



## The effect of alterations in salinity and temperature on neuroendocrine responses of the Antarctic fish *Harpagifer antarcticus*



L. Vargas-Chacoff<sup>a,b,\*,1</sup>, J.L.P. Muñoz<sup>c,\*\*,1</sup>, D. Ocampo<sup>c</sup>, Kurt Paschke<sup>b,d</sup>, Jorge M. Navarro<sup>a,b</sup>

<sup>a</sup> Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile

<sup>b</sup> Centro FONDAP de Investigación de Altas Latitudes (FONDAP IDEAL), Universidad Austral de Chile, Valdivia, Chile

<sup>c</sup> Centro de Investigación y Desarrollo i-mar, Universidad de los Lagos, Casilla 557, Puerto Montt, Chile

<sup>d</sup> Instituto de Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile

### ARTICLE INFO

#### Keywords:

Serotonin  
5HT  
5HIAA  
Dopamine  
*Harpagifer antarcticus*

### ABSTRACT

Increased levels of tissue monoaminergic neurotransmitters, as well as circulating catecholamines, appear to play a role in the regulation of the physiological responses of teleost fish. *Harpagifer antarcticus* is a stenothermic, Antarctic notothenioid fish. The aim of this study was to determine the effect of increased seawater temperature and decreased salinity on the levels of 5-HT, 5-HIAA, DA, and Noradrenaline in the brain, stomach, and gut of *H. antarcticus*. Wild-gathered fish were acclimatized to habitat conditions (2 °C, 33 PSU) prior to placement in aquaria with 4 temperatures (2, 5, 8 and 11 °C) and 3 salinities (23, 28 and 33 PSU) for 10 days. Fish exposed to 11 °C had higher levels of the brain neurotransmitters than those at 2 °C. Concomitant exposure to low salinity exacerbated the effect of exposure to 11 °C. At lower temperatures, concomitant alterations in salinity induced differential effects on brain neurotransmitters. When fish were exposed to 28 PSU, 5-HIAA, DA, and Noradrenaline levels at 5 and 8 °C presented no significant differences with those at 2 °C. In contrast, only 5HT and 5-HIAA levels in fish at 33 PSU were elevated at 5 and 8 °C respectively. Fish at 28 and 33 PSU had lower Gut 5HT levels at the 3 elevated temperatures, meanwhile fish at 23 PSU showed a biphasic effect when exposed to elevated temperatures. 5-HIAA levels decreased at 5 and 8 °C at 33 PSU. Stomach 5HT levels also showed a differential response at the 3 salinity levels when exposed to increased temperatures. At 11 °C, 5HT levels were markedly higher than those at 2 °C for fish at 33 PSU, moderately elevated for fish at 28 PSU, and lower for fish at 23 PSU, meanwhile 5-HIAA levels only increased with temperature at 33 PSU. These findings indicate that rapid exposure to alterations in temperature with or without concomitant changes in salinity is associated with differential responses in tissue monoaminergic neurotransmitter levels. The relatively high changes in neurotransmitter levels in fish exposed to moderate salinity and high temperature changes may indicate the physiological plasticity of *H. antarcticus* to possible changes in ocean temperature and salinity.

### 1. Introduction

Fish, as ectothermic animals are vulnerable to increased temperatures affecting metabolic rate, energy, osmotic, and physiological response (Oyarzún et al., 2018; Luis Vargas-Chacoff et al., 2009). To adapt to new environmental temperatures fish use different physiological mechanisms. Increased levels of tissue monoaminergic neurotransmitters as well as circulating catecholamines appear to play a role in the regulation of the physiological responses of teleost fish to changes in seawater temperature and salinity, as well as, other stressor. Øverli et al. (Øverli et al., 1999, 2001) described that monoaminergic

cerebral neurotransmitters such as NA (noradrenaline), DA (dopamine), or 5HT (serotonin) are involved in the control and integration of the fish stress response. Brain serotonergic activity is increased after exposure to different types of stressors, such as handling, isolation, predator exposure, pollutant exposure, social stress, or crowding (Gesto et al., 2008, 2009, 2013; Weber et al., 2012; Winberg and Nilsson, 1993). In this regard, the serotonergic system shows complex reciprocal interactions with the Hypothalamus-Pituitary-Interrenal “HPI” axis, affecting and being affected by other elements of the stress response (Chaouloff, 2000; Heisler et al., 2007; Winberg et al., 1997).

The neuroendocrine system is involved in certain adaptive

\* Corresponding author at: Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile.

\*\* Corresponding author: Centro de Investigación y Desarrollo i-mar, Universidad de los Lagos, Casilla 557, Puerto Montt, Chile.

E-mail addresses: [luis.vargas@uach.cl](mailto:luis.vargas@uach.cl) (L. Vargas-Chacoff), [joseluis.munoz@ulagos.cl](mailto:joseluis.munoz@ulagos.cl) (J.L.P. Muñoz).

<sup>1</sup> These authors contributed equally to this work.

adjustments in ionic regulation that occur during exposure to changes in salinity and/or temperature. DA levels vary in the brains of *Carassius auratus* when subjected to different salinities (Luz et al., 2008). Furthermore, De Boeck et al., (Boeck et al., 1996) reported that DA levels were increased in the brain stem of common carp after 1 week of salinity stress at 30 °C. In Caspian roach *Rutilus rutilus caspicus* a half migratory fish and bream *Abramis brama orientalis* the serotonergic system activity in brain, measured as the serotonin-modulating anticonsolidation protein, changes in response to the ambient salinity (Mustafayev and Mekhtiev, 2008).

However, there is little information on the neuroendocrine responses of Antarctic fish to changes in sea temperatures or salinity. According to IPCC (IPCC, 2014), climate change occurs and becomes effective at global, regional, and local levels. Presently, temperature changes are evident, yet not similar, on all continents and in the oceans (Bozinovic and Pörtner, 2015). In particular, the Antarctic and Sub-Antarctic regions have been freshened over decades during the second half of the 20th century (Wong et al., 1999). Some report on temperature of the surface seawater of the Western Antarctic Peninsula (WAP) has warmed nearly 1 °C in the last half century, and salinity has experienced strong changes, especially in coastal surface waters, due to melting Antarctic sea ice (Haumann et al., 2016; Meredith and King, 2005; Turner et al., 2005). Cardenas et al. (Cárdenas et al., 2018) reported that temperatures up to 3 °C for shallow benthic environments in the Antarctic Peninsula. Changes in environmental temperature-salinity can have profound effects on the physiological processes of ectotherm animals (Oyarzún et al., 2018; Pörtner et al., 2015; Sandblom et al., 2014; Schulte, 2015; Somero, 2010). The Antarctic area, or high latitude areas, have a highly endemic coastal ichthyofauna which is dominated by fish belonging to the Notothenioids suborder (Andriashv, 1965). The model species in this study was the intertidal Antarctic notothenioid fish, *Harpagifer antarcticus*, a stenothermic species (Brodeur et al., 2003). This species inhabits shallow waters (0–20 m) exposed to various environmental variations as salinity and temperature, and can be found from the Antarctic Peninsula to the South Sandwich Islands (White and Burren, 1992); being considered an important trophic resource for Antarctic coastal fish. Here, we test the hypothesis that warmer seawater and lower salinity induced by climate change will affect the fish (*H. antarcticus* as model) in this Antarctic coastal ecosystem. This hypothesis led to the prediction that significant drops in salinity will reduce the thermal tolerance range of the notothenioid fish, *H. antarcticus*, and therefore the aim of this study was to determine the effect of increased seawater temperature and decreased salinity on during the initial steps of the stress response, measure levels of 5-HT, 5-HIAA, DA and Noradrenaline in the brain, stomach and gut of *H. antarcticus*.

## 2. Methods

### 2.1. Animals and experimental protocols

Adult *Harpagifer antarcticus* ( $n = 60$ ,  $14 \pm 1$  g of body weight and  $9.5 \pm 1$  cm of length) were caught by turning over rocks from the lower intertidal zone (2 °C and 33 PSU) of the South Shetland Islands, Fildes Bay, King George Island (62° 11'S, 58° 59' W), Antarctic, during 2 days of sampling. Fish were acclimatized for one week to natural conditions: 2 °C, 33 PSU (the temperature and salinity was measurement *in situ* and to repeated in lab), a natural photoperiod (we kept the same environmental conditions), and fed *ad libitum* with their natural diet which was collected every two days and was composed of the amphipod *Gondogeneia antarctica*. After the acclimation period, each fish was placed in individual aquariums (5 L volume). The experimental design comprised of an orthogonal combination of four temperatures (2 “control groups”, 5, 8 and 11 °C) and three salinities (23, 28, or 33 PSU “control groups”) for a 10-day period ( $n = 5$  per combination). Fifty percent of the water was renewed every day.

All experiments were performed under the guidelines for the use of laboratory animals established by the Chilean National Commission of Scientific and Technological Research (CONICYT) and the Universidad Austral de Chile.

### 2.2. Sampling procedure

Fish were netted, submitted to a lethal dose of 2-fenoxiethanol (1 mL/L), and euthanized by spinal sectioning before tissue removal (Vargas-Chacoff et al., 2016). The brain, gut, and stomach were rapidly removed and were snap-frozen in liquid N<sub>2</sub> and stored at –80 °C in Chilean base “Escudero Base”. The samples was transported by liquid nitrogen with Dry Shipper until our lab in Valdivia and keep at –80 °C until analysis.

### 2.3. Brain, gut, and stomach monoamines

The tissues were weighed and then homogenized by ultrasonic disruption in 0.5 mL of HPLC mobile phase. The homogenates were then centrifuged (16,000 ×g, 10 min) and supernatants were further diluted 1:2 (supernatant: mobile phase) prior to HPLC analysis. Data were expressed as ng for g of tissue. Serotonin (5-hydroxytryptamine, 5HT) and 5-hydroxyindole-3-acetic acid (5HIAA, a major 5HT oxidative metabolite) were analyzed in brain, gut, and stomach, only dopamine and noradrenaline were quantified in brain, and all of them were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-EC), as previously described [29] with some modifications. The HPLC system was equipped with a Dionex ultimate 3000 pump, a 5 μm analytical column (Waters, Atlantis C18, 150 mm length × 4.6 mm diameter), and a ESA Coulochem III detector. The detection system included a double analytical cell (M5011) with oxidation potentials set at +40 mV (first electrode) and +340 mV (second electrode). The mobile phase was composed of 63.9 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.1 mM Na<sub>2</sub>EDTA, 0.80 mM sodium 1-octanesulfonate and 15.3% (v/v) methanol; its pH was adjusted to 2.95 with ortho-phosphoric acid, and was filtered (0.20 μm filter, Millipore, Bedford, USA) and degasified with a vacuum before use. The analytical run time was 15 min with an isocratic flow rate of 1.0 mL/min at room temperature. The detection limit for the amines and their metabolites ranged between 0.5 and 1.5 pg per injection, with a signal-to-noise ratio of 3. Acquisition and integration of chromatograms were performed using the software Chromeleon 6.80 (Thermo Scientific).

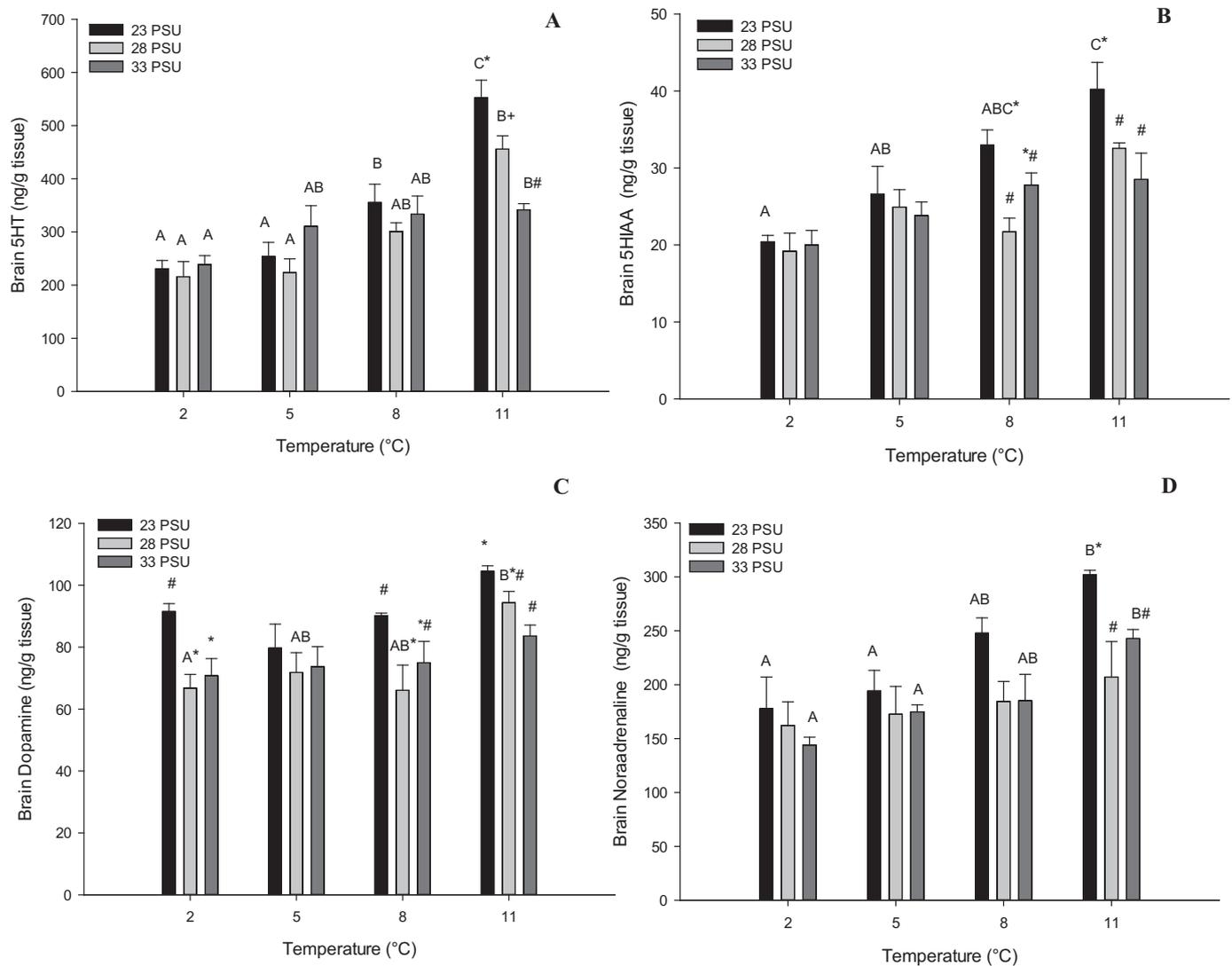
### 2.4. Statistical analysis

Assumptions of normality, independence, and homogeneity for the variances were tested. For each neuroendocrine variable, a two-way analysis of variance was performed. The factors of variance were salinity and temperature. A post-hoc Tukey-test was used to identify significantly different groups. Differences were considered significant at  $P \leq .05$ .

## 3. Results

### 3.1. Brain tissue

Serotonin concentrations were higher in both low salinity groups, but not in the control group (33 PSU) reaching the highest levels at 11 °C in all salinity groups. While no differences between salinity groups were found in the control animals. Salinity produced an effect at 8 and 11C in the 23 PSU group ( $P < .05$ ). Serotonin concentration at the lowest salinity concentration doubled the control values at 11 °C ( $230 \pm 16$  and  $552 \pm 33$  ng/g tissue respectively). (Fig. 1 A). A similar pattern was observed for 5-hydroxyindole-3-acetic acid (5HIAA). The highest levels of 5HIAA were observed in fish exposed to 11 °C and 23 PSU ( $40 \pm 3$  ng/g tissue), almost two-fold higher than the control



**Fig. 1.** Brain levels of A) Serotonin (5HT), B) 3. 5-hydroxyindole-3-acetic acid (5HIAA), C) Dopamine (DA), D) Noradrenaline (NA), the fish were sampled at 10 days post-transfer (dpt). Values are expressed as the mean  $\pm$  SEM (n = 5). Different letters indicate significant differences between salinity groups at different temperatures. Symbols (\*, #, +) indicate significant differences between the same temperature group at different salinities (two-way ANOVA, post-hoc Tukey's test,  $P < .05$ ).

group (Fig. 1 B). The lowest salinity concentration produced an important effect on brain Dopamine levels at all tested temperatures, meanwhile control and 28 PSU treatments showed similar responses. The highest temperature (11 °C) increased the dopamine concentration at 28 PSU, however, no changes were observed with 23 and 33 PSU. Meanwhile salinity produced changes to dopamine at 2, 8, and 11 °C presenting the highest levels at 23 PSU (Fig. 1 C). A significant increase in Noradrenaline levels was observed at 23 and 33 PSU in highest temperature (11 °C) compared to the control group (Fig. 1 D).

### 3.2. Gut tissue

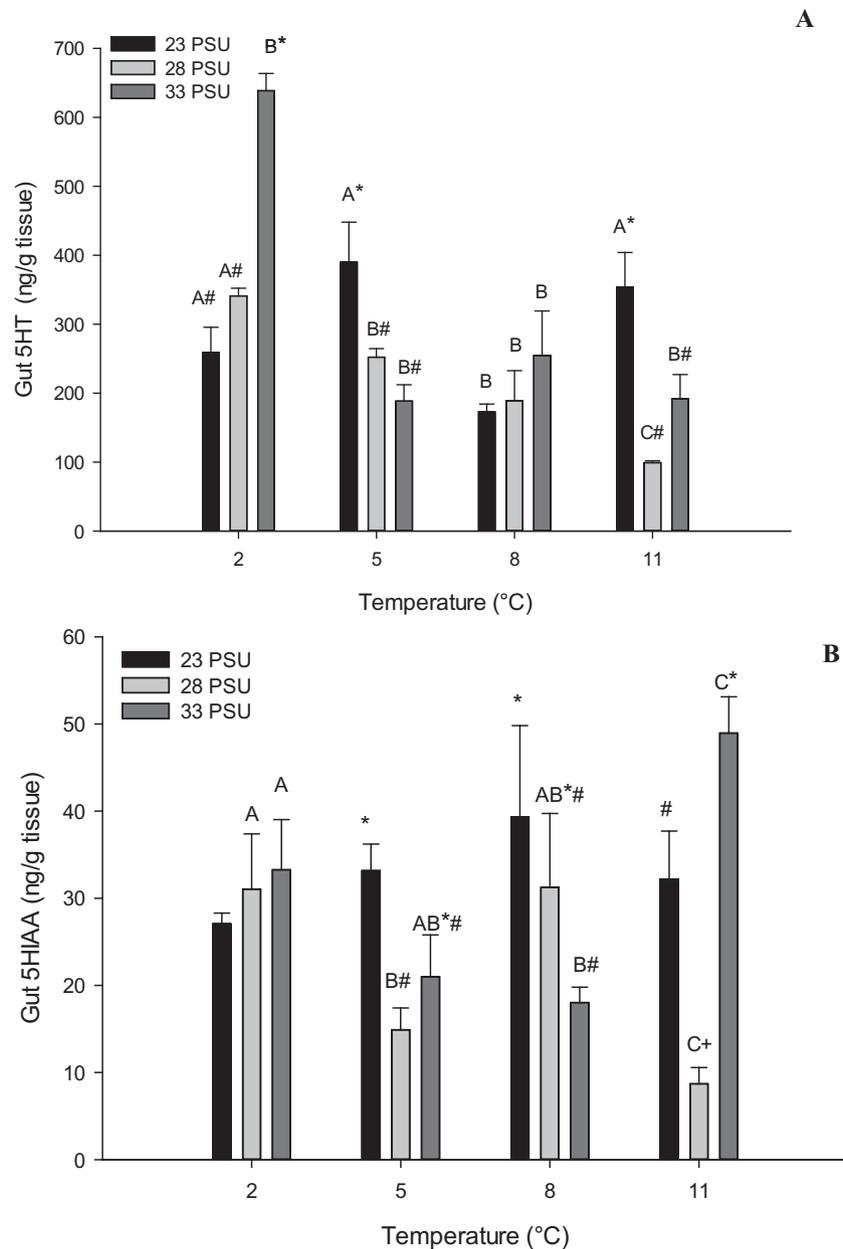
5HT levels were highest in fish maintained at 33PSU and 2 °C. At 28 and 33 PSU, fish exposed to higher temperatures had lower 5HT levels than those maintained at 2 °C ( $638 \pm 24$  and  $99 \pm 2$  ng/g tissue respectively) (Fig. 2 A). 5-hydroxyindole-3-acetic acid (5HIAA) levels were highest at 11 °C in the 33 PSU salinity group and were the lowest at 11 °C in the 28 PSU salinity group ( $48 \pm 4$  and  $8 \pm 1$  ng/g tissue respectively) compared to the control group at 2 °C at 33 PSU ( $33 \pm 6$  ng/g tissue) (Fig. 2 B).

### 3.3. Stomach tissue

Serotonin (5HT) levels were highest at 8 °C in the 33 PSU salinity group and the lowest levels were observed at 11 °C in the 23 PSU group ( $224 \pm 13$  and  $93 \pm 6$  ng/g tissue respectively) compared to the 2 °C control group 33 PSU ( $143 \pm 17$  ng/g tissue) (Fig. 3 A). 5-hydroxyindole-3-acetic acid (5HIAA) levels were highest at 8 and 11 °C in the 33 PSU salinity group ( $45 \pm 3$  and  $44 \pm 2$  ng/g tissue respectively) compared to the 2 °C control group at 33 PSU ( $24 \pm 1$  ng/g tissue) (Fig. 3 B).

### 3.4. 5-HIAA/5-HT ratios

Variations in the 5HIAA / 5HT ratio at brain level increased serotonergic activity at 5 °C to salinities of 23 and 28 PSU and also at 11 °C at 33 psu. In the stomach the ratio was increased to 23 psu at 8 and 11 °C, while in gut the ratio increased to 23 and 28 PSU at 8 °C (Table 1).



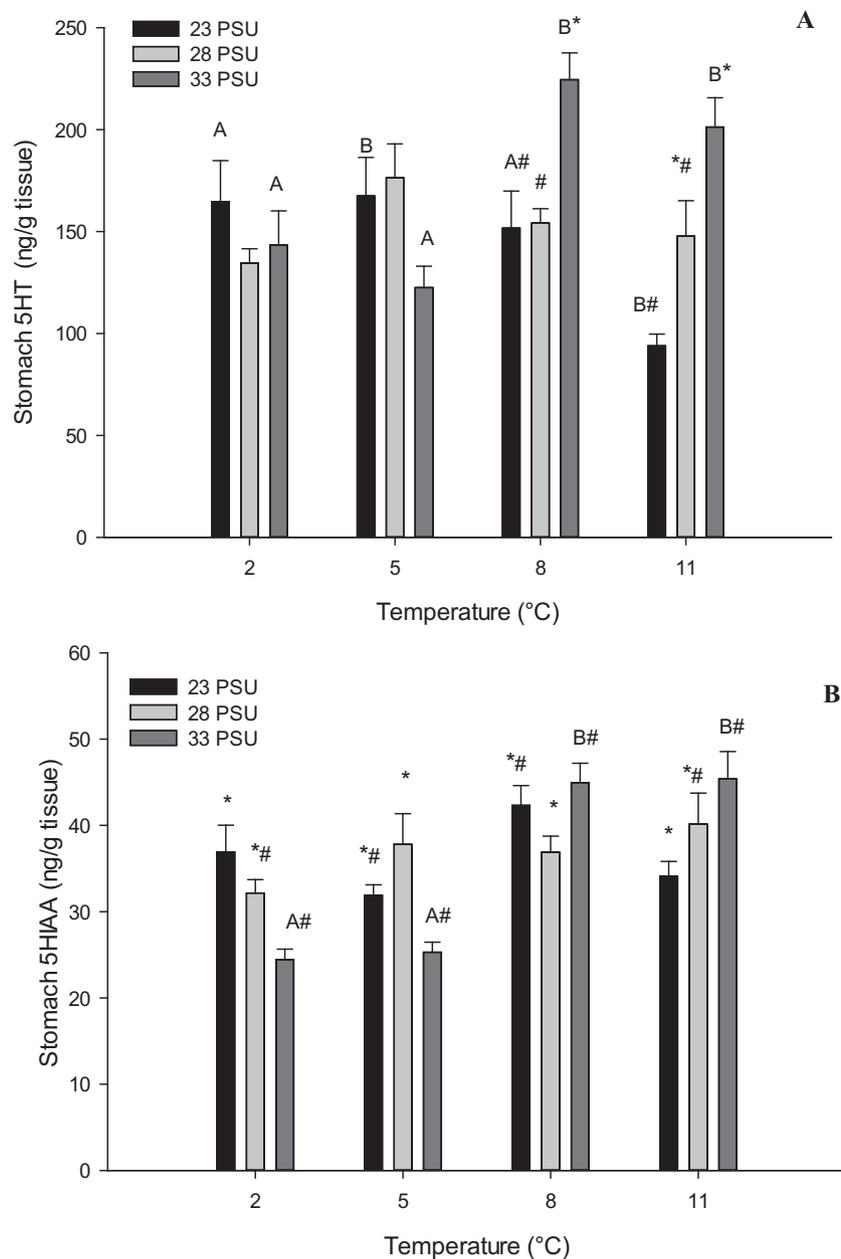
**Fig. 2.** Gut levels of A) Serotonin (5HT), B) 5-hydroxyindole-3-acetic acid (5HIAA), the fish were sampled 10 days post-transfer (dpt). Values are expressed as the mean  $\pm$  SEM (n = 5). Different letters indicate significant differences between salinity groups at different temperatures. Symbols (\*, #, +) indicate significant differences between the same temperature group at different salinities (two-way ANOVA, post-hoc Tukey's test,  $P < .05$ ).

#### 4. Discussion

The neuroendocrine response in *H. antarcticus* is modified by temperature and salinity. Our results showed that the neuroendocrine response was different over the endpoint experiment, which is consistent with studies conducted in other fish and abiotic variables.

These findings indicate that rapid exposure to alterations in temperature with or without concomitant changes in salinity are associated with differential responses in tissue monoaminergic neurotransmitter levels. The relatively high changes in neurotransmitter levels for the fish exposed to moderate salinity and high temperature changes may indicate the physiological plasticity of *H. antarcticus* to possible changes in ocean temperature and salinity, as a potential climate change effect. Our results after 10 days post-transfer indicated that 5HT (serotonin) levels in the brain were at their highest at 23 PSU-11 °C, meanwhile in the stomach they were highest in seawater (33 PSU) at 8–11 °C, but in

gut reached their highest levels at 2 °C in the control group. It is important to note that serotonin levels at the intestinal-gut level are not comparable with brain levels, since in the intestinal-gut tissue this serotonin can have two origins that are from the enterochromaffin cells and those from serotonergic neurons associated with the musculature, unlike the cerebral serotonin that is of neuronal origin (Dong et al., 2018). Brain serotonergic levels of the Antarctic fish *Harpagifer antarcticus* showed responses that are comparable to those reported as a physiological response to stress in fish and other vertebrates (Chaouloff, 2000; Summers et al., 2005; Winberg and Nilsson, 1993). Forster et al. (1998) studied two Antarctic fish species (*Pagothenia borchgrevinki*, *Trematomus bernacchii*) and described the importance and sensitivity of serotonergic mechanisms for cardiovascular and branchial control, comparing to other teleost species. A circulating catecholamine concentration has been reported in rainbow trout (*Onchorynchus mykiss*); a response to heat shock treatment over a wide range of values (Currie



**Fig. 3.** Stomach levels of A) Serotonin (5HT), B) 5-hydroxyindole-3-acetic acid (5HIAA), the fish were sampled 10 days post-transfer (dpt). Values are expressed as the mean ± SEM (n = 5). Different letters indicate significant differences between salinity groups at different temperatures. Symbols (\*, #, +) indicate significant differences between the same temperature group at different salinities (two-way ANOVA, post-hoc Tukey's test, P < .05).

**Table 1**

The 5-HIAA/5-HT ratios. Values are expressed as the mean ± SEM (n = 5). Different letters indicate significant differences between salinity groups at different temperatures. Symbols (\*, #, +) indicate significant differences between the same temperature group at different salinities (two-way ANOVA, post-hoc Tukey's test, P < .05).

	Salinity	Temperature			
		2 °C	5 °C	8 °C	11 °C
Brain	23 PSU	0.0455 ( ± 0.003)A	0.1103 ( ± 0.008)B*	0.0390 ( ± 0.010)A	0.0584 ( ± 0.006)A
	28 PSU	0.0694 ( ± 0.012)A B	0.0909 ( ± 0.016)B*	0.0396 ( ± 0.006)A	0.0347 ( ± 0.003)A
	33 PSU	0.0430 ( ± 0.010)	0.0409 ( ± 0.006)#	0.0555 ( ± 0.009)	0.0802 ( ± 0.006)
Stomach	23 PSU	0.1131 ( ± 0.02)AB*	0.0777 ( ± 0.014)B	0.2506 ( ± 0.057)A*	0.1519 ( ± 0.021)A
	28 PSU	0.0920 ( ± 0.014) *	0.0672 ( ± 0.013)	0.1089 ( ± 0.007) #	0.1160 ( ± 0.009)
	33 PSU	0.0225 ( ± 0.005)A#	0.0441 ( ± 0.010)A	0.1110 ( ± 0.007)B#	0.1316 ( ± 0.027)B
Gut	23 PSU	0.1143 ( ± 0.019)*	0.1021 ( ± 0.030)	0.2232 ( ± 0.050)*	0.0900 ( ± 0.001)
	28 PSU	0.0909 ( ± 0.018)AB*#	0.0610 ( ± 0.013)B	0.1665 ( ± 0.033)A*	0.0875 ( ± 0.009)AB
	33 PSU	0.0527 ( ± 0.009)A#	0.1184 ( ± 0.029)AB	0.0616 ( ± 0.013)A#	0.1505 ( ± 0.033)B

et al., 2008; LeBlanc et al., 2011). Recently, studies on rainbow trout concluded that circulating catecholamines influence the cellular heat shock response (Templeman et al., 2014). Our results in brain presented high levels of noradrenaline which is in line with high temperature treatment, but without differences according to salinity, demonstrating that salinity at 23 and 28 PSU are not stressors, but 23 PSU salinity produces stress in synergy with temperature only when the temperature was higher (8 and 11 °C), as reported by Vargas-Chacoff et al. (Vargas-Chacoff et al., 2009a, 2009b) in plasma of *Sparus auratus*, Fiess et al. (2007) in *Oreochromis mossambicus* or Handeland et al. (2000) in *Salmo salar*.

There are few antecedent in fish of serotonergic activity and salinity effects, has been described, an elevation of the serotonergic system activity, evaluated by the content of serotonin-modulating anticonsolidation protein, in the Caspian roach and Bream brain under the effect of the water salinity increase, reflects the corresponding activation of the serotonergic system (Mustafayev and Mekhtiev, 2008). The ratio 5HIAA/5HT is an estimator of the serotonergic activity that could be useful under some circumstances. The ratio give us information about the catabolic rate that could be linked with serotonergic activity under several assumptions. One of them is that MAO activity is not impaired by any factor in our experiment. In our experiment we use temperature as a treatment, in goldfish has been describe MAO displays changes in functional activity in response to a change in environmental temperature (Hall et al., 1982).

The endocrine system is involved in certain adaptive adjustments in ionic regulation that occur during thermal acclimation of fish (McCormick et al., 2013; Vargas-Chacoff et al., 2009a, 2009b; Wendelaar Bonga, 1997). Ionic regulation is dependent on the temperature of the ion uptake mechanism. The anterior brainstem heating increases gill ventilation and heart rate (McCormick et al., 2013; Vargas-Chacoff et al., 2009a, 2009b; Wendelaar Bonga, 1997). Some hormones are involved in the ionic balance of fish, including growth hormone, prolactin, and cortisol (Bozinovic and Pörtner, 2015; McCormick et al., 2013; Vargas-Chacoff et al., 2009a; Wendelaar Bonga, 1997). The major function of prolactin is the retention of ions using several tissues (gills, skin, the intestine, kidney and bladder), at a level required for survival in low water salinity (McCormick et al., 2013; Vargas-Chacoff et al., 2009a, 2009b; Wendelaar Bonga, 1997), in mammals (Mains and Eipper, 2012), as well as in fish (Nihon Dōbutsu Gakkai et al., 1988). Dopamine (DA) is a potent inhibitor of prolactin secretion, and it has been suggested that DA is the prolactin-release-inhibiting factor, being PRL is an inhibitor of the NKA pump and when fish are at 23 PSU this could be meaning that the NKA pump must work harder in order to maintain the ionic balance, increasing DA and impeding PRL release (Forster et al., 1998), although in this study the NKA activity was not measurement many report shown this relationship.

## 5. Conclusion

This is the first neuroendocrine study in Antarctic fish where two variables as temperature and salinity were did together and how is the synergetic response. Our results indicate that rapid exposure to alterations in temperate with or without concomitant changes in salinity is associated with differential responses in tissue monoaminergic neurotransmitter levels. The relatively high changes in neurotransmitter levels for the fish exposed to moderate salinity and high temperature changes may indicate the physiological plasticity of *H. antarcticus* to possible changes in ocean temperature and salinity. The enhanced serotonergic neurotransmission could also act as an early signal during the initial steps of the stress response or during the recognition of the stressor by the central nervous system. As future researches approach is necessary to complement these measurements with other experiment and parameters associated with HPI axis.

## Acknowledgments

This work was funded by FONDAP-IDEAL Grant N°15150003, and supported by the Instituto Chileno Antártico (INACH) and the Vicerrectoría de Investigación y Creación Artística (ex DID), Universidad Austral de Chile.

## Conflict of interests

The authors declare that there are no conflicts of interest.

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