



The Antarctic fish *Harpagifer antarcticus* under current temperatures and salinities and future scenarios of climate change

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ARTICLE INFO

Keywords:

Warming
Salinity decrease
Harpagifer
Physiology
Antarctic
Magellan Region
Climate change

ABSTRACT

Antarctic coasts are highly vulnerable environments where temperature have remained very constant along millions of years. These unique environmental conditions have generated a large number of stenotic species that could be highly sensitive to future scenarios of climate change. We investigated the separate and interactive effects of increasing seawater temperature and decreasing salinity on the physiological performance of the notothenioid fish, *Harpagifer antarcticus*. Adult individuals were exposed to an orthogonal combination of five temperatures (2, 5, 8, 11, 14 °C) and three salinities (23, 28, 33 psu) for a 10-day period. A drastic increment in mortality was observed with seawater warming; the pattern in response to lower salinity was less clear. No fish died at the two lowest temperatures (2 and 5 °C); however, mortality increased significantly at the two highest temperatures across the salinity treatments (33.3% at 11 °C; 93.3% at 14 °C). No data were obtained at 14 °C that could be included in the physiological analyses. Ingestion and absorption rates were significantly affected by temperature and salinity, but not by the interaction of the two. Finally, we observed a negative effect of temperature but not of salinity or the interaction of both on the scope for growth of *H. antarcticus*. These results suggest that this species could cope with a moderate temperature increase (5 °C) in the Antarctic. However, the higher metabolic rates observed at 8 and 11 °C are associated with conditions beyond the natural thermal window of this species, representing a disadvantage in the face of climate change. Therefore, and even in the hypothetical case that *H. antarcticus* were able to disperse to sub-Antarctic areas such as the Magellan Region, current and projected scenarios of seawater temperatures might be unsuitable for the development of effective populations of this species. The results confirm the stenothermal nature of *H. antarcticus*, considering its high vulnerability to environmental changes and its limited ability to cope with the more severe global warming models projected for the Antarctic and Magellan regions for the end of the century (mainly temperature).

1. Introduction

Antarctic coasts are highly vulnerable environments and sensitive to climate changes and the environmental conditions (e.g., seawater temperature, dissolved oxygen) there have remained very constant along millions of years. Ectotherms living in these unique environmental conditions have generated a large number of stenotic species that could be highly sensitive to environmental changes such as those expected by the end of the century (Ficke et al., 2007; IPCC, 2014). It should be noted that coastal Antarctic communities are characterized by high endemism (Hogg et al., 2011; Griffiths and Waller, 2016) and that the limited ability to move to colder latitudes as the ocean warms,

makes them potentially vulnerable to warming scenarios.

The Antarctic Peninsula has been described as one of the environments most affected by climate change in the world, which can disrupt local interactions and, thus, ecosystem functioning and stability (Duffy et al., 2017). Changes in temperature and salinity in the Southern Ocean have been described as a prominent signal of climate change. On average, surface seawater of the Western Antarctic Peninsula 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 (Szafranski and Lipski, 1982; Meredith and King, 2005; Turner et al., 2005; Haumann et al., 2016). Currently, temperatures up to 3 °C have already been reported for shallow benthic

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<https://doi.org/10.1016/j.pocean.2018.09.001>

environments in the Antarctic Peninsula (Cárdenas et al., 2018). Despite these rapid abiotic changes, the responses of Antarctic organisms and consumer-resource interactions to ocean warming and reduced salinity remain unclear.

The ability of an organism to face changes in temperature and salinity is related to its level of tolerance. Stenohaline organisms have low tolerance and can only survive in a narrow thermal range. Therein, their aerobic performance is maximal and covers all physiological costs. When the organism is exposed to either extreme of the temperature range, its capacity for aerobic performance is reduced (Pejus temperatures; Pörtner, 2002). Beyond those critical temperatures, the mitochondrial demand for oxygen cannot be met.

Stenohaline organisms also have a rather narrow range of tolerance for salinity, and survival decreases above and below the optimal range (Kinne, 1964). In terms of the relationship between temperature and salinity, a reduction in salinity can heighten the sensitivity of an organism, reducing its thermal tolerance range as well as its capacity to respond to certain levels of environmental change (Kinne, 1970).

Temperature is one of the most important environmental variables for ectotherms as it controls all biochemical reactions within the body tissues (Hochachka and Somero, 2002). Because many marine organisms live close to their thermal compensatory capacity (Somero, 2002), they are highly affected (i.e., behavioral and physiological responses) by environmental temperatures that fluctuate beyond their species-specific optimum, with effects extending to survival and ecological interactions (Pörtner, 2006; Godbold and Solan, 2013; Sandblom et al., 2014). Thus, physiological plasticity has been described as one of the main factors influencing survival in ectothermic organisms, and the capacity to acclimate has been recognized as a key process for coping (or not) with climate change drivers (Calosi et al., 2008; Peck et al., 2010).

The coastal ichthyofauna of Antarctica is highly endemic and dominated by fish belonging to the suborder Notothenioids (Andriashev, 1987). The physiological characteristics of this group of fish are related to its isolation as well as the physical characteristics of the environment (Clarke, 1983): stable low temperatures, high salinity, and seasonal changes in the ice sheet. The model species for the present study was the Antarctic notothenioid fish, *Harpagifer antarcticus*. This stenothermic species (Brodeur et al., 2003) inhabits shallow waters (0–20 m) from the Antarctic Peninsula to the South Sandwich Islands (White and Burren, 1992). *Harpagifer antarcticus* consumes mobile preys, principally gammarid amphipods, of which *Gondogeneia antarctica* is the most abundant species (Duarte and Moreno, 1981; Daniels, 1982). Because amphipods can form dense assemblages of up to 300,000 ind m⁻² (Amsler et al., 2014), they constitute an important trophic resource for Antarctic coastal fish. Thus, in order to improve our understanding of the consequences of climate change for Antarctic coastal ecosystems, we must determine whether and how climate change factors (e.g., seawater warming, salinity changes) affect the capacity of Antarctic notothenioid fish to prey on gammarid amphipods.

Here, we test the hypothesis that warmer seawater and lower salinity induced by climate change will affect predator-prey interactions 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*, leading to a reduction in its aerobic performance capacity and a concomitant decline of predation rates on the gammarid amphipod, *G. antarctica*. To test this prediction, we investigate the effects of a suite of temperature-salinity combinations (current and projected conditions in the Antarctic and Sub-Antarctic Magellan Region) on the physiological performance of *H. antarcticus* and its predation rate on *G. antarctica*. This study will help to prove why there are separate species of *Harpagifer* in the Magellan and Antarctic regions and why there could be no gene flow from south to north, even if oceanographic conditions allowed it – that is, the role of local environmental filtering in the structuring of local Antarctic and

Sub-Antarctic communities.

2. Materials and methods

2.1. Collection site and experimental design

Adult *Harpagifer antarcticus* (n = 75) were caught by turning over rocks in the lower intertidal zone off the South Shetland Islands, Fildes Bay, King George Island (62° 11' S, 58° 59' W). Fish were maintained for one week at 2 °C, 33 psu, under a natural photoperiod (period to recover from the stress of collection, in situ environmental conditions were preserved), and fed *ad libitum* with their natural diet. For this, amphipods, *Gondogeneia antarctica* (length = 6.8 ± 0.67 mm, dry weight = 0.91 ± 0.06 mg), were collected every two days.

After the recovery period, the fish were placed in individual aquaria (one fish per aquarium; 5-L volume) and exposed to different combinations of temperature (2, 5, 8, 11, 14 °C) and salinity (23, 28, 33 psu) for 10 days (n = 5 aquaria per combination). The control group corresponded to the specimens exposed to 2 °C and 33 psu and the other combinations of temperature/salinity to experimental conditions that simulated current environmental conditions of the Magellan region and possible future scenarios of climate change for Antarctica and Magallanes. Seawater was changed every other day (regardless of temperature) and maintained within a range of variation of ± 0.5 °C.

2.2. Fish mortality, ingestion, and absorption rates

All aquaria were monitored daily, and dead fish were removed each day. The number of dead individuals was pooled for a period of 10 days and expressed as accumulated mortality for each treatment combination.

Ingestion rates of *H. antarcticus* were determined every two days. Twenty new amphipods were supplied to each aquarium every day and consumed preys were determined by collecting all uneaten amphipods. To evaluate the daily ingested ration, regressions between total length (anterior margin of head to telson) of 80 individuals of each prey (L = cm) versus dry tissue weight (W = mg) were carried out using the allometric equation $W = aL^b$. Ingestion rates (mg·h⁻¹) were converted to energy units using the conversion factors 1 g dry tissue weight of the related gammarid amphipod *Corophium insidiosum* (Nair and Anger, 1979) = 15.76 J·mg dry weight⁻¹.

The absorption efficiency was determined using the Conover (1966) method. For this, we determined the organic fraction of representative samples of the faeces from *H. antarcticus* and the diet supplied (i.e., *G. antarctica*). Samples of food and faeces were filtered through pre-ashed, pre-weighed, 25-mm glass fiber filters, rinsed with isotonic ammonium formate, dried to a constant weight at 80 °C, weighed, combusted at 450 °C for 3 h, and weighed again to estimate the organic and inorganic fraction contained in the food and faeces. The absorption rate was calculated as the product of the organic ingestion rate and absorption efficiency. For each fish, ingestion and absorption rates were averaged over time (n = 5) before conducting the statistical analyses.

2.3. Oxygen consumption and scope for growth

Oxygen consumption was determined every time the rate of food consumption was measured (every third day). For this, the fish were gently transferred from the experimental aquaria to 800-mL glass respiration chambers and incubated for ca. 1 h. Oxygen uptake (routine metabolism) was measured using a Fiber Optic Oxygen Transmitter (FIBOX 3, PreSens) and oxygen sensor spots (PreSens GmbH, Regensburg, Germany) attached to the inner wall of the chambers. This process guarantees high temporal resolution and measurement without drift, oxygen consumption, or gas exchange between the incubation chamber and the environment (Warkentin et al., 2007). During each measurement, two chambers of similar volume without specimens were

used as the control (closed cell respirometry). Data were recorded using OxyView 3.51 software (PreSens GmbH). During the experiments, glass chambers were placed in a temperature-controlled water bath and the dissolved oxygen in the seawater was recorded; the oxygen concentration was not allowed to fall below 80% saturation. After the respiration measurements, the fish were weighed at nearest 0.1 g (wet weight). Considering a density of 1.34 for each fish, the volume of water in the chamber was calculated as the difference of the total volume of each chamber and the estimated volume of each fish, using its wet weight.

Measurements of the energy available for growth, termed scope for growth (SFG), provide a rapid and quantitative assessment of an organism's energy status (Widdows, 1985). SFG was calculated after converting the oxygen consumption rates and diet to energy equivalents ($\text{J}\cdot\text{h}^{-1}$): $1 \text{ mL O}_2 = 19.9 \text{ J}$ (Elliot and Davison, 1975), and $1 \text{ mg of diet} = 15.76 \text{ J}$ (Nair and Anger, 1979).

$$\text{SFG} = \text{A} - \text{R}$$

where A = energy absorbed (J d^{-1}), R = oxygen uptake ($\text{mL O}_2 \text{ d}^{-1}$). Energy lost in ammonia excretion was not determined as it represent a low fraction of the absorbed energy (Widdows, 1985).

2.4. Statistical analysis

We tested the independent and interactive effects of warming and lower salinity on each dependent variable by means of two-way analyses of variance (ANOVAs). Homogeneity of variances and normality were verified with Cochran's test and quantile-quantile plots, respectively. Each response variable was estimated as the mean of three subsamples of each experimental unit. Fish size was included in the full model as a covariate. Since the effect of fish size on each dependent variable was not statistically significant ($p > 0.05$), we selected the reduced model without covariates. In addition, we used a one-way ANOVA with 12 levels (i.e., all treatment combinations after removing the highest temperature) to corroborate that fish size was homogenous across the experimental groups. The analysis indicated that the experimental groups did not vary in terms of fish size between treatment ($F_{11, 48} = 1.42$, $P = 0.194$).

3. Results

3.1. Collection site

The temperature in the Ardley isthmus varied between 1 and 3.1°C , and salinity was constant at 33 psu during the collection of fish. However, salinity fell to 27 psu when small icebergs were present following a glacial detachment.

3.2. Fish mortality

During the experiment, we observed a drastic increment in mortality with warmer seawater, but a less clear pattern in response to lower salinity (Fig. 1). No fish died at the two lowest temperatures (2°C and 5°C) during the 10-day experiment. Only one fish (20%) died at 8°C and 28 psu. However, mortality increased at the two highest temperatures across the salinity treatments, with the deaths of 5 out of 15 individuals (33.3%) at 11°C and 14 out of 15 individuals (93.3%) at 14°C (Fig. 1). Only in the group exposed to 11°C we did detect a trend of increasing mortality due to lower salinity; the mortality rate for the 23 psu treatment was 3-fold that observed for 28 and 33 psu. Given the high number of fish that died during the first days (60% by day 2, 86% by day 5) of exposure to 14°C at all three salinities, this experimental temperature was not included in further analyses.

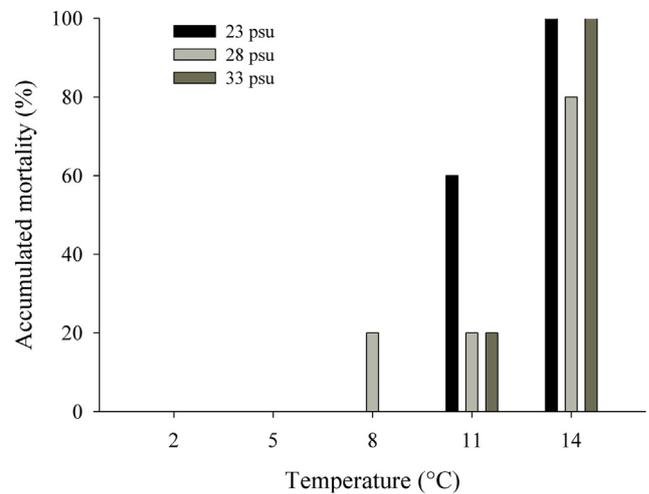


Fig. 1. Cumulative mortality measured for a period of 10 days for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity.

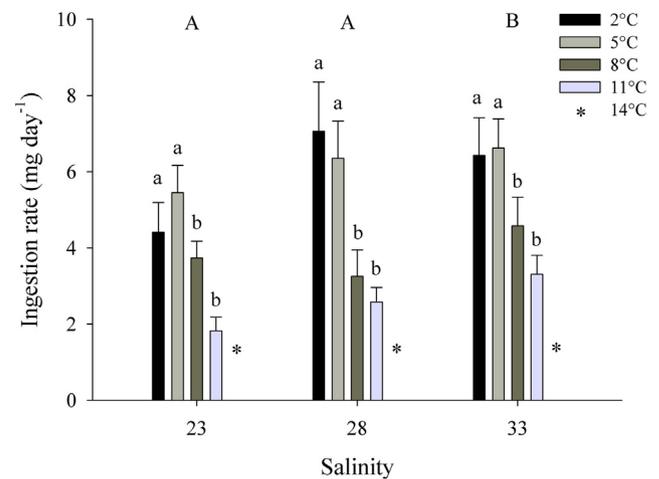


Fig. 2. Ingestion rate measured for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity. Values represent the mean \pm standard error. * No data were obtained for the combinations of 14°C and the three salinities; fish mortality was near 100%. Different upper (salinity) and lower (temperature) cases stand for statistically significant differences ($p < 0.05$) between treatments (Tukey's HSD post-hoc tests). No temperature by salinity statistically significant interaction was detected.

3.3. Ingestion and absorption rates

The ingestion rate (IR) of the fish, *H. antarcticus*, on the prey, *G. antarctica*, decreased with warming and lower salinity (Fig. 2). Individuals exposed to 11°C and 23 psu ($1.78 \pm 0.06 \text{ mg day}^{-1}$, mean \pm standard error of the mean, SEM) showed a lower mean IR than those in the control (2°C and 33 psu; $6.43 \pm 0.92 \text{ mg day}^{-1}$). Similar results were obtained for 28 and 33 psu of salinity at the extreme experimental temperatures (Fig. 2). These responses indicated that IR was significantly affected by temperature and salinity but not by their interaction (Table 1).

Absorption efficiency (AE) fluctuated between 60 and 80%, independently of temperature and salinity treatments (Fig. 3). Accordingly, AE was not affected by temperature, salinity, or their interaction (Table 1). In contrast, absorption rate (AR) decreased linearly with increasing temperature and decreasing salinity (Fig. 4). The analysis of variance supported this pattern as both factors, but not their interaction, had statistically significant effects on AR (Table 1).

Table 1
Two-way ANOVA for the different physiological variables measured in *Harpagifer antarcticus*. Significant P values are in *italic*.

Physiological rate	d.f.	MS	F value	P value
<i>Ingestion rate</i>				
Temperature	3	0.29	17.83	<i>0.001</i>
Salinity	2	0.06	3.80	<i>0.029</i>
Temperature * salinity	6	0.01	0.77	0.601
Error	48	0.02		
<i>Absorption efficiency</i>				
Temperature	3	449.52	2.22	0.098
Salinity	2	76.60	0.38	0.687
Temperature * salinity	6	258.82	1.28	0.284
Error	48	202.19		
<i>Absorption rate</i>				
Temperature	3	0.23	18.70	<i>0.001</i>
Salinity	2	0.06	5.13	<i>0.010</i>
Temperature * salinity	6	0.01	1.15	0.350
Error	48	0.01		
<i>Oxygen uptake</i>				
Temperature	3	0.14	25.03	<i>0.001</i>
Salinity	2	0.01	1.73	0.189
Temperature * salinity	6	0.02	2.85	<i>0.019</i>
Error	48	0.01		
<i>Scope for growth</i>				
Temperature	3	10933.17	12.63	<i>0.001</i>
Salinity	2	1707.44	1.97	0.150
Temperature * salinity	6	1819.79	2.10	0.070
Error	48	865.76		

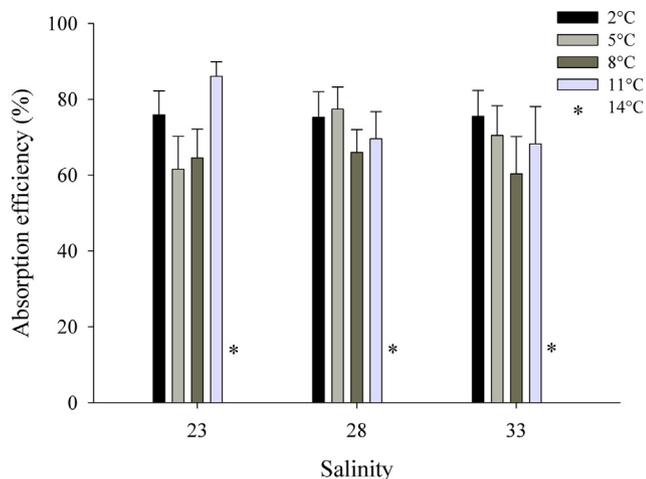


Fig. 3. Absorption efficiency measured for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity. Values represent the mean \pm standard error. * No data were obtained for the combinations of 14 °C and the three salinities; fish mortality was near 100%. No statistically significant effect of temperature, salinity, or interaction was detected.

3.4. Oxygen consumption and scope for growth

Oxygen uptake (VO_2) more than doubled as temperatures increased from 2 to 11 °C (Fig. 5; $1.78 \pm 0.25 \text{ mL d}^{-1}$ at 2 °C and 33 psu to $5.83 \pm 0.87 \text{ mL d}^{-1}$ at 11 °C and 33 psu). This response to temperature was notably stronger in the group exposed to 33 psu (Fig. 5), which led to a statistically significant interactive effect of temperature and salinity on VO_2 (Table 1). In addition, we observed a statistically significant effect of temperature on VO_2 , but no effect of salinity (Table 1).

Scope for growth (SFG) showed a strong response to the temperature treatment (Fig. 6). Positive SFG values were obtained only at the two lowest temperatures (2 and 5 °C), and large negative SFG were observed at all other experimental temperatures. Thus, in general, this

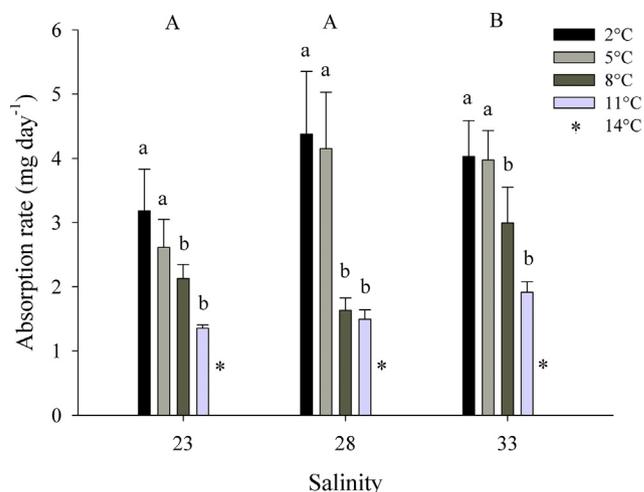


Fig. 4. Absorption rate measured for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity. Values represent the mean \pm standard error. * No data were obtained for the combinations of 14 °C and the three salinities; fish mortality was near 100%. Different upper (salinity) and lower (temperature) cases stand for statistically significant differences ($p < 0.05$) between treatments (Tukey's HSD post-hoc tests). No temperature by salinity statistically significant interaction was detected.

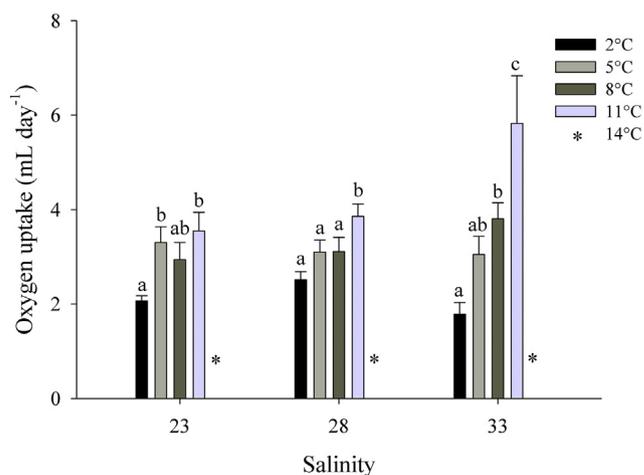


Fig. 5. Oxygen uptake measured for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity. Values represent the mean \pm standard error. * No data were obtained for the combinations of 14 °C and the three salinities; fish mortality was near 100%. Different lowercase letters stand for significant differences ($p < 0.05$) between treatments (Tukey's HSD post-hoc tests). Post-hoc analyses were conducted between temperature treatments and within each level of salinity after a statistically significant interaction between both factors was detected.

factor had a statistically significant effect on SFG (Table 1). Although salinity did not have a statistically significant effect on SFG, negative values were observed when individuals were exposed to the lowest salinity (23 psu), independently of the temperature (Fig. 6). Albeit the interaction resulted to be statistically non-significant, we observed a strong tendency of temperature to affect SFG in the 23 psu level of salinity (Fig. 6).

4. Discussion

The results of our study showed significant and independent effects of seawater warming and falling salinity on an endemic species of Antarctic coastal communities. On one hand, both factors significantly and linearly decreased the rate by which the notothenioid fish,

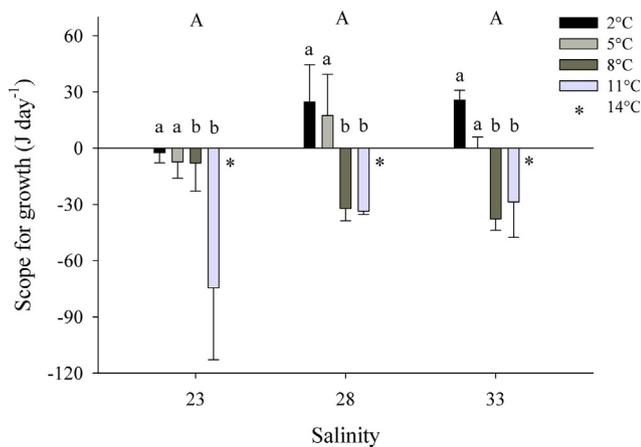


Fig. 6. Scope for growth measured for the fish, *Harpagifer antarcticus*, exposed to different combinations of temperature and salinity. Values represent the mean \pm standard error. * No data were obtained for the combinations of 14 °C and the three salinities; fish mortality was near 100%. Different upper (salinity) and lower (temperature) cases stand for significant differences ($p < 0.05$) between treatments (Tukey's HSD post-hoc tests). No temperature by salinity statistically significant interaction was detected.

Harpagifer antarcticus, preyed on the gammarid amphipod, *Gondogeneia antarctica*. These effects were accompanied by concomitant decreases in absorption rates of the predator, hinting at severe physiological consequences for this species. On the other hand, only oxygen uptake showed a systematic response to the interactive effect of warming and lower salinity. This reinforces the previous observation of independent effects of both climate change stressors. Finally, scope for growth (i.e., the energy available for growth and reproduction) responded significantly to warming, suggesting that this factor had an overriding effect on *H. antarcticus* relative to that of decreased salinity. However, the temperature by salinity interaction showed a significant marginal effect on the scope for growth, suggesting that salinity also has an impact on the warming-related reduced ingestion rate of *H. antarcticus*. In the following, we discuss the physiological mechanisms by which sub-lethal effects of warming and salinity changes can significantly impair the ability of this model species to cope with current temperatures and salinities conditions and projected climate change scenarios.

Our results showed that the ingestion rate of *H. antarcticus* significantly decreased with warming and decreasing salinity, in agreement with the stenothermal nature of Antarctic marine ectotherms (Peck and Conway, 2000). Several fish species live within a narrow temperature window, between -1.86 °C and $+4$ °C (Eastman, 1993; Somero et al., 1998). Targett et al. (1987), working during wintertime, described high daily *ad libitum* feeding rates for *H. antarcticus* at temperatures of 0 and -1.7 °C. Other studies with Antarctic ectotherms have also reported that, when temperature variations are within the natural range, the values of physiological processes remain similar; however, when the temperature increases over that range unexpected responses (increases or decreases) can occur (Peck, 2002). The question remains of what the functional consequences are when the prey is affected by temperature and salinity. Despite this shortcoming, our work indicates that, even when the effects of warming on predator physiology are sub-lethal, small increments in temperature can cause strong effects on the trophic structure of, at least, an important part of the marine coastal community.

Our results show that warming had an overriding effect on *H. antarcticus* relative to that of falling salinity. At the lowest experimental temperatures (2 and 5 °C), no mortality occurred at any of the experimental salinities. This can be explained by the summer temperatures experienced by this species in their collection habitat, which reached values of 3.0 – 3.5 °C (personal observation, January 2017). At the same time, salinity dropped from 33 to 27 psu due to a glacial detachment,

which left numerous small icebergs floating at the site inhabited by *H. antarcticus* for several days.

Exposure of the fish to higher temperatures had the opposite effect: the fish began to die at 8 °C, reaching 100% mortality at the highest experimental temperature (14 °C) and at 23 and 33 psu. Slightly lower mortality (93%) was observed at 28 psu and 14 °C. According to these results, a moderate increase of the temperature in this region of the Antarctic could be tolerated by this species. However, during the present study, the fish were exposed to the experimental conditions for only 10 days, and there may be a more drastic effect when exposing this species to the same experimental conditions for a longer period of time.

Antarctic marine ectotherms are characterized by their low acclimation capacities. Indeed, Polar stenotherm organisms may require particularly long periods (i.e. months) to achieve the complete acclimation (Peck et al., 2010). *Harpagifer antarcticus* was kept under controlled conditions (2 °C and 33 psu) for one week before the experiment, which corresponds to a period of recovery after the stress of the collection. The present study did not include a gradual acclimation and the results described the acute response of *H. antarcticus* to a wide range of temperature and salinity. Peck et al (2009) demonstrated that rate of warming has a marked effect on the upper temperature limit reached by Antarctic marine species. Thus, a gradual acclimatization to the experimental temperatures and salinities used could result in a less drastic effect, depending on the physiological plasticity of this species. Despite this limitation, our study is one of the first studies reporting the combined effects of ocean warming and multiple salinities for predation rates and the physiology of Antarctic organisms (see Gomes et al., 2013 for an example of absorption efficiency).

In our study, exposure of *H. antarcticus* to different temperature/salinity combinations resulted in a lower feeding rate but had no effect on the absorption efficiency. Boyce et al. (2000) studied the digestion rate and absorption efficiency of *Harpagifer antarcticus* collected near Rothera Point, Adelaide Island, Antarctica – the authors used amphipods, the most abundant component of the natural diet of this fish (Duarte and Moreno, 1981) as preys in those experiments. Boyce et al. (2000) determined the absorption efficiency by the Conover ratio method, with results that varied between 63 and 80%. These are very similar to the values described in the present study using the same method (60–86%). Other studies on marine mollusks have also described the capacity of the digestive system to process food within a wide range of temperatures and salinities (Winter et al., 1984; Navarro et al., 2016). Thus, the experimental temperatures and salinities experienced by *H. antarcticus* did not affect the absorption efficiency, suggesting that this physiological process is independent of these environmental factors along the range tested. As temperature and salinity did not affect the absorption efficiency of *H. antarcticus*, the absorption rate followed the same pattern as the food ingested: higher temperatures and lower salinity had significant negative effects.

As expected, the oxygen uptake of *H. antarcticus* (routine metabolism) increased with increasing temperatures. Although the salinity factor alone did not have an effect on oxygen uptake, the temperature/salinity interaction did, and a metabolic depression was observed when this species was exposed to combinations of lower salinities/higher temperatures. Similar interactions have been described in some invertebrates. The Antarctic crustacean, *Serolis polita*, was more vulnerable to lower salinities when exposed to higher temperatures, affecting the time spent swimming (Janecki et al., 2010). The salinity tolerance of the copepod, *Gladioferens pectinatus*, decreased at higher temperatures, affecting survival and egg production (Hall and Burns, 2002).

The significant increase in energy spent by *H. antarcticus* at the higher experimental temperatures (8 and 11 °C) suggests that under current and future warming scenarios (mainly for the Magellan Region), the energy budget of this species will experience a negative impact. In the hypothetical case that its geographical distribution could reach the Magellan Region, the energy absorbed might not be enough to supply the basic metabolic requirements of this fish. The different

physiological processes related to energy gain and expenditure largely depend on an organism's environmental temperature and salinity (Bayne and Newell, 1983). Scope for growth represents the integration of all these processes varying as a function of environmental factors.

Animals that can maintain their aerobic capacity at warmer temperatures have a higher thermal tolerance and will be able to grow and reproduce as the temperature increases (Pörtner, 2001; Sandersfeld et al., 2017). However, growth can be reduced under conditions of low salinity due to osmoregulatory limitations that can negatively impact the feeding rate and efficient use of the ingested material (Kinne, 1964; Navarro and Gonzalez, 1998; Vargas-Chacoff et al., 2016). Sandersfeld et al. (2015) studied the effect of elevated temperature on the energy budget of the high-Antarctic notothenoid fish, *Trematomus bernacchii*, from the Ross Sea around McMurdo Sound. These authors described a reduction in mass growth up to 84% after long-term acclimation to 2 and 4 °C compared with the control group at 0 °C. This difference was explained by reduced food assimilation rates at warmer temperatures and suggests a decrease in production of a similar magnitude for this species according to the growth estimates of Hureau (1970). Brodte et al. (2006) described maximum scope for growth of the Antarctic eelpout *Pachycara brachycephalum* at 4 °C, when exposed to a temperature range of 0 to 6 °C. This may appear to be relatively high for an Antarctic species, but the authors explain that these results may reflect the deep-sea origin of the genus *Pachycara* (Anderson, 1984).

The present study showed a negative effect of temperature but not of salinity or the interaction of the two on the scope for growth of *H. antarcticus*. The higher values of SFG observed at 2 °C coincide with the lower metabolic rates measured in *H. antarcticus*. These results are in line with the studies of Frederich and Pörtner (2000) and Pörtner (2001), which conclude that the thermal range where positive growth occurs corresponds to the tolerance window set by oxygen limitations.

The 8 and 11 °C treatments represented the warmest conditions reported for the Magellan Region (González et al., 2016; personal observation), and 14 °C reflected the possible future scenario of climate change at the end of the century in this region. The higher metabolic rates observed at 8 and 11 °C are associated with conditions beyond the natural thermal window of this species and represent a disadvantage for this Antarctic fish when facing the challenge of climate change. Should *H. antarcticus* reach the geographic zone of Magellan, this species would not be able to tolerate the region's current temperatures and would be less able to tolerate the future warming scenarios to which they may be exposed as a result of climate change.

Lower salinity can also be an important factor to consider in the Magellan Region due to “freshening” caused by the higher glacial melting rate as result of the warming conditions. Although salinity did not have a significant effect on scope for growth, this index was always negative in fish exposed to the lowest salinity (23 psu). The intermediate salinity (28 psu) is not critical at the lower temperatures, which can be explained by the experience these fish have with being exposed to salinities of 27 psu (personal observation) during the presence of icebergs after the release of glaciers. Antarctic ectothermic organisms are defined as stenothermic and highly sensitive to warming (Pörtner, 2006). The present study is in agreement with these authors, and our results confirm the stenothermal nature of the notothenoid fish *H. antarcticus*, considering its high vulnerability to environmental changes and limited ability to cope with global warming models projected for the end of the century. Finally, and despite the fact that amphipods are very abundant in Antarctic coastal habitats, future scenarios of temperature and salinity could also affect amphipod assemblages, increasing the deleterious effects of climate change on coastal fish in these ecosystems.

In summary, our results suggest that seawater warming and decreasing salinity can have significant, but independent, effects on the physiological energetics of the endemic Antarctic notothenoid fish, *H. antarcticus*. Moreover, the results indicate that projected climate change scenarios can be even more deleterious should this species be

introduced into sub-Antarctic regions. The combined effects of warming and decreasing salinity on the physiology and predation rates of Antarctic coastal organisms have rarely been assessed. With this study, we hope to shed light and stimulate further experimental research on the physiological and ecological consequences that climate change has on marine organisms.

Acknowledgements

Thanks to the Instituto Antártico Chileno (INACH) for their logistic support during the experiments at the Base Profesor Julio Escudero, Antártica, Chile. Funding was provided by Center FONDAP – IDEAL, CONICYT – CHILE (Grant 15150003). While writing JMN was financially supported by FONDECYT grant 1161420 and NV by FONDECYT grants 1181300 and 1161699.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.poccean.2018.09.001>.

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