

Non-structural carbohydrate content in cryptogamic Antarctic species after two years of passive warming on the Fildes Peninsula

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Abstract

Cryptogamic vegetation dominates the ice-free areas of the maritime Antarctic. This particular flora grows slowly due to extreme environmental conditions, typically low temperature that may limit growth during a short summer. Over the last 50 years, the Antarctic Peninsula has undergone the highest registered temperature increases in Antarctica. As a consequence of higher temperatures, we hypothesized that lichens and mosses would produce more carbohydrates. To test this, open top chambers (OTCs) were installed in an *Usnea-Himantormia* community on Fildes Peninsula of King George Island. After two years, lichen thalli and plant tissues were collected to quantify non-structural carbohydrates in three lichens and two mosses. Responses contrasted between species. While non-structural carbohydrates were higher in the OTC for the lichen *Himantormia lugubris*, the values decreased in the moss *Polytrichastrum alpinum*. No marked responses to experimental warming were observed in the other three species. A significant species-specific increase in soluble sugar was observed inside the OTCs, while polyols content were not markedly different due to OTC treatment. In general, the obtained results indicate that warming does not increase carbohydrate content in all cryptogams, instead suggesting a strong species-specific response to a scenario of global warming. Further long-term warming experiments are needed to assess the responses of target species in the terrestrial Antarctic ecosystem.

Key words: Antarctica, OTC, bryophytes, lichens, carbohydrate metabolism

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Introduction

Cryptogamic vegetation grows on ice-free areas along the Antarctic Peninsula and other mainland areas. Lichen and moss stands extend until 86°S, near the South Pole (Olech 2004, Øvstedal et Smith 2001, Ochyra et al. 2008), and these species establish where fog or liquid water is available. The highest cryptogamic biodiversity occurs in the Northwest of the Antarctic Peninsula and the South Shetland Island Archipelago.

Fildes Peninsula, including Ardley Island off the coast of King George Island, is the second largest ice-free area along the western Antarctic Peninsula. Here, non-vascular cryptogamic vegetation dominates vegetation communities, expanding from north to south, whereas the only vascular plant to grow on Fildes Peninsula is the grass *Deschampsia antarctica* Desv. This is in contrast to other ice-free areas of the Shetland Island Archipelago, where, at few kilometers distance, *e.g.* Potter cove, both native vascular plants, *D. antarctica* and *Colobathus Quitensis* Kunth) Bartl. can be found. This growth pattern suggests that the environmental conditions of the Fildes Peninsula are harsher than other areas of the King George Island. Of the more than 350 lichen species described for Antarctica, 109 grow on the Fildes Peninsula (Andreyev 1989), representing 54 genera and 25 families. Similarly, 50% of the identified Antarctic mosses grow here (Ochyra et al. 2008). Together, mosses and lichens occupy a wide area of the peninsula and give color to the arid territory.

As in other Antarctic regions, cryptogam species must withstand severe polar conditions that include cold temperature, low water availability, frequent daily freeze-thaw cycles, strong and dry wind, high or low radiation, and high or low nutrient input, depending on presence/absence of nearby penguin colonies (Robinson et al. 2000). All of these conditions contribute to the very slow growth rate of

cryptogams. Crustose lichen grows between 0.1-2.0 mm y^{-1} , foliose lichens between 0.5-4.0 mm y^{-1} (Sancho et al. 2007), and mosses present similarly low growth rates (Green et al. 2007).

As poikilohydric organisms, lichens and mosses must accumulate and maintain relatively high levels of cell solutes to withstand the extreme polar conditions, including lipids, amino acids, and soluble carbohydrates (Melick et Seppelt 1994). Soluble carbohydrates confer frost resistance to cells by increasing tolerance to osmotic and thermal stress (Levitt 1980), as has been shown in Antarctic vascular plants (Zúñiga et al. 1996). However, a year-long study in Antarctic lichens and mosses did not reveal significant changes in sugar content (Melick et Seppelt 1994), which is probably a result of the extreme climate and rapid temperature fluctuations during the austral summer, where a relatively short period of metabolic activity does not ensure the storage of high sugar quantities. Cryptogams must produce enough photoassimilates during the growth season to maintain frost and drought resistance during the winter. However, carbohydrate metabolism is indirectly sensitive to temperature since photosynthetic CO_2 assimilation is dependent on this environmental factor. Soluble sugars are the first product of photosynthesis, and sucrose plays several roles in carbohydrate metabolism in plant cells (Zúñiga et al. 1996).

Over the last five decades, Antarctica has undergone the most intense warming of the world. Recent measurements from the Faraday/Vernadsky Station on the Argentine Islands showed an increase in surface temperature of 0.56 K decade⁻¹, or $3.7 \pm 1.6^\circ C$ per last century (Vaughan et al. 2003, 2006, Turner et al. 2005, 2009, 2014, Steig et al. 2009) and an increase in precipitation (Turner et al. 1997, 2005). Intensified glacier retreat along the west-

ern coast of the Antarctic Peninsula (Cook et al. 2005) and the break-up and retreat of ice shelves (Scambos et al. 2000) are understood direct effects of climate warming. Over the last three years (2010-2013), the average rate of ice thinning in western Antarctica has also continued to rise, and glacier mass losses from this sector are now 31% greater than during the period 2005-2011 (McMillan et al. 2014).

The effect of climate warming on cryptogamic vegetation has been studied extensively in other parts of the world, such as the Arctic and in high alpine ecosystems. However, these passive warming studies yielded varied results among bryophyte and lichen communities and have principally measured plant coverage (Lang et al. 2012). Elmendorf et al. (2012) analyzed 61 experimental warming studies on tundra vegetation in the alpine and Arctic and found that lichens and mosses were the most negatively impacted functional groups.

However, few studies have analyzed the responses of Antarctic cryptogams to global climate change (Convey et Smith 2006). The first warming experiment using open top chambers (OTCs) found no significant decrease of cover of mosses and lichens (Bokhorst et al. 2007). On the other hand, *in situ* passive warming experiments by Day et al. (2008, 2009) resulted in decreased moss coverage after four years of long-term growth on Anvers Island off the Antarctic Peninsula. Earlier passive warming experiments, reviewed by Kennedy (1995), suggest that on substrate without plants, moss coverage can

increase by 40% in two years. Moreover, the vertical accumulation rates of *Chorisodontium aciphyllum* peat moss have increased in the maritime Antarctic since the industrial revolution, suggesting that global warming is a cause of increasing moss growth rates (Royles et al. 2012). However, Wasley et al. (2006) has documented increased moss drying over the last decades for eastern Antarctica, as an effect of higher aridity as a part of climate change. This drying has been accompanied by an increased growth of lichens (*Buellia* sp.), which frequently colonize moribund mosses. Despite these studies, there are virtually no data about lichen and moss metabolism under global climate change. Moreover, the slow growth rates of these plants mean that changes in the coverage of cryptogams in manipulation experiments require long observation periods of several seasons, the logistical feasibility of which is complicated due to the harsh Antarctic environment.

In 2008, warming experiments began on the Fildes Peninsula to analyze the responses of cryptogamic biota to increasing temperatures. Taking into account the very slow growth rates of lichens and mosses, research was focused on using specific carbohydrates as response markers to experimental warming. We hypothesized that warming would increase the production and accumulation of soluble carbohydrates in cryptogams. We here present the results of the first two years of OTC-treatment to evaluate the effects of warming on five common cryptogam species, including two mosses and three lichens.

Material and Methods

Study site

The warming experiments were established on Fildes Peninsula, King George Island (62° 00' S, 58° 15' W), which is part of the South Shetland Archipelago.

The climate of the Fildes Peninsula is mild by Antarctic standards, with a maritime climate in the summer and polar conditions in the winter (Carrasco et Gonzalez

2007). Mean air temperature in summer (December-February) is between 0.6-1.5°C and mean temperature in winter (July-August) is -6.5°C, as recorded for the period 1970-2004 (Carrasco et Gonzalez 2007). Mean minimal air temperature is -13.5°C in July, the coldest month, while the lowest absolute minimum is -28.7°C.

Experimental warming

In 2008, *in situ* warming experiments were set up on Fildes Peninsula (Fig. 1) using OTCs according to the general model established by the International Tundra Experiments during the last two decades (Henry et Molau 1997). Open top chambers are valid tools to assess the effects of passive warming on Antarctic terrestrial ecosystems (Bokhorst et al. 2007, 2011). The OTCs were assembled from 3 mm thick, 40 cm high, transparent acrylic panels with a basal area of 0.93 m² forming a hexagonal frustum with open top. The panels were provided with small perforations to permit air exchange and avoid excessive warming. On La Cruz Plateau (62° 12' S, 58° 57' W, 41 MASL), close to the Julio Escudero Station of the Instituto Antartico Chileno (INACH), nine OTCs were installed over cryptogamic communities domi-

nated by the lichens *Usnea aurantiaco-atra* (Jacq.) Bory and *Himantormia lugubris* (Hue) I. M. Lamb. (Olech 2002). To assess chamber effect on air temperature, HOBO Pro v2 loggers (Onset, Bourne, MA, USA) were used to register air temperature and relative humidity every hour in one OTC and one control plot at 20 cm above the canopy. Air temperature was also recorded throughout the year. To assess the effect of temperature, all monthly air temperature values (February 2008-March 2010) were considered, but for relative humidity analyses, only temperature values for the spring-summer period (November-March) were used as these values included the majority of viable temperature data; the logger did not work correctly under snow.

Quantifying non-structural carbohydrates (NSC)

After two years of OTC treatment, samples of five species were taken. These included three fruticose lichens (*Usnea antarctica* Du Rietz, *U. aurantiaco-atra* (Jacq.) Bory, and *H. lugubris* (Hue) I. M. Lamb.) and two mosses, the cushion-forming *Polytrichastrum alpinum* (Hedw.) G.L. Sm., and the carpet-forming *Sanionia uncinata* Hedw. Loeske. Tissue samples were immediately dried at 40°C and transferred to the Faculty of Chemical Sciences and Pharmacy at the University of Chile in Santiago, Chile. Three dry tissue samples weighing 0.1 g each were collected from

each species, and the extraction and quantification of total soluble sugars was performed according to Zúñiga et al. (2012). The samples were incubated at ambient temperature in 1 ml of 80% ethanol for 96 hours. Then, 480 µl of aliquots were taken, concentrated in the Savant™ DNA SpeedVac™ (Thermo Fisher, MN, USA), and resuspended in 0.1 mM of calcium-EDTA buffer before being filtered (0.45 µm) and used for high performance liquid chromatography analysis. A final volume of 20 µl per sample was injected into an Agilent 1100 series chromatograph

equipped with a 300 mm x 6.5 mm Sugar-Pak I Column (Waters Corp., MA, USA) at 75°C and with an Agilent 1100 series refractive index detector at 55°C. Isocratic elution consisted in a mobile phase of 0.1 mM calcium-EDTA with a flow of

0.35 ml min⁻¹ and pressure of 38 bars per 40 minutes. The soluble carbohydrate standards of glucose, fructose, galactose, sucrose were identified, as well as several polyols (arabitol, pinitol, ribitol, erythritol, and mannitol) (Sigma-Aldrich, MO, USA).

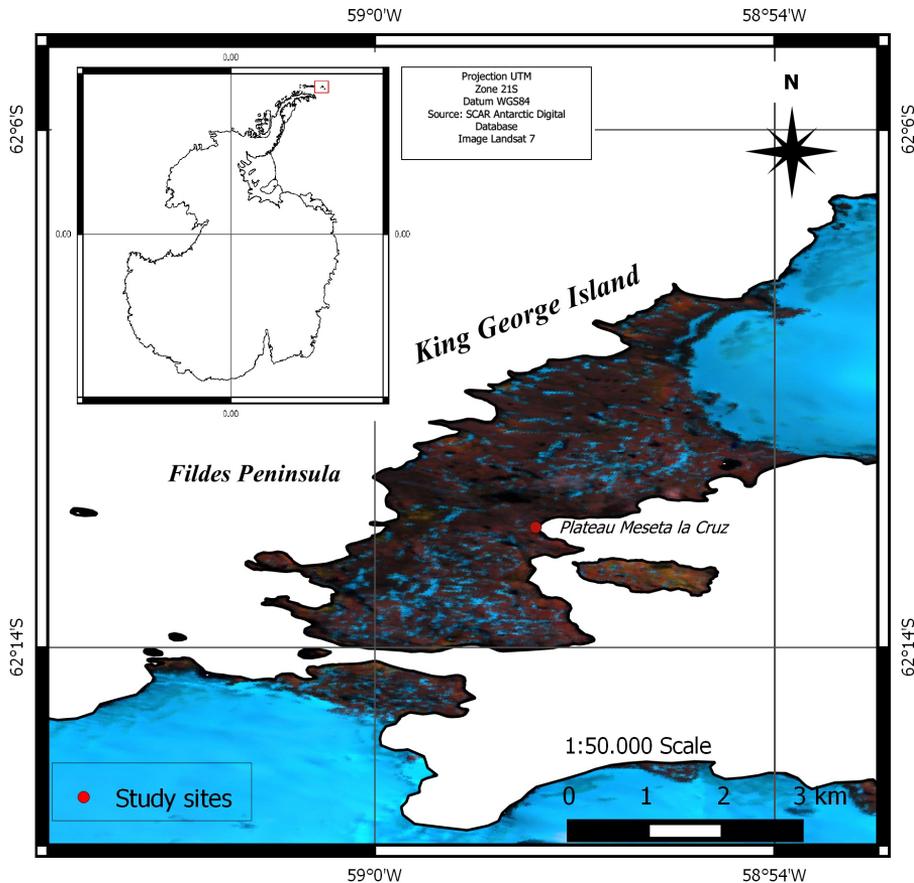


Fig. 1. Map of Fildes Peninsula on King George Island showing the site where open top chambers were installed in 2008. King George Island is part of the South Shetland Archipelago, located to the north of the Antarctic Peninsula.

Statistical analysis

A Kruskal-Wallis test was used to analyze significant differences in carbohydrate content between treatments (con-

trols and OTCs), considering a $P < 0.05$ or, in a few cases, $P < 0.1$. Analyses were performed with INFOSTAT.

Results and Discussion

Two years of OTC-induced warming not only modified temperatures, but also influenced relative humidity. Mean air temperature was slightly higher inside the OTCs (2.31°C) as compared to the controls (1.7°C) during the three summer months (December-February). No marked differences in the mean values for maxi-

imum and minimum air temperatures were found in winter or autumn between the OTCs and controls. However, during spring (September-November), OTCs were slightly warmer, with a higher minimum temperature (-6.86°C) than the controls (-11.3°C). Maximum air temperature was also higher in the OTCs for this period (Table 1).

	Summer		Fall		Winter		Spring	
	OTC	Control	OTC	Control	OTC	Control	OTC	Control
T° Mean	2.31 (2.0)	1.7 (1.6)	-0.8 (2.9)	-0.73 (2.7)	-7.1 (5.1)	-7.22 (5.9)	-0.91 (2.9)	-1.3 (3.1)
T° Min	-3.35	-3.37	-8.48	-7.96	-19.37	-20.03	-6.86	-11.3
T° Max	12.74	12.48	4.25	3.99	3.06	3.3	6.56	3.68
RH (%)	84.3 (13.0)	90.4 (11.6)	82.2 (18.3)	94.6 (4.0)			88.8 (8.7)	85.8 (16.7)

Table 1. Microclimate record of air temperature (°C) and relative humidity (RH; %) during experimental *in situ* warming with open top chambers (OTCs) on the La Cruz Plateau of Fildes Peninsula. Values are represented as the mean for each season between February 2008-December 2010.

Relative humidity, measured 20 cm above the canopy, was 6% and 12% lower during the summer and autumn, respectively, inside the OTCs as compared to controls and differences were slightly higher during spring (Table 1). During the winter, the weather sensor did not work properly due to snow fall, so these values were not used. Clearly, the OTCs affected microclimatic variables, as has been reported in other regional studies (Molau et Mølgaard 1996, Marion et al. 1997) and, recently, in Antarctica (Bokhorst et al. 2007, 2013). However, OTCs are the most suitable tool for assessing warming effects on Antarctic vegetation (Bokhorst et al. 2011). Moreover, the most important microclimatic changes occurred during spring, which is probably the best period for active metabolism in cryptogams.

Total non-structural carbohydrate (NSC) content showed insignificant differences between the species in and outside of the OTCs (Table 2). In fact, the only statistically significant variable, according to the Kruskal-Wallis test, was the interaction between species and treatment ($P < 0.0059$; $H 23.15$, $fd_{1,4}$) for the contents of NSC, soluble sugar (sum of sucrose, fructose, glucose, and galactose) ($P < 0.0299$; $H 18.49$, $fd_{1,4}$) and polyols (sum of galactinol, mannitol, pinitol, arabitol, ribitol, erithritol) ($P < 0.0329$; $H 18.2$; $fd_{1,4}$) (Table 2).

Only *H. lugubris* exhibited a slightly significant ($P < 0.10$) increase in NSC due to OTC treatment, ranging from 22.0 ± 2.2 to $28.4 \pm 2.7 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$ (Table 2). When comparing the soluble sugars pool with the polyols pool in the two lichen species, only the soluble sugar produced by the al-

ga were increased, expressed as an increased fructose content, which suggests that sugar production improved under the microclimatic conditions of the OTC. In contrast, the moss *P. alpinum* presented a marked decrease of NSC in the OTC, which was probably due to the significant ($P < 0.0317$) reduction in soluble sugar content (see Fig. 2), the most significant of which was observed for the fraction of galactose ($P < 0.0237$). These results suggest that this endohydric moss -which forms cushion-, can be affected by decreased relative humidity, as occurred inside the OTC during the summer. Related to this, *P. alpinum* dehydrates relatively quickly compared to *S. uncinata*, which grows as a compact carpet to avoid water loss (Zúñiga et al. 2015). Finally, polyol analysis showed that ribitol, a sugar alcohol produced by the photobiont and transferred to the microbiont in lichen species, was strongly decreased in *U. antarctica* growing in the OTC, which could suggest modifications in the sugar metabolism of this lichen as a result of OTC warming.

There are few works evaluating lichen species under global climate change conditions. Bokhorst et al. (2007) was the first to show that *Usnea* species, and particularly *U. antarctica*, underwent a decrease in

coverage after four years inside an OTC on Signy Island. However, no reports exist on changes in lichen carbohydrate contents under climate change. Lichens are the most successful organisms colonizing Antarctica, reaching as far as 86° S, near the pole. Nevertheless, these species are slow growing and should respond positively to warming, but water availability may limit photosynthetic metabolism. Data from all studies on lichens and mosses register decreased cover after long-term experimental warming (Elmendorf et al. 2012, Lang et al. 2012), and this is probably due to extended exposure to conditions above biological thresholds, which is likely to affect the life cycle of cryptogams in terms of growth rate, development, and reproduction (Green et al. 2007).

Our results suggest that there is not a common response to experimental warming across different functional groups and species, but that metabolic markers, such as carbohydrates, can be a useful tool for understanding the individual effects of warming on cryptogams. However, long-term studies that include specimen sampling during different seasons are required to integrate the responses of cryptogams to global climate change in polar regions.

Carbohydrates	Mosses						Lichens					
	<i>Polytrichastrum alpinum</i>		<i>Sanionia uncinata</i>		<i>Usnea antarctica</i>		<i>Usnea aurantiaco-atra</i>		<i>Himantormia lugubris</i>			
	Control	OTC	Control	OTC	Control	OTC	Control	OTC	Control	OTC		
Glucose	0.90 (0.9)	0	1.50 (1.0)	2.17 (1.9)	0.30 (0.1)	0	0	0.07 (0)	0.73 (0.56)	0.07 (0.03)		
Fructose	3.27 (1.8)	0	2.30 (1.1)	2.77 (1.4)	0.60 (0.4)	8.57 (4.2)	5.53 (5)	0.11 (0.1)	0.43 (0.3)	6.4 (0.3)		
Sucrose	2.27 (1.1)	3.60 (0.1)	3.80 (1.4)	1.97 (1.1)	5.20 (0.2)	5.55 (0.6)	2.37 (1.9)	1.36 (0.7)	0.83 (0.3)	2.3 (0.06)		
Galactose	48.5 (8.8)	12.07 (6.4)	2.23 (1.0)	7.97 (3.1)	0.30 (0.1)	0.83 (0.4)	0	0	3.23 (2.8)	1.2 (0.21)		
Galactinol	0	2.10 (1.2)	2.40 (1.5)	1.53 (0.8)	0.83 (0.2)	8.7 (0)	0.27 (0.2)	1.41 (1)	0.63 (0.6)	0		
Manitol	0	0	0	0	3.07 (0.1)	8.3 (0)	0	0.0	5.2 (1.8)	10.3 (2.4)		
Pinitol	9.57 (3.4)	11.87 (1.6)	3.75 (3.4)	5.17 (2.8)	1.17 (0.8)	0.6 (0)	0.05 (0)	0.07 (0.0)	2.08 (1.6)	0.57 (0.5)		
Arabitol	7.20 (2.0)	26.60 (9.9)	13.0 (10)	18.63 (7.6)	8.93 (0.3)	4.5 (0)	1.03 (0.9)	5.09 (1.4)	4.63 (1.2)	5.6 (3.7)		
Ribitol	0	0	5.38 (5)	0	73.5 (1.3)	30.73 (17.7)	2.5 (1.3)	5.81 (4)	4.03 (2.1)	2.03 (2.03)		
Eritrhitol	0	0.20 (0)	0	0	0.07 (0.1)	2 (0)	0	0.03 (0.0)	0.18 (0.1)	0		
Total	71.73 (9.2)	56.43 (9)	40.48 (11)	40.47 (5)	68.9 (24)	51.83 (16.7)	11.73 (3.4)	13.96 (3.8)	21.9 (2.2)	28.37 (2.7)		

Table 2. Non-structural carbohydrate content (mg *g⁻¹ DW) in Antarctic cryptogam species exposed to long-term growth in open top chambers (OTCs) and control conditions on the Fildes Peninsula. Values are means (n=3) ± SE in brackets.

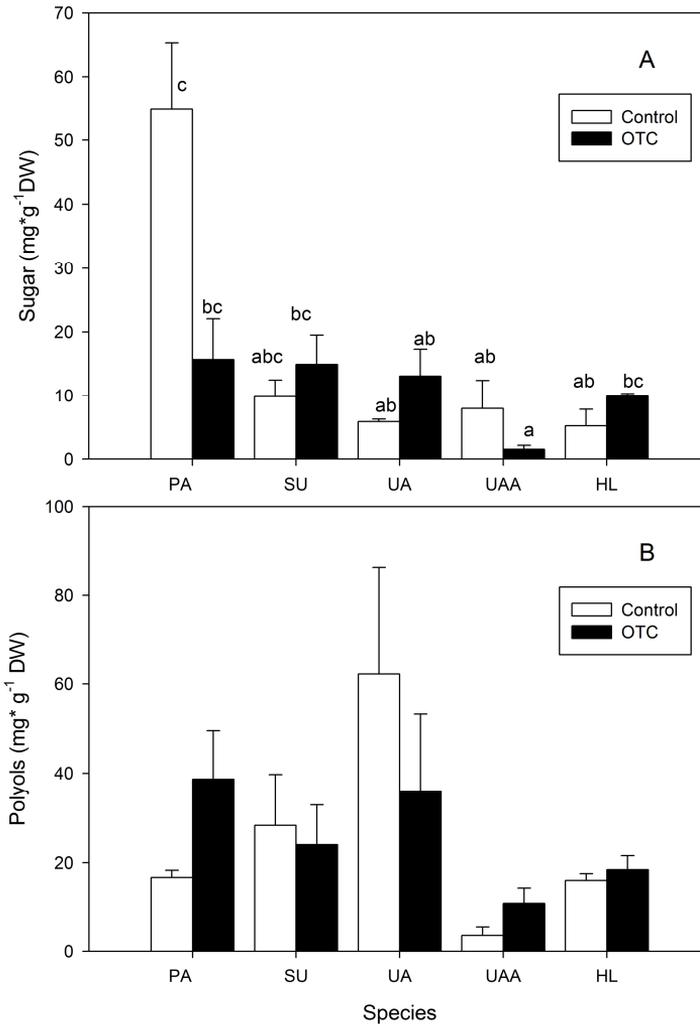


Fig. 2. Carbohydrate contents (mg * g⁻¹ DW) in mosses and lichens grown for two years under experimental warming conditions with open top chambers (OTCs) on Fildes Peninsula. PA, *Polytrichastrum alpinum*; SU, *Sanionia uncinata*; UA, *Usnea antarctica*; UAA, *Usnea aurantiaco-atra*; and HL, *Himantormia lugubris*. Values are means (n=3) ± SE. Different letters indicate significant differences (P < 0.05) according to posthoc test in Kruskal Wallis analysis.

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