentina. Available in: <http://sedici.unlp.edu.ar/handle/10915/62009>
- Rohlf, F. J., 2013. TPSdig version 2.17, State University at Stony Brook, New York:
- Rohlf, F. J. & D. Slice, 1990. Extensions of the Procrustes methods for the optimal superimposition of landmarks. *Systematic Zoology* 39: 40–59.
- Rueda, E. C., K. A. Mullaney, C. Conte-Grand, E. M. Habit, V. Cussac & G. Ortí, 2017. Displacement of native Patagonian freshwater silverside populations (*Odontesthes hatcheri*, Atherinopsidae) by introgressive hybridization with introduced *O. bonariensis*. *Biological Invasions* 19: 971–988.
- Sayer, M. D. J., J. P. Reader & T. R. K. Dalziel, 1993. Freshwater acidification: effects on the early life stages of fish. *Reviews in Fish Biology and Fisheries* 3: 95–132.
- Schunter, C., T. Ravasi, P. L. Munday & G. E. Nilsson, 2019. Neural effects of elevated CO₂ in fish may be amplified by a vicious cycle. *Conservation Physiology* 7: coz100.
- Talmage, S. C. & C. J. Gobler, 2010. Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proceedings of the National Academy of Sciences* 107: 17246–17251.
- Weiss, L. C., L. Pötter, A. Steiger, S. Kruppert, U. Frost & R. Tollrian, 2018. Rising pCO₂ in freshwater ecosystems has the potential to negatively affect predator-induced defenses in *Daphnia*. *Current Biology* 28: 327–332.
- West, D. W., J. A. Boubée & R. F. Barrier, 1997. Responses to pH of nine fishes and one shrimp native to New Zealand freshwaters. *New Zealand Journal of Marine and Freshwater Research* 31: 461–468.

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