Ocean freshening and acidification differentially influence mortality and behavior of the Antarctic amphipod *Gondogeneia antarctica*

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ABSTRACT

The Western Antarctic Peninsula (WAP) has experienced rapid atmospheric and ocean warming over the past few decades and many marine-terminating glaciers have considerably retreated. Glacial retreat is accompanied by fresh meltwater intrusion, which may result in the freshening and acidification of coastal waters. Marian Cove (MC), on King George Island in the WAP, undergoes one of the highest rates of glacial retreat. Intertidal and shallow subtidal waters are likely more susceptible to these processes, and sensitive biological responses are expected from the organisms inhabiting this area. The gammarid amphipod *Gondogeneia antarctica* is one of the most abundant species in the shallow, nearshore Antarctic waters, and it occupies an essential ecological niche in the coastal marine WAP ecosystem. In this study, we tested the sensitivity of *G. antarctica* to lowered salinity and pH by meltwater intrusion following glacial retreat. We exposed *G. antarctica* to four different treatments combining two salinities (34 and 27 psu) and pH (8.0 and 7.6) levels for 26 days. Mortality, excluding cannibalized individuals, increased under low pH but decreased under low salinity conditions. Meanwhile, low salinity increased cannibalism, whereas low pH reduced food detection. Shelter use during the daytime decreased under each low salinity and pH condition, indicating that the two stressors act as disruptors of amphipod behavior. Under low salinity conditions, swimming increased during the daytime but decreased at night. Although interactions between low salinity and low pH were not observed during the experiment, the results suggest that each stressor, likely induced by glacial melting, causes altered behaviors in amphipods. These environmental factors may threaten population persistence in Marian Cove and possibly other similar glacial embayments.

1. Introduction

The Western Antarctic Peninsula (WAP) has undergone rapid oceanic and atmospheric warming since the end of the twentieth century (Jones et al., 2017). Many grounded marine-terminating glaciers of the WAP have retreated considerably over the past several decades (Cook et al., 2016). Floating ice shelf thinning may also trigger acceleration in retreat and increase the discharge of freshwater from marine-terminating glaciers (Shepherd et al., 2018), both of which are predicted to influence long-term freshening of surface water in the Southern Ocean (de Lavergne et al., 2014; Goddard et al., 2017; Purich et al., 2018). Oceanic water pH levels have decreased since the late 18th century because of the absorption of atmospheric CO_2 produced by

human activities, and it is predicted to decrease by an additional 0.3–0.4 units by 2100 (Orr et al., 2005; Sabine et al., 2004; Yamamoto-Kawai et al., 2009). Although the WAP coastal waters fringed with marine-terminating glaciers experience increased carbonate mineral saturation owing to strong primary production from freshwater intrusion during the austral summer, the subsurface water in the region has a low pH level through the remineralization of organic matter (Hauri et al., 2015; Jones et al., 2017). The vulnerability of Southern Ocean coastal and surface waters to acidification is predicted to be influenced by freshwater input from glacial melts, low temperatures that promote the uptake of CO_2, and strong winds that promote both the pumping of relatively acidic deep water up to the surface and episodic mixing events (Jones et al., 2017; Takahashi et al., 2014).

Marian Cove (MC) is one of the tributary embayments of Maxwell Bay off King George Island in the WAP (Ahn et al., 2016). In MC, tide-water glaciers have retreated approximately 1.9 km from 1956 to 2017 (Ha et al., 2019; Moon et al., 2015). Glacial meltwater has resulted in distinct gradients in temperature, salinity, and suspended particles in the MC water column during the austral summer (Ahn et al., 2004; Kim et al., 2015; Moon et al., 2015; Yoo et al., 2015). Inflows of freshwater also occur as streams and rapids from the surrounding terrigenous snowfields, although the relative contributions of these different fresh-water sources are yet to be determined. These terrigenous sources of meltwater have been found to have very low pH levels during the austral summer (personal observations from Dec 2017 to Feb 2018 showed pH of 6.45 ± 1.09 [mean ± SD]; Lim et al., 2014).

Freshening and acidification of the Antarctic coastal waters following the retreat of marine-terminating glaciers over the past several decades could affect the individual performance of marine organisms, thereby resulting in ecological shifts (Cowart et al., 2009; Harley et al., 2006). Although there are a large number of studies on the behavioral or physiological responses of marine organisms to ocean freshening (Aarset and Aunaas, 1987; Cowart et al., 2009; Fanini et al., 2012; Wolcott and Wolcott, 2001) and acidification (Jakubowska and Normant-Saremba, 2016; Karelitz et al., 2017; Kim et al., 2016; Lee and Kim, 2017), studies on the effects of both stressors concurrently and the effect on polar marine invertebrates are less common (Dickinson et al., 2013; Manno et al., 2012). The mortality and swimming patterns of the pteropod *Limacina retroversa*, captured in the northern Norwegian Sea, was influenced by the combined effects of low salinity and pH (Manno et al., 2012). Salinity may also be an important factor in regulating the effects of acidification on estuarine and coastal organisms, such as *Mercenaria mercenaria*, a hard-shell clam species in the western Atlantic (Dickinson et al., 2013).

In this study, we investigated the effects of ocean freshening and acidification on *Gondogeneia antarctica*, one of the most abundant amphipod species of the WAP, that is widely distributed throughout the intertidal and shallow subtidal waters of the region (Amsler et al., 2005; Doyle et al., 2012). Similar to other amphipod species, *G. antarctica* feeds on algae, small crustaceans, and organic detritus, and it is a food source for fish and macroinvertebrates (Amsler et al., 2009; Aumack et al., 2011; Dauby et al., 2001; De Broyer et al., 2001; Jażdżewski et al., 2000; Nyssen et al., 2005). Thus, it is a key component of the Antarctic coastal food web. Moreover, *G. antarctica* is a suitable species for incubation experiments because of its tolerance in captivity (Gomes et al., 2009, 2013). A few studies have been conducted to determine the effects of combined climatic stressors on *G. antarctica*; low salinity amplified the negative effects of warming on the metabolism of *G. antarctica* (Gomes et al., 2013), and survival was more severely affected by low pH than by high temperatures (Schram et al., 2016).

Here, we measured the mortality, cannibalism, and molting of *G. antarctica*, to evaluate the effects of ocean freshening and acidification. Furthermore, we repeatedly observed *G. antarctica* food detection, shelter use, and swimming during exposure to low salinity and pH conditions. These behaviors are indicators of energy intake, escape, and hiding from predators, as well as important predictors of individual survival and population persistence (Nagelkerken and Munday, 2016; Vellinger et al., 2012). We hypothesized that low salinity or low pH will have negative effects on amphipod mortality, as well as their behavioral and physiological patterns. We also predicted that the combination of low salinity and low pH will have a more severe effect than either single stressor (Fabry et al., 2008; Whiteley, 2011).

2. Materials and methods

2.1. Collection and maintenance of G. antarctica

We collected more than 200 *G. antarctica* individuals on January 7 and 8, 2018 from the intertidal zone in front of King Sejong Station

(62.22° S 58.79° W) in MC. At low tide, the amphipods were caught using a handheld net (1 mm mesh size) from tidal pools and placed in plastic jars half-filled with seawater from the collection sites. Within 20 min of capture, the jars were immersed in an outdoor flow-through aquarium, where they were kept from January 7 to 10, with seawater continuously pumped from MC. On January 10, five actively swimming amphipods of similar sizes (length: 5.05 ± 0.24 mm) were randomly allocated to thirty-six 500 mL beakers in four groups (n = 9, 180 individuals in total). The amphipods were then preconditioned in a control treatment (34 psu, pH 8.0) for 7 days. Dead or unhealthy amphipods were immediately replaced with healthy individuals during this period. Before exposure to the different treatments, all individuals in the four groups were observed for molting and assumed to be of the same molt state (one-way ANOVA, $F_{3,32} = 0.056$, $p = 0.982$).

Each beaker contained a shelter and 250 mL of treatment seawater. The shelter, made of a PVC pipe that was cut in half (3 × 4 × 1.5 cm), was provided as a hiding and resting place for the amphipods. The 36 beakers were divided into two groups, and each group of 18 beakers was placed in a water bath (80 × 45 × 20 cm). In the water baths, seawater temperature was maintained at 1.3 ± 0.3 °C under a 19:5 h light:dark cycle, with LED lights simulating the diurnal cycle of austral summer. To avoid unknown bias, some beakers were randomly moved between aquariums every day. The amphipods were fed for 2 h once per week with solid round-shaped food (The Aqua Crustacea®; Aqua plus Inc., Republic of Korea), to perform the food detection experiments (detailed below).

2.2. Salinity and pH treatments

The experiment was conducted for 26 days (Jan 17 to Feb 11). The salinity and pH values for the control (34 psu and pH_NBS 8.0) and the treatment (27 psu and pH_NBS 7.6) were determined from in-situ measurement of the ambient seawater in MC (Fig. 1) and predicted future values in the Southern Ocean owing to climate change (Kapsenberg et al., 2015). To determine the different salinity treatments, we partially froze the ambient seawater, filtered it with 0.2 and 0.07 μm filters into clean plastic containers, and separated the ice and unfrozen seawater. In this way, we obtained both high- (unfrozen part) and low-salinity water
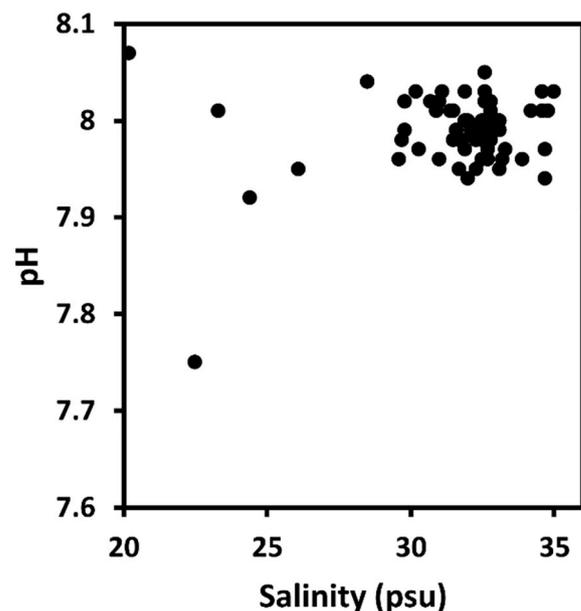

Fig. 1. Salinity and pH data from a station located in the coastal area of Marian Cove, Maxwell Bay, King George Island. The parameters were measured daily at low and high tide, January 1 to February 10, 2018, using a YSI Pro30 and InLab Expert Go-ISM.

from the ice at the same time. After the low-salinity ice melted, it was mixed with the high-salinity water to create different salinities (34 and 27 psu). The completed treatments were stored in four LDPE (Low Density Polyethylene) reservoirs (20 L) located outside (at low ambient temperatures) and completely used and refilled within 2 days.

The pH treatments (pH 8.0 and 7.6) were performed by dissolving CO_2 tablets (Sera CO_2-Tabs plus®; Sera Inc., Germany) into the different saline seawater treatments before performing seawater exchanges to maintain the pH levels under stable conditions relative to salinity. One-quarter of the CO_2 tablet was dissolved in conical tubes (50 mL) filled with water of differing salinities to generate extremely acidic water. Then, minute amounts of the acidic water were precisely dropped into the different salinity treatments and stirred to obtain the target pH levels. The treated seawater was directly utilized to replenish the water in the replicates, and thus, all individuals were exposed to their designated treatment at the same time. The salinity and pH_{NBS} levels of the treatment water in the four reservoirs were measured using a YSI Pro 30 (Yellow Spring Instruments Inc., USA). Furthermore, we randomly chose two replicates per treatment to measure the pH and temperature before and after the daily replenishing processes, by using InLab Expert Go-ISM (Mettler-Toledo International Inc., Germany) with a pH resolution of 0.01, calibrated once in 2 weeks (Supplemental table 1). Dissolved oxygen (DO) in each replicate was measured using a DO meter YSI 5000 connected to YSI 5010 BOD probe (Yellow Spring Instruments Inc., USA) but only before the seawater treatment exchanges because of the self-stirring process of the probe. During the experimental period, we sampled and fixed the seawater in each treatment with $HgCl_2$ in a DO bottle to determine the total alkalinity (TA); this was replicated three times (Table 1). To calculate the seawater carbonate chemistry variables with the CO_2SYS Calc XLS program (v2.1) (Pierrot et al., 2006), we used the aforementioned daily measurements of temperature, salinity, and pH values, and the TA values from the fixed seawater samples, with the CO_2 dissociation constants (Dickson and Millero, 1987; Mehrbach et al., 1973) and the KSO_4 source (Dickson, 1990). We used the mixed water from all replicates of each treatment that was removed during the replenishing process of the TA analysis. There was no significant difference in TA between the control and low pH treatments (paired t-test, $t_2 = -1.054$, $p = 0.402$); therefore, we concluded that CO_2 tablets did not significantly affect TA. We also confirmed no significant difference in TA between the low salinity and combined treatments ($t_2 = 0.237$, $p = 0.835$, Table 1). To maintain the salinity and pH within the nominal limits and thereby minimize the stress on *G. antarctica*, 150 mL out of 250 mL of seawater was exchanged every day. During the water exchange, feces at the bottom of the beakers was removed. We also replenished the total amount of water in the beakers every week after feeding.

2.3. Mortality, cannibalism, and molting

During the experimental period, the mortality, proportion of cannibalized individuals, and cumulative molting frequency of the amphipods were recorded for each replicate. The status of each individual (alive or dead) was determined by gently prodding the inactive amphipods with a plastic pipette. Molts were collected at least twice per day. When an individual disappeared, or its body was missing, it was regarded as cannibalized. To distinguish between death caused by treatment stress vs. cannibalism, we calculated mortality, excluding cannibalized individuals, by subtracting the proportion of cannibalized individuals from the total mortality. Individuals that appeared inactive and nearly dead were rarely observed in the low salinity treatment, where cannibalism was clearly evident (see the Results and Fig. 2 below). Therefore, there is only a low possibility that "cannibalized" individuals were actually consumed after they had succumbed to the treatment.

2.4. Food detection

The food detection experiments were performed four times in all replicates, during the day and with approximately 1-week intervals between trials. A piece of crustacean food (The Aqua Crustacea®; Aqua plus Inc., Republic of Korea) was supplied simultaneously using tweezers to a random spot in each beaker. Every 10 min, the number of individuals on the surface of the food was recorded; this was performed 12 times. When the last measurement was completed 2 h after food provision, all of the remaining food was removed, and the seawater was exchanged.

2.5. Swimming and shelter-use behavior

G. antarctica is a nocturnal species that shelters in the daytime and forages at night (Aumack et al., 2011). To assess the activities of *G. antarctica* during the day and night, observations and recordings were performed by the same researcher each day and every third or fourth night (detailed below). The number of individuals performing each behavior was recorded and all beakers were observed in less than 8 min. The behaviors were categorized into the following 3 indices: (1) using the shelter, defined by staying on the outer or internal part of the shelter, (2) swimming, or (3) staying at the bottom of the beaker and/or away from the shelter. *G. antarctica* have been shown to prefer to shelter on unpalatable (chemically defended) macroalgae to protect themselves from visual predators during the day (Aumack et al., 2011; Zamzow et al., 2010). Such behaviors in animals are closely related to the balance between survival and growth (Nagelkerken and Munday, 2016); therefore, amphipods must migrate for food and shelter in nature.
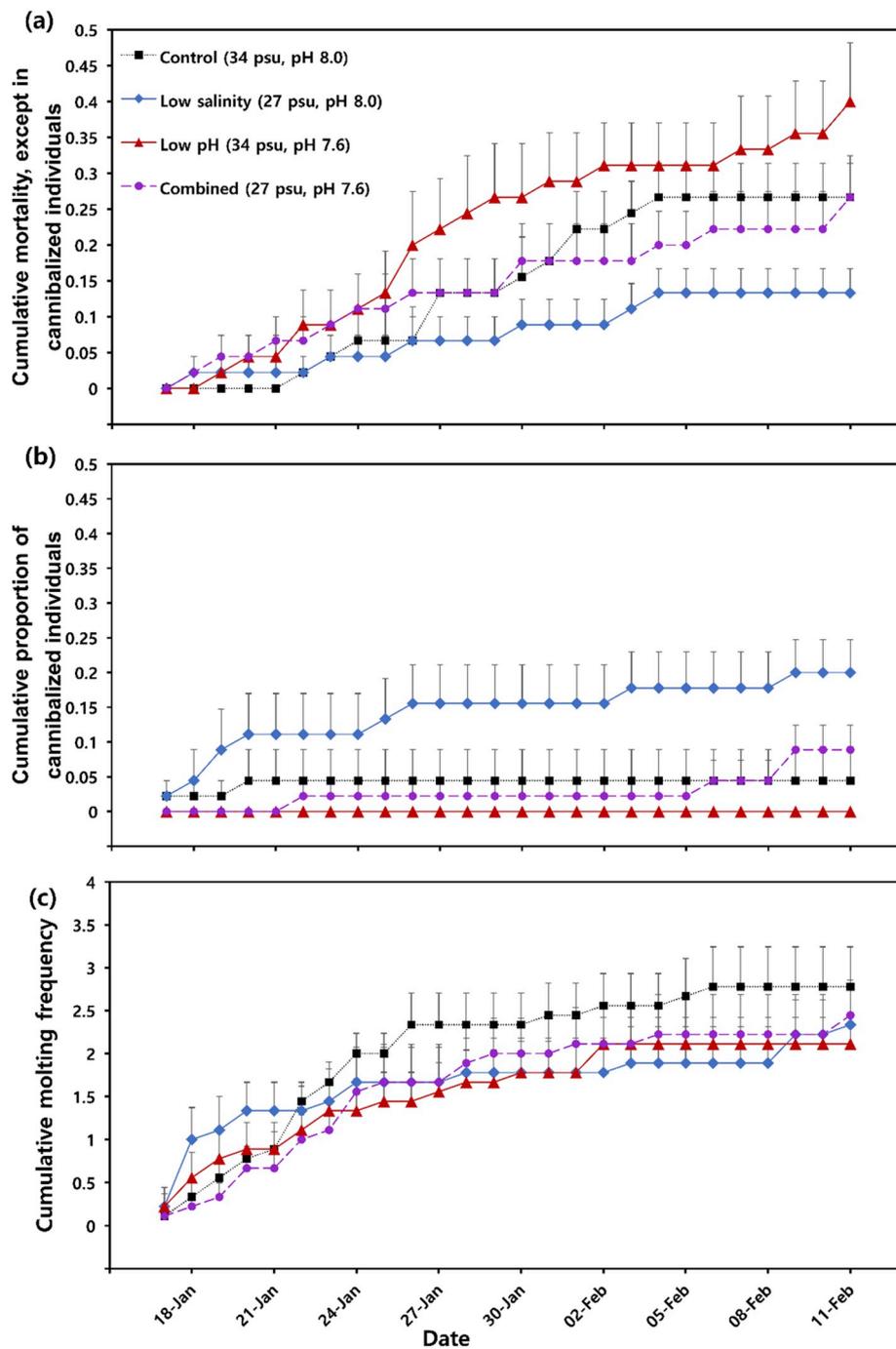
Table 1

Seawater chemistry values (mean ± SD) of the four experimental treatments. Salinity, pH, and temperature were measured daily, and dissolved oxygen (DO) was measured once every 2–3 days. Total alkalinity (TA), partial pressure of carbon dioxide (pCO_2), total inorganic carbon or dissolved inorganic carbon (DIC), calcite saturation, and aragonite saturation were calculated using the CO_2SYS Calc XLS program (v2.1) (Pierrot et al., 2006) from the seawater samples that were fixed during the experimental period.

	Control	Low salinity	Low pH	Combined
Salinity (psu)	34.00 ± 0.05	27.01 ± 0.03	34.02 ± 0.05	27.03 ± 0.05
pH (NBS scale)	8.00 ± 0.07	7.97 ± 0.07	7.64 ± 0.13	7.6 ± 0.21
Temperature (°C)	1.4 ± 0.65	1.28 ± 0.68	1.27 ± 0.68	1.42 ± 0.69
DO (mg l^{-1})	10.81 ± 1.16	10.85 ± 1.12	9.69 ± 1.16	9.44 ± 1.27
TA (mmol kg^{-1} SW)	2512.13 ± 138.75	2155.41 ± 232.58	2618.56 ± 240.02	2132.8 ± 235
pCO_2 (µatm)	593.03 ± 50.47	567.01 ± 54.89	1616.63 ± 217.36	1488.11 ± 238
$[CO_2]$ (µmol kg^{-1} SW)	34.40 ± 2.52	33.42 ± 2.16	92.89 ± 15	88.23 ± 17.74
$[HCO_3^-]$ (µmol kg^{-1} SW)	2306.36 ± 132.61	2008.86 ± 212.73	2526.54 ± 239.37	2072.61 ± 234.41
$[CO_3^{2-}]$ (µmol kg^{-1} SW)	82.62 ± 3.43	58.78 ± 9.52	36.98 ± 1.31	23.79 ± 1.9
TCO_2 (µmol kg^{-1} SW)	2423.39 ± 138.49	2101.06 ± 224.15	2656.41 ± 255.07	2184.64 ± 251.88
Calcite saturation	1.98 ± 0.08	1.46 ± 0.24	0.89 ± 0.03	0.59 ± 0.05
Aragonite saturation	1.25 ± 0.05	0.9 ± 0.15	0.56 ± 0.02	0.36 ± 0.03

Fig. 2. The cumulative (a) mortality, excluding cannibalized individuals; (b) proportion of cannibalized individuals; and (c) molting frequency of *Gondogeneia antarctica* during exposure to the different salinities and pH levels. Data are expressed as means ± SE.

Table 2

Results of two-way ANOVA for the effects of low salinity and low pH on mortality, cannibalism, and molting of *G. antarctica*. ↑ or ↓ indicates increase or decrease in each observed parameter, respectively.

	Mortality including cannibalized individuals	Mortality excluding cannibalized individuals	Cannibalism	Molting
Between-subject effect				
Salinity	$F_{1,32} = 0.015$ $p = 0.905$	$F_{1,32} = 5.336$ $p = 0.027↓$	$F_{1,32} = 15.025$ $p < 0.0001↑$	$F_{1,32} = 0.019$ $p = 0.891$
pH	$F_{1,32} = 0.501$ $p = 0.484$	$F_{1,32} = 5.210$ $p = 0.029↑$	$F_{1,32} = 4.037$ $p = 0.053$	$F_{1,32} = 0.476$ $p = 0.495$
Salinity × pH	$F_{1,32} = 0.505$ $p = 0.483$	$F_{1,32} = 0.044$ $p = 0.835$	$F_{1,32} = 0.840$ $p = 0.366$	$F_{1,32} = 0.933$ $p = 0.341$

Table 3

Results of repeated measures two-way ANOVA for the effects of low salinity and low pH on the food detection of *G. antarctica*. ↑ or ↓ indicates increase or decrease in each observed item, respectively.

	Food detection
Within-subject effect	
Time	$F_{3,96} = 15.602$ $p < 0.0001$
Time × Salinity	$F_{3,96} = 0.630$ $p = 0.597$
Time × pH	$F_{3,96} = 5.103$ $p = 0.003↓$
Time × Salinity × pH	$F_{3,96} = 1.041$ $p = 0.378$
Between-subject effect	
Salinity	$F_{1,32} = 1.006$ $p = 0.323$
pH	$F_{1,32} = 4.853$ $p = 0.035↓$
Salinity × pH	$F_{1,32} = 1.238$ $p = 0.274$

At night, a weak light (a smartphone flashlight covered by 3 mm thick wipers [WYPALL® L25 wiper; Yuhan-Kimberly, Republic of Korea]) was used to observe amphipod behavior; this did not seem to alter their behavior. Nighttime measurements were performed on 11 haphazardly selected days from Jan 18 to Feb 11.

2.6. Statistical analysis

Arcsine square root transformations were performed on all proportional data. We used a two-way ANOVA to determine whether salinity, pH, or their interaction influenced cumulative mortality, cannibalism, and molting frequency. To determine the effects of exposure time, as well as salinity and pH, a repeated measures two-way ANOVA was conducted on the proportion of individuals that detected food or were observed swimming and using their shelter. When sphericity (equality of the variance of the data among the experimental exposure days) was violated (Mauchly's test, $p < 0.05$), we used Huynh–Feldt corrections. When there was a significant within-subject (interaction with time) effect, we analyzed data on each day with two-way ANOVA for the post-hoc test.

3. Results

The results of the analyses are summarized in Tables 2–5. Together, they demonstrate the different effects of low salinity and low pH on the mortality and behaviors of *G. antarctica*. Detailed information on the post-hoc tests of the repeated measures is presented in Figs. 3–5. All of the experimental results consist of four treatments, with nine replicates of five amphipods each.

3.1. Mortality, cannibalism, and molting

Mortality, including cannibalized individuals, was not influenced by low salinity or low pH. However, mortality excluding the cannibalized individuals significantly decreased under low salinity but increased under low pH conditions (Fig. 2a and Table 2). In contrast, cannibalism significantly increased in the low salinity treatment, whereas low pH did not affect cannibalism (Fig. 2b). The interaction between low salinity and low pH also did not affect cannibalism (Table 2). The molting frequency was not significantly influenced by low salinity, low pH, or the combination of the two stressors (Fig. 2c and Table 2). Sudden slight increases in molting in the early phase of the experiment (Jan 17 to 20)

Table 4

Results of repeated measures two-way ANOVA for the effects of low salinity and low pH on the swimming, shelter-using, and staying at the bottom and/or away from the shelter behavior of *G. antarctica* during the daytime. ↑ or ↓ indicates increase or decrease in each observed item, respectively.

	Swimming (day)	Shelter use (day)	Staying at the bottom and/or away from the shelter (day)
Within-subject effect			
Time	$F_{13.602,435.250} = 3.928$ $p < 0.0001$	$F_{17.944,574.224} = 6.813$ $p < 0.0001$	$F_{16.478,527.309} = 5.585$ $p < 0.0001$
Time × Salinity	$F_{13.602,435.250} = 1.369$ $p = 0.167$	$F_{17.944,574.224} = 1.715$ $p = 0.033↓$	$F_{16.478,527.309} = 1.325$ $p = 0.174$
Time × pH	$F_{13.602,435.250} = 1.173$ $p = 0.294$	$F_{17.944,574.224} = 0.800$ $p = 0.701$	$F_{16.478,527.309} = 0.712$ $p = 0.788$
Time × Salinity × pH	$F_{13.602,435.250} = 1.598$ $p = 0.079$	$F_{17.944,574.224} = 1.144$ $p = 0.305$	$F_{16.478,527.309} = 0.677$ $p = 0.822$
Between-subject effect			
Salinity	$F_{1,32} = 10.785$ $p = 0.002↑$	$F_{1,32} = 8.384$ $p = 0.007↓$	$F_{1,32} = 1.807$ $p = 0.188$
pH	$F_{1,32} = 0.077$ $p = 0.783$	$F_{1,32} = 6.909$ $p = 0.013↓$	$F_{1,32} = 7.394$ $p = 0.010↑$
Salinity × pH	$F_{1,32} = 0.010$ $p = 0.920$	$F_{1,32} = 2.301$ $p = 0.139$	$F_{1,32} = 2.446$ $p = 0.128$

Table 5

Results of repeated measures two-way ANOVA for the effects of low salinity and low pH on the swimming, shelter-using, and staying at the bottom and/or away from the shelter behavior of *G. antarctica* during the nighttime. ↑ or ↓ indicates increase or decrease in each observed item, respectively.

	Swimming (night)	Shelter use (night)	Staying at the bottom and/or away from the shelter (night)
Within-subject effect			
Time	$F_{9.356,299.406} = 4.532$	$F_{10,320} = 4.901$	$F_{8.965,286.889} = 4.041$
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Time × Salinity	$F_{9.356,299.406} = 2.605$	$F_{10,320} = 2.115$	$F_{8.965,286.889} = 0.477$
	$p = 0.006↓$	$p = 0.023↑$	$p = 0.889$
Time × pH	$F_{9.356,299.406} = 1.278$	$F_{10,320} = 1.225$	$F_{8.965,286.889} = 0.734$
	$p = 0.246$	$p = 0.274$	$p = 0.678$
Time × Salinity × pH	$F_{9.356,299.406} = 1.034$	$F_{10,320} = 0.930$	$F_{8.965,286.889} = 0.405$
	$p = 0.414$	$p = 0.506$	$p = 0.931$
Between-subject effect			
Salinity	$F_{1,32} = 4.168$	$F_{1,32} = 6.516$	$F_{1,32} = 1.533$
	$p = 0.050↓$	$p = 0.016↑$	$p = 0.225$
pH	$F_{1,32} = 0.111$	$F_{1,32} = 0.509$	$F_{1,32} = 0.206$
	$p = 0.741$	$p = 0.481$	$p = 0.653$
Salinity × pH	$F_{1,32} = 0.932$	$F_{1,32} = 1.021$	$F_{1,32} = 0.499$
	$p = 0.342$	$p = 0.320$	$p = 0.485$

in the low salinity treatment coincided with intense cannibalism (Fig. 2b and c). However, there was no significant effect of low salinity on cannibalistic behavior (two-way ANOVA, $F_{1,32} = 0.980$, $p = 0.330$) or the cumulative molting frequency (two-way ANOVA, $F_{1,32} = 0.391$, $p = 0.536$) during that period.

3.2. Food detection

The interaction between time and low pH significantly affected food detection, and low pH had a significant influence after 2 weeks of exposure (on Jan 30, Fig. 3 and Table 3). However, both low salinity and the interaction between low salinity and low pH did not have a significant effect on *G. antarctica* food detection (Table 3).

3.3. Swimming and shelter-use behavior

During the daytime, low salinity significantly increased the

swimming behavior, but there was no significant interaction between time and low salinity (Fig. 4 and Table 4). Low pH did not influence daytime swimming behavior. Daytime shelter use by *G. antarctica* significantly decreased under low pH conditions and was significantly affected by the interaction between salinity and time (Fig. 4 and Table 4). However, neither the low pH and time nor the low salinity and low pH interactions influenced daytime shelter use (Fig. 4 and Table 4). The individuals that stayed at the bottom of the beaker and/or away from the shelter did so significantly more in low pH than salinity, but there was no effect of low salinity or the interaction between time and low pH (Fig. 4 and Table 4).

At night, neither the swimming behavior nor shelter use of *G. antarctica* was influenced by low pH (Fig. 5 and Table 5), but the interaction between salinity and time significantly influenced both night swimming behavior and shelter use (Fig. 5 and Table 5). Low salinity and low pH did not affect those individuals that did not swim or use the shelter (Fig. 5 and Table 5).
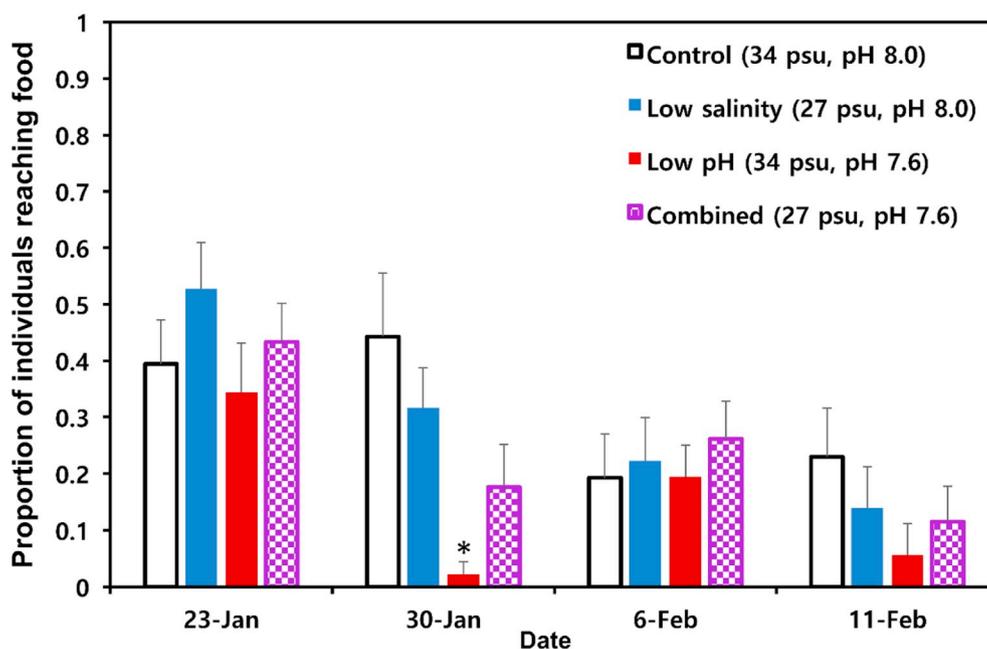

Fig. 3. Proportion of *Gondogeneia antarctica* individuals that found food during exposure to the different salinities and pH levels. Data are expressed as means ± SE. The asterisk (*) indicates the time when there was a significant effect of the pH, since there was significant interaction between time and low pH.
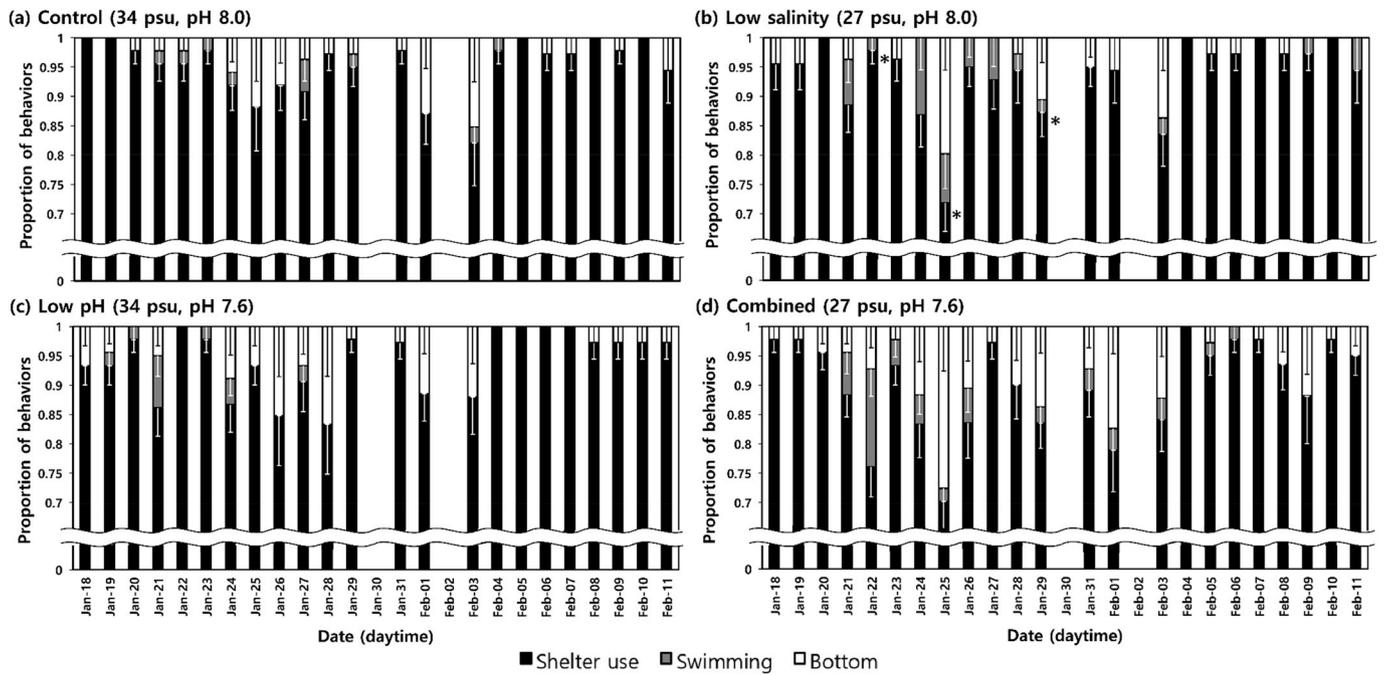
Fig. 4. The proportion of behaviors in the (a) control, (b) low salinity, (c) low pH, and (d) combined treatment groups in the daytime. Data are presented with stacked bars (means ± SE) in each time. The asterisk (*) on the upper right side of the stacked bar indicates when there is a significant effect of the stressor.

4. Discussion

Low salinity and low pH altered the behavior of *G. antarctica*, one of the most dominant amphipod species in the shallow coastal waters around the WAP. Under low salinity conditions, cannibalism and proportion of individuals that exhibited altered swimming and shelter-use increased. Low pH conditions increased mortality (excluding cannibalized individuals) and also caused altered behaviors, such as a reduction in daytime shelter use and food detection within a given period. The interaction between these two climatic stressors did not influence

G. antarctica. Our results suggest that decreased pH or salinity acts as independent stressors of mortality and behavior.

Increased cannibalism under low salinity may be a way to compensate for extra energetic costs. Low salinity induces changes in osmoregulation, which raises the metabolic rates and energy demands of amphipods (Aarset and Aunaas, 1990; Freire et al., 2003). A previous study showed that low salinity (25 psu) significantly increased the oxygen consumption and ammonia excretion of *G. antarctica* because of the increased energy demand and metabolic stress induced by ionic exchange and osmoregulation (Gomes et al., 2013).
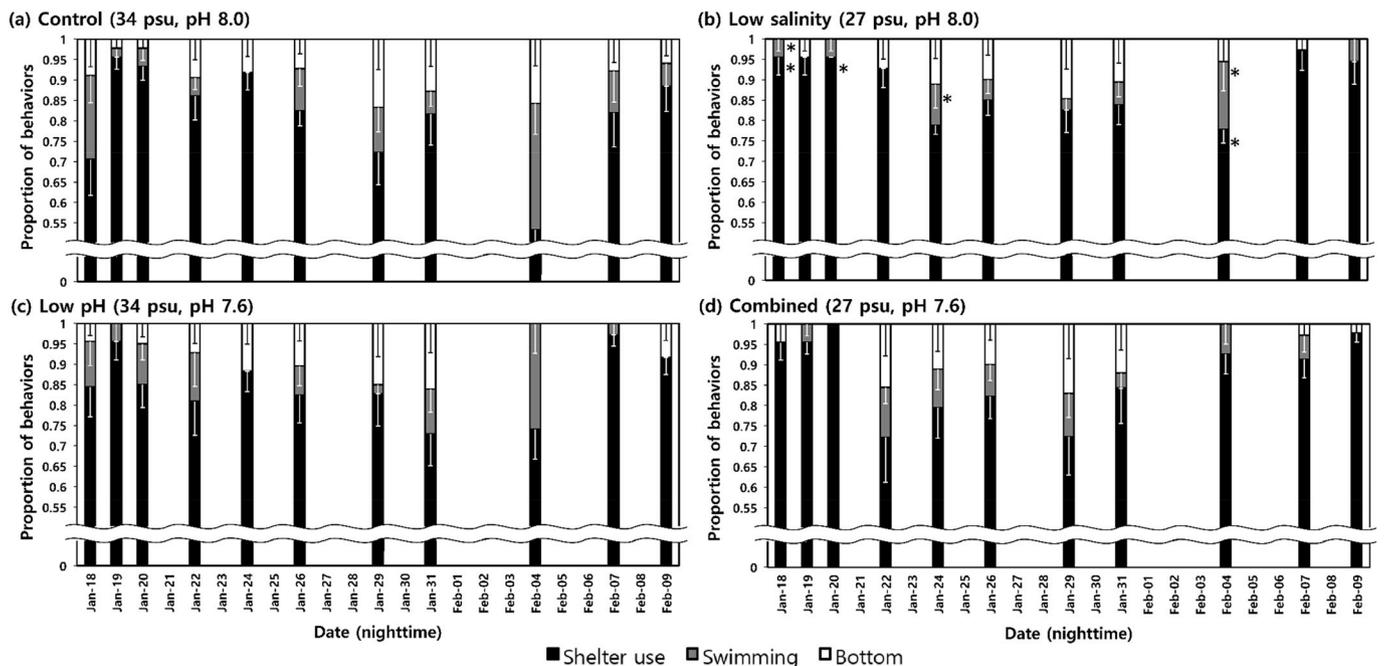

Fig. 5. The proportion of behaviors in the (a) control, (b) low salinity, (c) low pH, and (d) combined treatment groups in the nighttime. Data are presented with stacked bars (means ± SE) in each time. The asterisk (*) on the upper right side of the stacked bar indicates when there is a significant effect of the stressor.

Low salinity also induced unusual behavior, such as increased swimming activity during the day and shelter use at night. Increased swimming during the daytime could be seen as an attempt to escape the osmo-stressful environment (Fanini et al., 2012; Wolcott and Wolcott, 2001). Indeed, in the field, the population density of *G. antarctica* declined significantly with decreased salinity, suggesting that they moved to escape the sub-optimal conditions (personal observations from Dec 2018 to Feb 2019 by Jibin Im and Boongho Cho). Increased daytime activity, even to escape low salinity conditions, may increase predation risks. During the nighttime, however, low salinity conditions lead to a significant decrease in swimming behavior and a significant increase in shelter use. Increased metabolic rates are likely to result in energy deficiencies, from the excessive consumption and reallocation of energy to maintain physiological functions such as osmoregulation (Aarset and Aunaas, 1990; Gomes et al., 2013; Vellinger et al., 2012). This process may have hindered the nocturnal activity of *G. antarctica*. However, low salinity (27 psu) did not have a direct lethal effect on the amphipods during the 26 days of exposure, and it altered nighttime behaviors only within particular times. This might be because they naturally experience wide salinity ranges in the shallow nearshore waters (Freire et al., 2003; McGaw et al., 1999), especially during the austral summer when a considerable amount of glacial and snow meltwater is introduced (Yoo et al., 2015). *G. antarctica* may be able to tolerate low-salinity conditions without any severe effect on their survivorship by raising their metabolic rate against hypo-osmotic stress.

Low pH, on the other hand, not only increased mortality but also deteriorated the normal activity of *G. antarctica*, such as food detection and shelter use during the daytime. Moreover, a greater number of amphipods were observed at the bottom of the beakers during the day. Changes in the ion regulation of *G. antarctica* under low pH conditions in our study (pH 7.6) may have exceeded their tolerance, disrupted the maintenance of homeostasis, and further suppressed their metabolism (Parker et al., 2017; Seibel et al., 2012). A previous study showed that acidic environments triggered the loss of $[Na^+]$ and $[Cl^-]$ in the hemolymph of an amphipod species, thereby decreasing the survival rate (Felten and Guerold, 2004). Another study showed that low pH (7.6) significantly decreased the survival of *G. antarctica* (Schram et al., 2016), and other amphipod and crustacean species (Poore et al., 2013). Moreover, hypercapnic environments may hinder their ability to locate food (de la Haye et al., 2012; Kim et al., 2016), which contributes to energy deficiencies. The negative influence of low pH on the ability of *G. antarctica* to detect chemical cues also implies a higher predation risk in the wild owing to poor habitat choice and failed predator chemodetection (Zamzow et al., 2010). However, a previous study of Baltic amphipods inhabiting coastal eutrophic habitats with CO_2-driven pH variations of 8.1–7.5 showed resistance to acidification by stimulated acid–base regulation (Jakubowska and Normant-Saremba, 2016). Therefore, it appears that *G. antarctica* inhabiting MC had not been exposed to large pH variations or low pH levels during our experiments.

A previous study on the effects of pH and warming on *G. antarctica* showed that low pH (7.6) increased the rate of food consumption, regardless of warming, and this was presumed to be compensation for the increased energy demands of acid–base regulation (Schram et al., 2016). This result appears to conflict with our results, but some key differences should be considered. First, food detection and food consumption rates are different measurements. In contrast to Schram et al. (2016), who provided food *ad libitum*, the individuals in our experiment initially detected their food using sensory organs and then approached it. Second, there were experimental differences between the studies, such as the environmental conditions (i.e., seawater treatments), the seasons when the experiments were conducted (Doyle et al., 2012), and the type of food available. Finally, there is a possibility that cannibalism can influence food detection behavior, but for our results, this was not the case. Food detection was significantly low under low pH treatment, where cannibalism was absent.

Although it is widely known that amphipods in the intra-molting or newly molted period are highly susceptible to conspecific predation (Dick and Platvoet, 2000), we did not find any indication that molting influenced cannibalism. Additionally, we did not find any interaction between the two climatic stressors that influenced the behaviors of *G. antarctica*. Rather, we found counteracting effects of low salinity and pH on amphipod cannibalism and mortality (excluding cannibalism). Under the combined stressor treatments, *G. antarctica* cannibalism was observed more than in the low pH treatment but less than in the low salinity treatment. In contrast, mortality under the combined stressors was higher than in the low salinity treatment and lower than in the low pH treatment. Although low salinity and low pH both negatively influenced behavior, we speculate that there were differential effects on the *G. antarctica* physiology, as metabolism is boosted by low salinity and decreased by low pH. Since stenothermal organisms have a low physiological capacity to deal with such stressors, owing to an inherent limitation to the metabolic energy supply (Clarke, 2003; Clarke et al., 2007), concurrent climatic stressors may limit the capacity to elevate metabolism, despite high metabolic plasticity in *G. antarctica* (Doyle et al., 2012).

In conclusion, the results from this study showed that ocean freshening and acidification occurring in MC, a rapidly warming and deglaciating fjord in the WAP, can adversely affect the behavior and, therefore, the survival of *G. antarctica*. The Antarctic continent and its islands are isolated by the strong Antarctic Circumpolar Current and maintain relatively stable conditions despite thermal disturbances from the lower latitudes (Martinson, 2012). Thus, ectotherms inhabiting the Southern Ocean maintain their low energy budget with a low metabolism (Gomes et al., 2013). However, metabolic rates are subject to environmental stressors, and metabolic variations resulting in physiological changes may induce behavioral responses. Moreover, continuous climatic stressors will force organisms to adapt, migrate to a more suitable environment, or risk extinction (Clarke et al., 2007). The continuous accumulation of climatic stressors could eventually affect the entire marine ecosystem (Harley et al., 2006). Owing to the lack of time-series data and the complexity of ecological food webs, however, our understanding of rapid changes in the polar marine ecosystem due to climatic stressors remains insufficient (Schofield et al., 2010; Yang et al., 2016).

Declaration of competing interest

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

CRediT authorship contribution statement

Seojeong Park: Methodology, Investigation, Formal analysis, Writing - original draft. **In-Young Ahn:** Resources, Project administration, Writing - review & editing. **Eunchong Sin:** Methodology, Investigation. **JeongHee Shim:** Formal analysis. **Taewon Kim:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.104847.

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