Interpopulational differences in the nutritional condition of *Aequiyoldia eightsii* (Protobranchia: Nuculanidae) during austral summer at the Western Antarctic Peninsula

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The Western Antarctic Peninsula (WAP) has been a hotspot of warming in the Southern Hemisphere since the mid-20th century. Warming has particularly occurred in the central and southern regions of the WAP, with greater rates of change and impacts on marine-terminating glaciers than in the northern WAP. The current work investigates potential contrasting effects of this on the biology of benthic marine invertebrate species. Here, for the first time we used adult individuals of the bivalve *Aequiyoldia eightsii* to evaluate large-scale spatial variation in the biochemical composition (measured as lipid, protein and fatty acids) and energy content, as a proxy of nutritional condition, of three populations along the WAP: O'Higgins Research Station in the north (63°S), Yelcho Research Station in mid-WAP (64°S) and Rothera Research Station further south (67°S). The results reveal significantly higher quantities of lipids (L), proteins (P), energy (E) and total fatty acids (FA) in the northern population (O'Higgins) (L: 8.33 ± 1.32%; P: 22.34 ± 3.16 %; E: 171.53 ± 17.70 Joules; FA: 16.33 ± 0.98 mg g) than in the mid-WAP population (Yelcho) (L: 6.23 ± 0.84%; P: 18.63 ± 1.17%; E: 136.67 ± 7.08 Joules; FA: 10.93 ± 0.63 mg g) and southern population (Rothera) (L: 4.60 ± 0.51%; P: 13.11 ± 0.98%; E: 98.37 ± 5.67 Joules; FA: 7.58

± 0.48 mg g). We hypothesize these differences in the nutritional condition could be related to the capacity of this species to adjust their biochemical composition depending on the prevailing environmental conditions at each site within their broad latitudinal distribution gradient.

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Abstract

The Western Antarctic Peninsula (WAP) has been a hotspot of warming in the Southern Hemisphere since the mid-20th century. Warming has particularly occurred in the central and southern regions of the WAP, with greater rates of change and impacts on marine-terminating glaciers than in the northern WAP. The current work investigates potential contrasting effects of this on the biology of benthic marine invertebrate species. Here, for the first time we used adult individuals of the bivalve *Aequiyoldia eightsii* to evaluate large-scale spatial variation in the biochemical composition (measured as lipid, protein and fatty acids) and energy content, as a proxy of nutritional condition, of three populations along the WAP: O'Higgins Research Station in the north (63°S), Yelcho Research Station in mid-WAP (64°S) and Rothera Research Station further south (67°S). The results reveal significantly higher quantities of lipids (L), proteins (P), energy (E) and total fatty acids (FA) in the northern population (O'Higgins) (L: 8.33 ± 1.32%; P: 22.34 ± 3.16 %; E: 171.53 ± 17.70 Joules; FA: 16.33 ± 0.98 mg g) than in the mid-WAP population (Yelcho) (L: 6.23 ± 0.84%; P: 18.63 ± 1.17%; E: 136.67 ± 7.08 Joules; FA: 10.93 ± 0.63 mg g) and southern population (Rothera) (L: 4.60 ± 0.51%; P: 13.11 ± 0.98%; E: 98.37 ± 5.67 Joules; FA: 7.58 ± 0.48 mg g). We hypothesize these differences in the nutritional condition could be related to the capacity of this species to adjust their biochemical composition depending on the prevailing environmental conditions at each site within their broad latitudinal distribution gradient.

Introduction

Since the mid-20th century, the Western Antarctic Peninsula (WAP) has shown profound warming of both atmosphere and ocean, and associated changes in both ice from terrestrial sources (glaciers and ice shelves) and sea ice (Vaughan et al., 2003; Meredith & King, 2005; Henley et al., 2019; Morley et al., 2020). Some of the main reported changes in the cryosphere have been: (1) a multi-decade decrease in sea ice concentration and extent (Stammerjohn et al., 2008; Massom & Stammerjohn, 2010; Holland & Kwok, 2012; Hobbs et al., 2016), (2) retreat and collapse of ice shelves (Cook & Vaughan, 2010; Paolo, Fricker & Padman, 2015) and (3) increase in glacier melting and retreat (Cook et al., 2005; Kunz et al., 2012; Cook et al., 2016; Konrad et al., 2018). Contemporaneously, some studies have shown a north-south gradient in ocean temperature change along the WAP, whereby Circumpolar Deep Water (CDW) layers that upwell on to the shelf of the central and southern areas have warmed by up to 1 °C (Cook et al., 2016). Water masses at the same depth along the northern WAP have not shown comparable warming. They are renewed primarily by the inflow of cold waters from the Weddell Sea as opposed to onshelf flow of CDW from the deep levels of the Antarctic Circumpolar Current (Cook et al., 2016). The differential warming trends have exacerbated the meridional contrast in deep ocean temperature along the WAP, and have resulted in a pattern of deep ocean change that differs greatly from what might be expected based purely on latitude.

Nutritional condition is a key property when investigating biological response to environmental change, since it is a factor that is expected to limit the efficiency of response of diverse

biological mechanisms to biotic or abiotic variability (Somero, Lockwood & Tomanek, 2017). On the one hand, in aquatic species of aquaculture and fishery importance, nutritional condition has generally been analyzed through multiple methods such as DNA: RNA, proximate composition and fatty acids (Tacon & Metian, 2013; Tan et al., 2021). In these studies, nutritional condition has been used as an indicator of food quality for human consumption, since it allows detection of the presence of molecules with high nutritional value (Lah et al., 2017; Lorenzo et al., 2021). On the other hand, nutritional condition has great potential to be used as an indicator of the energy reserves of aquatic organisms for ecological purposes (Vesterinen et al., 2020; Bascur et al., 2020). For instance, a recent study on the Antarctic bivalve *Nuculana inaequisculpta* found differences in the nutritional condition of individuals on a distance gradient from a retreating glacier in a small WAP fjord (Bascur et al., 2020), and consistent with variation in other population attributes such as genetic diversity (Muñoz-Ramírez et al., 2021). However, understanding of how nutritional condition of different populations of marine invertebrate species vary in response to prevailing environmental conditions at large spatial scales is still very limited in Antarctic ecosystems.

Biochemical composition has been widely investigated in a range of marine bivalve species from high and low latitude ecosystems (e.g. Ahn et al., 2003; Pogoda et al., 2013). Lipids, protein, carbohydrates and fatty acids all have crucial roles in development, metabolism and functioning of marine organisms (Somero, Lockwood & Tomanek, 2017). In species with a wide geographic distribution, these biochemical reserves can be modified by abiotic factors such as sea temperature or food availability (Guzmán-Rivas et al., 2021). However, biotic factors can also play an important role. For example, it has been indicated there is a close relationship between biochemical composition and reproduction in marine invertebrates, since there is a significant expenditure of biochemical reserves in the production of gametes, which are released at the time of spawning (Mathieu & Lubet 1993; Darriba, Juan & Guerra, 2005; Ngo et al., 2006; Li et al., 2011). In this way, it is necessary to consider reproductive status when the reproductive cycle is not known with certainty or there is no information available on gonad maturity of the samples. Accordingly, species biochemical composition and energy content can be cautiously used as an indicator to compare the nutritional condition among different benthic populations. For this purpose, the WAP constitutes a valuable natural laboratory in which to evaluate biological variables on a large spatial scale, since it allows the understanding of biological patterns across a contrasting environmental gradient (Brante et al., 2003; Fischer et al., 2009; Barnes et al., 2020).

Aequiyoldia eightsii (Jay, 1839) is an infaunal bivalve mollusk species of the Protobranchia subclass, distributed patchily in Antarctic and sub-Antarctic areas with muddy sediments (Dell, 1991; González-Wevar et al., 2012). *A. eightsii* is a long-lived species with a maximum lifespan around 60 years (Nolan & Clarke, 1993; Peck & Bullough, 1993; Román-González et al., 2017). This abundant species can be found from intertidal to deep waters, although it is more frequent at depths less than 100 m, with densities of up to 1540 individual m^{-2} (Peck & Bullough, 1993). It has been described as an opportunistic species, since it feeds mainly on organic sediment

deposits (Zardus, 2002). However, it can modify its feeding habits by ingesting suspended particles when phytoplankton is available (Davenport, 1988). Recent studies found an endogenous growth rhythm in this species, likely related to reallocation of energy investment towards growth or reproduction (Román-González et al., 2017). This species shows a 1:1 male: female sex ratio and a lecithotrophic pericalima larva (Zardus, 2002). At South Orkney Islands (61°S), individuals of this species reach their sexual maturity when shell length is > 20 mm (Peck, Colman & Murray, 2000). Further south, at Rothera Station, this species showed continuous oogenesis throughout the year with spawning in austral winter (Lau et al., 2018).

Here, we used biochemical and energetic data obtained from adult body mass of *A. eightsii*, an abundant benthic bivalve species with a pivotal role as a nutrient recycler (Cattaneo-Vietti et al., 2000; Lovell & Trego, 2003; Gordillo, Malvé & Moran, 2017), to determine nutritional condition differences among three populations that inhabit contrasting environments along the range of the WAP. Our biological results on the nutritional condition are considered in the context of the physical oceanography along the WAP.

Materials & Methods

Sample collection

To assess nutritional condition of a key benthic species among localities with contrasting environmental conditions in Antarctica, adult individuals of the bivalve mollusk *A. eightsii* were collected. Samples were collected during austral summer at three localities along the WAP by SCUBA diving at 10-15 m depth (Fig 1). The individuals of the O'Higgins (63°19′S, 57°53′W; n = 24) and the Rothera stations (67°34′S, 68°07′W; n = 15) were collected during January 2018, while the individuals of the Yelcho station (64°52′S 63°35′W; n = 19) were collected during March 2017. After collection, all the samples were immediately preserved in 99% ethanol and maintained at –80 °C. Then, samples were transported to the UCSC Hydrobiological Resources laboratory at Concepción, Chile and kept under the same conditions until further analysis. The collection permits were granted by the UK Government for JR17001 and JR18003 expeditions: 31/2017 and S6-2018/01. Also, a permit was granted for collection adjacent to Rothera Research Station: 33/2017.

Recently, cryptic speciation has been described in *A. eightsii*, suggesting it is composed of two different lineages in the WAP (González-Wevar et al., 2019). In this context, to avoid biases in the biological response, samples from the same lineage have been used in the present study, following genetic analyses (Muñoz-Ramírez et al., 2020). However, it is inevitable that sampling could not be perfectly simultaneous; thus this caveat is factored into our analysis and interpretation of data below.

Shell length and body mass

Using Vernier calipers with 0.01 mm precision, we determined the individuals' sizes, measured as the distance between the anterior and posterior edges of the shell (i.e. shell length). To determine the body mass of each individual, the soft tissue was separated from the shells and

washed with abundant distilled water on a 0.2 mm sieve in order to remove salt and sediment. Then, samples in independent labeled Eppendorf tubes were frozen at –20 °C and dried for 48 hours at –80 °C by sublimation in a lyophilizer (FDU-7012, Operon). Finally, using an analytical balance with a sensitivity of 0.1 mg, body mass was determined as dry mass of the individuals (LA230S, SARTORIUS).

Proximate biochemical composition (lipid and protein content)

The proximate composition was measured in 20 mg of homogenized dry mass for each individual and expressed in absolute values (mg 20 mg^{-1}), and then calculated in relative values of dry mass for each biochemical component [% dry mass, (DM)= (mg of component × 100)/ mg of DM]. In order to improve the performance of the tests, samples were exposed for 15 minutes at 6 °C in an ultrasonic bath (AC-120H, MRC) with distilled water (protein content) or dichloromethane: methanol (lipid content), and then were analyzed using the methods outlined below.

Lipid content was quantified both in the dry samples and in the ethanol in which the samples were preserved, following the gravimetric method of Folch, Lees & Stanley (1957), modified by Cequier-Sánchez et al., (2008). Each dried sample was homogenized in amber tubes with 5 mL of dichloromethane: methanol (2:1). Then, samples were combined with 4 mL of 0.88% potassium chloride, mixed for 15 seconds in a vortex (SBS100-2, Select Vortexer) and centrifuged (S-8, Boeco) for 5 minutes at 6 °C and 1500 rpm. The precipitate of each sample was transferred to pre-weighed vials and dried through evaporation using ultrapure nitrogen gas (109A YH-1, Glass Col). Total lipid extract obtained by evaporating the solvent was weighed on a precision balance (120A, Precise) and was calculated by subtracting the weight of the empty vial from the weight of the vial with the lipid extract. Since we found a very low amount of lipid in the single ethanol extracts (8-10% of each individual total lipid content) we decided to pool the lipid content found in each ethanol sample with the lipid content found in each individual. Finally, lipid extract of each sample was preserved at –80 °C in dichloromethane: methanol (2:1) with butylhydroxytoluene (BHT) as an antioxidant to avoid the degradation of future fatty acid analyzes.

Protein content was quantified using a microplate adaptation of the BIO-RAD colorimetric assay of Lowry et al., (1951). This kit included three reagents: S, A and B. The dry samples of 4 mg for each individual were homogenized in 200 µL of ultrapure water (Mili-Q). Then, 5 µL of the mixture was transferred to a 96-well microplate with 200 µL of Reagent B and 25 µL of Reagent A' (mixture of 20 µL of Reagent S and 1 mL of Reagent A). Subsequently, the samples were shaken for 15 seconds in a vortex (SBS100-2, Select Vortexer) and incubated in the microplates for 15 minutes at room temperature. Finally, the absorbance was measured in a spectrophotometer at a wavelength of 750 nm (ELx808, Biotek). The concentration of each sample was obtained using a calibration curve for proteins, diluting different concentrations of bovine serum albumin (500-0111, Bio-Rad).

Energy content

The energy content (J 20mg DM^{-1}) was estimated using a bioenergetics equivalent from the biochemical composition data (lipid and protein). The bioenergetics equivalents were calculated through conversion coefficients: (a) 1 mg of lipids = 39.54 J, (b) 1 mg of protein = 23.64 J. An approximation of the total energy content for each individual was calculated by adding the energy equivalents of the biochemical composition (total energy = J mg lipid + J mg protein) (Winberg, 1971; Urzúa et al., 2012; Bascur et al., 2017).

Fatty acids composition

Fatty acid profile was determined through standard methods (Urzúa et al., 2011; Bascur et al., 2018). Fatty acid methyl esters (FAMEs) were measured after preparation using the samples lipid extract. Lipid extracts were esterified at 70 °C for 1 h in a Thermo-Shaker (DBS-001, MRC) using sulfuric acid (1% in methanol) incubations. Then, each sample was vortexed (SBS100-2, Select Vortexer) with 3 mL of n-hexane and centrifuged for 15 s. This process was repeated three times and the supernatant was transferred to labeled tubes. Finally, using a nitrogen evaporator (109A YH-1, Glass Col), fatty acids were concentrated. The measurement of FAMEs was performed using a gas chromatograph (Agilent, model 7890A) at set temperature equipped with a DB-225 column (J&W Scientific, 30 m in length, 0.25 internal diameter, and 0.25 μm film). Using chromatograph software (Agilent ChemStation, USA), individual FAMEs were identified by comparison to known standard fatty acids of marine origin (certificate material, Supelco 37 FAME mix 47885-U (Malzahn et al., 2007; Urzúa et al., 2011). Each sample was quantified using a calibration curve for fatty acids, diluting different concentrations of Supelco 37 FAME mix standard.

Statistical analysis

Statistical analyses were performed based on standard methods (Sokal & Rohlf, 1995; Clarke & Gorley, 2006; Zuur, Ieno & Graham, 2007) in the STATISTICA V8 and PRIMER V6 (+ PERMANOVA) software packages with a 95% confidence level ($p < 0.05$). The assumptions of the ANOVA analysis were evaluated with Kolmogorov-Smirnov tests for normality and Levene test for homogeneity of variances. When significant differences were detected for ANOVA or Kruskal-Wallis test, post hoc Tukey HSD or multiple range tests with a Bonferroni correction were performed to assess differences among localities, respectively. All analyzes were performed with the locality factor [with 3 levels: O'Higgins station (northern WAP), Yelcho station (middle WAP) and Rothera station (southern WAP)].

The shell length of *A. eightsii* individuals collected at the three study localities was analyzed through a one-way ANOVA as assumptions of normally distributed data and homogeneity of variances were fulfilled. Because these assumptions (normality and homogeneity) were not fulfilled for soft tissue dry mass, lipid and protein content (mg and %DM) and energy content of *A. eightsii* individuals captured at the three study localities, these variables were analyzed by non-parametric Kruskal-Wallis tests. Also, the assumptions of normality and homogeneity of

variances were evaluated for the quantity of each fatty acid (e.g. C16: 0) and for the total values of each group of fatty acids (e.g. total saturated fatty acids, SFA) among the three study localities. The vast majority of fatty acid comparisons were analyzed with a Kruskal-Wallis test because they did not fulfill ANOVA assumptions. The exceptions analyzed with a one-way ANOVA after a log (x + 1) data transformation, were C18:0 (normality: KS = 0.11, $p > 0.20$; homogeneity: $F = 2.91$, $p = 0.06$), C22:6n-3 (normality: KS = 0.14, $p > 0.20$; homogeneity: $F = 2.50$, $p = 0.09$) and the total of SFA (normality: KS = 0.12, $p > 0.20$; homogeneity: $F = 1.89$, $p = 0.16$). On the other hand, the fatty acids C18:2n-6c and C22:1n-9 were only found in two localities, and as they did not fulfill the assumptions of normality and homogeneity, they were analyzed with a Mann-Whitney U test.

In addition, multivariate analyses were conducted to compare fatty acid composition. A one-way permutational multivariate analysis of variance (PERMANOVA) analysis based on Bray-Curtis similarity and fourth root data transformation was performed to evaluate the complete fatty acids data set. Last, a similarity percentage analysis (SIMPER) was carried out to observe the percentage of contribution of each fatty acid to dissimilarity among localities.

Results

Shell length and body mass

After the analysis of the assumptions of normality (KS = 0.08; $p > 0.20$) and homogeneity of variances ($F_{2, 55} = 1.11$; $p = 0.34$), the shell length (mm ind.$^{-1}$) showed no significant differences among the three study localities ($F_{2, 54} = 3.09$; $p = 0.054$). Individuals were of similar size around O'Higgins (22.67 ± 2.13 mm), Yelcho (24.07 ± 1.88 mm) and Rothera stations (22.96 ± 1.62 mm) (Fig. 2a, Table S1). In contrast, soft tissue dry mass (mg ind.$^{-1}$) of individuals showed significant differences among the study localities. Individuals around the O'Higgins (162.31 ± 52.28 mg) and Yelcho stations (177.43 ± 37.78 mg) showed a higher soft tissue biomass than individuals from Rothera station (123.44 ± 20.08 mg) ($H_{2, 58} = 12.73$; $p < 0.01$) (Fig. 2b, Table S2).

Proximate biochemical composition (lipid and protein)

Lipid content (mg 20 mg DM^{-1}) showed significant differences among the localities analyzed ($H_{2, 58} = 43.95$; $p < 0.001$), with O'Higgins station individuals having a higher lipid content (1.67 ± 0.26 mg) than those from Yelcho (1.25 ± 0.17 mg) and Rothera station (0.92 ± 0.10 mg) (Fig. 3a, Table S2). Lipid proportions (% DM) were also significantly different ($H_{2, 58} = 43.95$; $P < 0.001$) between individuals of the three localities, showing higher values in individuals near O'Higgins station (8.33 ± 1.32%) than individuals from Yelcho (6.23 ± 0.84%) and Rothera stations (4.60 ± 0.51%) (Fig. 3b, Table S2).

Protein content (mg 20 mg DM^{-1}) of *A. eigthsii* was significantly different between the three study localities ($H_{2, 58} = 41.93$; $p < 0.001$), with higher protein values around O'Higgins individuals (4.47 ± 0.63 mg) than individuals of Yelcho (3.73 ± 0.23 mg) and Rothera (2.62 ± 0.20 mg) (Fig. 3c, Table S2). In turn, protein percentages in relation to dry mass (% DM) also

showed significant differences among individuals of the three study localities ($H_{2, 58} = 41.93$; $p < 0.001$). Higher protein percentages were found in O'Higgins station individuals (22.34 ± 3.16%) than those from Yelcho (18.63 ± 1.17%) and Rothera stations (13.11 ± 0.98%) (Fig. 3d, Table S2).

Energy content

The energy content (J 20 mg DM^{-1}) showed significant differences between individuals collected at the three study localities ($H_{2, 58} = 49.25$; $p < 0.001$). A higher energy content was found in individuals from O'Higgins (171.53 ± 17.70 J), than individuals from Yelcho (136.67 ± 7.08 J) and Rothera (98.37 ± 5.67 J) (Fig. 4, Table S2).

Fatty acids composition

One-way ANOVA results showed some significant differences among the fatty acid profiles at the three study localities (Table 1). The amount of total fatty acids was higher in O'Higgins station individuals (16.33 ± 0.98 mg FA g DM) than those from Yelcho (10.93 ± 0.63 mg FA g DM) and Rothera station (7.58 ± 0.48 mg FA g DM) ($H_{2, 58} = 41.57$; $p < 0.001$). A higher quantity of saturated fatty acids was found in O'Higgins station individuals (10.01 ± 1.35 mg FA g DM) than in those from Yelcho (6.00 ± 0.76 mg g DM) and Rothera station (5.17 ± 0.65 mg g DM) ($F_{2, 55} = 58.27$; $p < 0.001$). Moreover, a greater quantity of monounsaturated fatty acids (3.44 ± 0.42 mg FA g DM, 3.34 ± 0.72 mg FA g DM, 1.04 ± 0.17 mg FA g DM; $H_{2, 58} = 32.80$; $p < 0.001$), polyunsaturated fatty acids n-6 (0.92 ± 0.06 mg FA g DM, 0.56 ± 0.04 mg FA g DM, 0.29 ± 0.08 mg FA g DM; $H_{2, 58} = 46.29$; $p < 0.001$) and polyunsaturated fatty acids n-3 (1.96 ± 0.33 mg FA g DM, 1.03 ± 0.09 mg FA g DM, 1.08 ± 0.23 mg FA g DM; $H_{2, 58} = 23.85$; $p < 0.001$) was observed in O'Higgins station individuals than Yelcho and Rothera station individuals, respectively. In addition, total polyunsaturated fatty acids also showed significant differences ($H_{2, 58} = 32.37$; $p < 0.001$) between the three study localities, with a greater amount in O'Higgins station individuals (2.88 ± 0.28 mg FA g DM) than in individuals from Yelcho (1.59 ± 0.10 mg FA g DM) and Rothera stations (1.37 ± 0.21 mg FA g DM) (Table 1).

PERMANOVA analysis, which compares the complete fatty acid data set of all populations, showed significant statistical differences between *A. eightsii* individuals in the three study localities (Pseudo-$F_{2, 55} = 206.68$; $p < 0.001$; 999 permutations; Table S3). Differences among the three groups was consistent with the SIMPER analysis, since the contribution to the dissimilarity of the individuals' fatty acids is driven by different fatty acids for each comparison between localities [O'Higgins vs. Yelcho: eicosenoic acid (C20: 1), arachidic acid (C20: 0), behenic acid (C22: 0) erucic acid (C22: 1n-9); O'Higgins vs. Rothera: eicosenoic acid (C20: 1), arachidic acid (C20: 0), linoleic acid (C18: 2n-6c) and erucic acid (C22: 1n-9); Yelcho vs. Rothera: behenic acid (C22: 0), oleic acid (C18: 1n-9) and linoleic acid (C18: 2n-6c)] (Table 2).

Discussion

The WAP is the strongest gradient in physical conditions and recent environmental change

around Antarctica and thus is an ideal location to explore biological responses. The present study provides the first record of interpopulational variability in the nutritional condition of a marine bivalve species along the WAP. We found that individuals of *A. eightsii* showed significant differences in biochemical composition among three study localities that are likely to have consequences for the populations. Individuals collected at O'Higgins (the northernmost of our study sites) showed a higher lipid, protein, energy content, and fatty acid levels (SFA, MUFA and PUFA) than individuals collected at Yelcho and Rothera stations. The observed differences in the nutritional condition may be due to each population's ability to adjust their biochemical composition in response to the prevailing environmental conditions at each site within their broad latitudinal distribution range (Guzmán-Rivas et al., 2021).

The biochemical composition of marine invertebrates has been shown to be influenced by oceanographic changes exhibited at different latitudes (Guzmán-Rivas et al., 2021). Such changes could be intensified by the contrasting effects of climate change along marine ecosystems of the WAP (Cook et al., 2016). Clear gradient patterns of temperature, primary productivity and other relevant factors as well as biological change along the WAP have been reported (Rogers et al., 2020). In terms of temperature, the clearest partition is between the northern part of the WAP and the central/southern part (Cook et al., 2016). In the northern part, the inflow of water masses from the Weddell Sea can maintain ocean temperatures below 0 °C throughout most of the water column (Moffat & Meredith, 2018). Further south, ocean temperature is dominated by diverse factors (e.g. glacial melt) that result in a water column that is particularly warm at depth (1 °C or higher), capped by a thin, transient, warm layer in summer (Cook et al., 2016). Also, there is significant spatial structure in oceanic primary productivity along the WAP, reflecting combined physical and biogeochemical drivers that include water column structure, upwelling and sea ice seasonality (see Rogers et al., 2020). Marked meridional contrasts are evident during summer, as higher concentrations of chlorophyll-a are present during December-March in the south, while the bloom in northern WAP is more limited to the period December-February (Montes-Hugo et al., 2009; Kim et al., 2018). This regional variability is driven by local environmental settings (Kavanaugh et al., 2015). We suggest that our study species would be likely to present intraspecific variability in nutritional characteristics along the WAP, driven by the gradient and contrasting oceanographic parameters. Future studies could assess the potential local adaptation of its populations (Sanford & Kelly, 2011; Segovia et al., 2020).

Food available in Antarctic sediments, consumed by detritivoral taxa, contains an important source of organic matter from both planktonic and benthic origin (Glover et al., 2008; Minks et al., 2008). Nevertheless, the amount of this food is not stable, since spatio-temporal variations have been observed in the amount of food available in Antarctic sediment (Isla et al., 2011). For instance, sediments with a higher content of lipids and proteins (high food quality) during the autumn and sediments with a higher content of carbohydrates (low food quality) during spring have been recorded (Isla et al., 2011). Moreover, recent spatial variability in total organic carbon (TOC) was found, as a proxy of food quantity, of sediment in a distance gradient from a

deglaciating fjord at WAP (Kim et al., 2021). Their results exhibit a larger proportion of TOC at farther sites than closer sites from the glacier edge (Kim et al., 2021). These TOC results are consistent with previous results of the nutritional condition of the bivalve *Nuculana inequisculpta* at different distances from the glacier in the same Antarctic fjord (Bascur et al., 2020). This research found that individuals captured at the closer site had a poor nutritional condition than individuals captured at the farther site from the glacier (Bascur et al. 2020). In this context, spatial changes in the food quality and quantity from sediment could be expected due to the contrasting pattern of warming along the WAP (Montes-Hugo et al., 2009; Cook et al., 2016). This could be a factor we should also consider in order to explain the high variability found in our results among populations from different geographical origin.

Recently, the reproductive cycle and ontogenetic growth rhythms have been studied in *A. eightsii* at the WAP (Román-Gonzalez et al., 2017; Lau et al., 2018). From these works, the idea of this bivalve species can exhibit different growth rhythms depending on the allocation of energy resources has arisen. This meant that some individuals (or populations) could be in different stages of their growth cycle, either gametogenesis or somatic growth. Based on this, it could be hypothesized that our Rothera population is allocating energy for its somatic growth phase while the O'Higgins and Yelcho populations could be in their gametogenesis energy allocation phase. Therefore, since the Rothera population had a lower nutritional condition, they could have a differential ecological characteristics through reproduction, recruitment, population stability and resilience to ecosystem perturbations compared to the other two populations of the WAP (Steinberg, 2018). Nevertheless, more studies on growth rhythms of different populations of this species along the WAP are highly necessary in order to support this argument.

Our analyses of biochemical composition of *A. eightsii* showed that protein content is the main component of the species dry mass (13.11-22.34% DM), which was almost triple the lipid content (4.60-8.33% DM). Our results are within the range of values previously described in Antarctic non-bivalve invertebrates (i.e. gastropod, ascidian, nemertean), measured between late spring and summer. These have wide protein (5.90-36% DM) and lipid content (4.9-18% DM) ranges (Heine et al., 1991; McClintock et al., 1991, McClintock et al., 1992). Additionally, our lipid results (4.60-8.30% DM) are lower than those observed in other Antarctic bivalve species (*N. inaequisculpta*: 12.20-17.10% DM, *Laternula elliptica*: 6.00-18.00% DM; Ahn et al., 2003; Bascur et al., 2020) and they are very similar with the lipid proportion recorded in sub-polar bivalves (*Nucula sulcata*: 4.95-8.74% DM, *Astarte montagui*: 4.19-5.86% DM; Ansell, 1974; Ansell, 1975). In turn, it is very difficult to make reliable comparisons for our protein results and other bivalve species because there is a lack of methodological standardization for their quantification (Mæhre et al., 2018). However, recent protein data obtained through the Lowry method in the Antarctic bivalve *N. inaequisculpta* (21.10-25.80% DM; Bascur et al., 2020) indicates our protein results (13.11-22.34% DM) present a higher variability, but some of our values are within their reported range.

Within lipid composition, fatty acids have pivotal relevance in the membrane function, nervous system development (Beltz et al., 2007), immune responses (Bell et al., 2006; Fritsche, 2006),

gonadal maturation (Hurtado et al., 2012; Bolognini et al., 2017), growth (Marshall, McKinley & Pearce, 2010) and as energy source in long-term starvation conditions (Auerswald et al., 2015). It is thought that most mollusks, including bivalves, lack the capacity to biosynthesize n-3 and n-6 PUFA de novo (Zhukova, 2019). That is, fatty acids such as EPA (eicosapentaenoic acid: C20: 5*n*-3) and DHA (docosahexaenoic acid: C22: 6*n*-3) are obtained exclusively through food. In this context, *A. eightsii* individuals from O'Higgins station showed a higher amount of total fatty acids (especially PUFA as EPA and DHA) than individuals collected at Yelcho and Rothera stations, likely influenced by different food quantity or quality, in sediment or from phytoplankton, among studied localities (Montes-Hugo et al., 2009; Schofield et al., 2017). Fatty acids can be used as biomarkers of trophic relationships (e.g. see Hughes et al., 2005). Fatty acid markers have proved highly successful in assessing the trophic ecology of Antarctic marine species (e.g. Yang et al., 2016; Servetto et al., 2017; Rossi et al., 2018). Considering the fatty acid profiles found in the present study and the use of fatty acid biomarkers available in the literature, we suggest that *A. eightsii* has an omnivorous feeding behavior, mainly consuming flagellates, detritus, different types of algae and meiofauna (Table 3). Such fatty acid results and diet profile represent a valuable contribution to baselines for (much needed) future studies on WAP marine food webs.

Our present study has three clear limitations. First, the absence of information on gonadal maturation of the analyzed individuals. Our samples were collected in summer, temporally distinct from the spawning season described for *A. eightsii* in the southern WAP during winter (Lau et al., 2018), suggesting gonad maturation would form a minor (if any) component of the variation between locations. A second consideration was what would be the impact of when the Yelcho sample collection is collected in a different year than the other two sample collections. This region may experience significant interannual variability, driven by the Southern Annular Mode (SAM) and El Niño-Southern Oscillation (ENSO) (Martinson et al., 2008; Santamaría-del-Ángel et al., 2021), which can translate into biotic variability. In this context, the oceanographic variables such as temperature and salinity at the southern area of Anvers Island (where Yelcho is located) indicated only limited (but significant) interannual variation between the summer seasons of 2017 and 2018 (Fig. S1). On the contrary, chlorophyll-a did not display significant differences between summers seasons of 2017 and 2018 (Fig. S1). Although we found significant differences in temperature and salinity between the two years, we think that these differences might not have an effect on the biology/physiology of the study species. Therefore, we think that the Yelcho samples can be validly used for comparison between localities. Third, we did not analyze glycogen content, which is a very important component in reproduction. In this context, despite the fact that proteins, lipids and fatty acids are also an important part of the biochemical composition of organisms, we suggest that related future studies prioritize evaluation of glycogen content and its relationship with the reproductive cycle of *A. eightisii*. However, we think the results shown here can be well considered since our biochemical data are within documented ranges in the published literature on Antarctic marine invertebrates (Heine et al., 1991; McClintock et al., 1991; McClintock et al., 1992). Furthermore, our data were obtained

through widely accepted and previously used methods across marine studies. Therefore, we suggest that this study advances a null hypothesis that there is a relationship between physiological and regional oceanographic processes, influencing the nutritional condition of benthic marine invertebrates of the WAP. Only testing with the other taxa will confirm whether *A. eightsii* proves to be a barometer of marine invertebrate responses to physical environment as many considered mollusks to be for reproductive strategies (Thorson's rule, see Mileikovsky, 1971).

Statistically different biochemical composition and energy content among different populations, lead us to postulate that there are modifications in the nutritional condition of *A. eightsii* along the WAP. *A. eightsii* individuals from Rothera present a different energy trade-off from the other two populations. This could be related directly to oceanographic conditions of their habitat. If so, our results not only reflect differences in the nutritional condition along the WAP, but also suggest an important environmental selective force that could be driving contrasting responses of *A. eightsii* populations along the WAP.

Conclusions

The current study provides novel and valuable information on large-scale spatial variation in the biochemical composition and energy content, as a proxy of nutritional condition, of three populations of the bivalve mollusk *A. eightsii* at the WAP. In this context, southern population (Rothera) had lower lipid, protein, energy and fatty acids content than individuals who inhabit at the middle (Yelcho) and northern localities (O'Higgins). It seems likely that these differences are driven by contrasting environmental conditions (e.g. temperature and food availability) at each study site in a latitudinal cline (albeit non-linear one) along the WAP. This could explain why we observed some degree of phenotypic plasticity or local adaptation in energy reserves characteristics among populations.

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Table 1(on next page)

Fatty acid composition (expressed in mg of fatty acid g dry mass^{-1} and in % of total FA pool in parentheses) of the soft tissue of *A. eightsii* collected in three different localities of Western Antarctica Peninsula. Values represent

Different letters in the superscript of each fatty acid (FA) indicate significant differences among localities ($p < 0.05$; parametric post-hoc Tukey HSD or non-parametric multiple range tests). Stat. value represent the statistical value obtained in each analysis (F of ANOVA for C18:0, C22:6*n*-3 and total SFA; U of Mann-Whitney for C18:2*n*-6c and C22:1*n*-9; H of Kruskal-Wallis for all the other comparisons).

Table 1. Fatty acid composition (expressed in mg of fatty acid g dry mass^{-1} and in % of total FA pool in parentheses) of the soft tissue of *A. eightsii* collected in three different localities of Western Antarctica Peninsula. Values represent mean ± SD, n = 58. Different letters in the superscript of each fatty acid (FA) indicate significant differences among localities ($p < 0.05$; parametric post-hoc Tukey HSD or non-parametric multiple range tests). Stat. value represent the statistical value obtained in each analysis (F of ANOVA for C18:0, C22:6*n*-3 and total SFA; U of Mann-Whitney for C18:2*n*-6c and C22:1*n*-9; H of Kruskal-Wallis for all the other comparisons).

	Locality				
Fatty acid	O'Higgins (63°S)	Yelcho (64°S)	Rothera (67°S)	Stat. value	*p* value
C11:0	0.24 ± 0.09 (1.47)^{a}	0.13 ± 0.02 (1.19)^{b}	0.19 ± 0.04 (2.51)^{a}	22.03	< 0.001
C12:0	0.29 ± 0.05 (1.78)^{a}	0.25 ± 0.03 (2.29)^{b}	0.25 ± 0.03 (3.30)^{b}	10.22	< 0.01
C13:0	0.26 ± 0.08 (1.59)^{a}	0.16 ± 0.02 (1.46)^{b}	0.27 ± 0.07 (3.56)^{a}	30.21	< 0.001
C14:0	0.65 ± 0.21 (3.98)^{a}	0.48 ± 0.04 (4.39)^{ab}	0.37 ± 0.10 (4.88)^{b}	20.71	< 0.001
C15:0	0.24 ± 0.07 (1.47)^{a}	0.20 ± 0.05 (1.83)^{a}	0.19 ± 0.05 (2.51)^{a}	6.38	< 0.05
C16:0	4.60 ± 1.42 (28.17)^{a}	2.58 ± 0.65 (23.6)^{b}	2.14 ± 0.55 (28.23)^{b}	30.18	< 0.001
C17:0	0.49 ± 0.14 (3.00)^{a}	0.32 ± 0.12 (2.93)^{b}	0.15 ± 0.03 (1.98)^{c}	39.25	< 0.001
C18:0	2.19 ± 0.47 (13.41)^{a}	1.30 ± 0.30 (11.89)^{b}	1.27 ± 0.38 (16.75)^{b}	36.65	< 0.001
C20:0	0.62 ± 0.12 (3.80)	0	0	-	-
C22:0	0	0.31 ± 0.11 (2.84)	0	-	-
C23:0	0.43 ± 0.09 (2.63)^{a}	0.27 ± 0.10 (2.47)^{b}	0.34 ± 0.03 (4.49)^{b}	26.39	< 0.001
Total SFA	10.01 ± 1.35 (61.30)^{a}	6.00 ± 0.76 (54.89)^{b}	5.17 ± 0.65 (68.21)^{b}	58.27	< 0.001
C14:1*n-5*	0.67 ± 0.28 (4.10)^{a}	0.72 ± 0.17 (6.59)^{a}	0.21 ± 0.02 (2.76)^{b}	28.31	< 0.001
C16:1*n-9*	0.72 ± 0.38 (4.42)^{a}	0.60 ± 0.27 (5.49)^{a}	0.22 ± 0.09 (2.90)^{b}	24.71	< 0.001
C18:1*n-9*	1.06 ± 0.39 (6.49)^{a}	1.85 ± 0.25 (16.93)^{b}	0.46 ± 0.11 (6.07)^{c}	45.04	< 0.001
C20:1	0.99 ± 0.33 (6.06)	0	0	-	-
C22:1*n-9*	0	0.17 ± 0.05 (1.56)^{a}	0.15 ± 0.03 (1.98)^{a}	97.00	0.12
Total MUFA	3.44 ± 0.42 (21.07)^{a}	3.34 ± 0.72 (30.56)^{a}	1.04 ± 0.17 (13.72)^{b}	32.80	< 0.001
C18:2*n-6*c	0.32 ± 0.12 (1.96)^{a}	0.21 ± 0.08 (1.92)^{b}	0	94.50	< 0.01
C18:2*n-6*t	0.24 ± 0.09 (1.47)^{a}	0.14 ± 0.02 (1.28)^{b}	0.15 ± 0.02 (1.98)^{b}	17.90	< 0.001
C18:3*n-6*	0.36 ± 0.13 (2.20)^{a}	0.21 ± 0.04 (1.92)^{b}	0.14 ± 0.02 (1.85)^{c}	36.61	< 0.001
Total *n-6* PUFA	0.92 ± 0.06 (5.63)^{a}	0.56 ± 0.04 (5.12)^{b}	0.29 ± 0.08 (3.83)^{c}	46.29	< 0.001
C20:3*n-3*	0.43 ± 0.13 (2.63)^{a}	0.25 ± 0.07 (2.29)^{b}	0.14 ± 0.03 (1.85)^{c}	42.81	< 0.001
C20:5*n-3*	1.03 ± 0.51 (6.31)^{a}	0.42 ± 0.19 (3.84)^{b}	0.59 ± 0.43 (7.78)^{b}	17.69	< 0.001
C22:6*n-3*	0.50 ± 0.18 (3.06)^{a}	0.36 ± 0.11 (3.29)^{b}	0.35 ± 0.22 (4.62)^{b}	5.33	< 0.01
Total *n-3* PUFA	1.96 ± 0.33 (12.00)^{a}	1.03 ± 0.09 (9.43)^{b}	1.08 ± 0.23 (14.25)^{b}	23.85	< 0.001
Total PUFA	2.88 ± 0.28 (17.64)^{a}	1.59 ± 0.10 (14.55)^{b}	1.37 ± 0.21 (18.07)^{b}	32.37	< 0.001
Total FA	16.33 ± 0.98 (100)^{a}	10.93 ± 0.63 (100)^{b}	7.58 ± 0.48 (100)^{c}	41.57	< 0.001

Abbreviations are the following= SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; SFA= sum of C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0 and C23:0; MUFA= sum of C14:1*n*-5, C16:1*n*-9, C18:1*n*-9, C20:1 and C22:1*n*-9; Total *n-6* PUFA= sum of C18:2*n-6*c, C18:2*n-6*t and C18:3*n-6*; Total *n-3* PUFA = sum of 20:3*n-3*, 20:5*n-3* and 22:6*n-3*; Total PUFA= sum of *n-3* and *n-6* PUFA; Total FA= sum of Total SFA, Total MUFA and Total PUFA.

Table 2(on next page)

Similarity percentage analysis (SIMPER) used to evaluate the contribution of each fatty acid found in *A. eightsii* individuals collected in three different localities at the WAP. n = 58.

The table shows fatty acids that contribute more than 4% to dissimilarity (Contr.%) of each comparison.

Table 2. Similarity percentage analysis (SIMPER) used to evaluate the contribution of each fatty acid found in *A. eightsii* individuals collected in three different localities at the WAP. n = 58. The table shows fatty acids that contribute more than 4% to dissimilarity (Contr.%) of each comparison.

Locality	Diss.%	FA	Av.Ab. 1	Av.Ab. 2	Av.Diss.	Diss./SD	Contr.%	Cum.%
OH vs. Ye	15.83	C20:1	0.99	0	2.97	12.59	18.76	18.76
		C20:0	0.89	0	2.67	15.97	16.85	35.62
		C22:0	0	0.74	2.23	11.48	14.07	49.69
		C22:1*n-9*	0	0.64	1.94	13.85	12.23	61.92
		C20:5*n-3*	0.98	0.79	0.65	1.64	4.13	66.05
OH vs. Ro	18.23	C20:1	0.99	0	3.20	12.56	17.55	17.55
		C20:0	0.89	0	2.87	15.40	15.77	33.32
		C18:2*n-6*c	0.74	0	2.41	9.85	13.24	46.56
		C22:1*n-9*	0	0.62	2.01	21.96	11.02	57.58
		C16:0	1.45	1.20	0.82	1.81	4.48	62.07
		C16:1*n-9*	0.90	0.67	0.74	1.77	4.04	66.11
Ye vs. Ro	11.33	C22:0	0.74	0	2.54	11.72	22.42	22.42
		C18:2*n-6*c	0.67	0	2.28	11.32	20.16	42.57
		C18:1*n-9*	1.16	0.82	1.18	5.76	10.44	53.01
		C14:1*n-5*	0.92	0.68	0.82	4.05	7.25	60.26
		C16:1*n-9*	0.86	0.67	0.67	1.72	5.94	66.20
		C20:5*n-3*	0.79	0.84	0.50	1.39	4.42	70.62

OH O'Higgins station, *Ye* Yelcho station, *Ro* Rothera station, *Diss.%* percentage dissimilarity of each comparison, *FA* fatty acid, *Av.Ab.* average abundance of each fatty acid, *Av. Diss.* the average similarity that each fatty acid contributes, *Diss./SD* the proportion of similarity and standard deviation, *Contr.%* the contribution of each fatty acid to the general dissimilarity, *Cum.%* General additive dissimilarity.

Table 3(on next page)

Fatty acid biomarkers used for trophic relationships in benthic and pelagic marine environments.

Table 3. Fatty acid biomarkers used for trophic relationships in benthic and pelagic marine environments.

Food source	Fatty acid biomarker	References
Bacteria in general	Odd numbered SFA	Volkman et al., 1998
Detritus	C16:0, C22:0, C18:0 + C18:1*n-9*	Dalsgaard et al., 2003
Green algae	C18:2*n-6*, C18:3*n-6*	Cañavate, 2018
Brown algae	C18:1*n-9*, C18:2*n-6*, C20:5*n-3*, C16:0	Zhukova, 2019
Phaeocystis	C18:1*n-9*, C18PUFA + C22:6*n-3*	Legeżyńska, Kędra & Walkusz, 2014
Heterotrophic flagellates	C18:2*n-6*, C22:6*n-3*	Zhukova, 2019
Flagellates in general	C18PUFA + C22:6*n-3*	Legeżyńska, Kędra & Walkusz, 2014
Red algae	C20:5*n-3*, C16:0	Legeżyńska, Kędra & Walkusz, 2014
Meiofauna	C22:6*n-3*, C18:1*n-9*	Zhukova, 2019
Zooplankton (e.g. copepods)	C20:1, C22:1*n-9*	Kelly & Scheibling, 2012
Diatoms and dinoflagellates	C22:6*n-3*, C20:5*n-3*	Dalsgaard et al., 2003; Cañavate, 2018

Abbreviations (SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid)

Figure 1

Map of the *A. eightsii* sampling along the West Antarctic Peninsula (WAP). Filled circles indicate the northern, middle, and southern WAP sampling localities: O'Higgins Base (OB), Yelcho (Ye) and Rothera (Ro), respectively.

Dashed arrows represent Southern Ocean currents, modified from Moffat & Meredith (2018): Antarctic Circumpolar Current (ACC), Antarctic Peninsula Coastal Current (APCC), Coastal Current (CC).

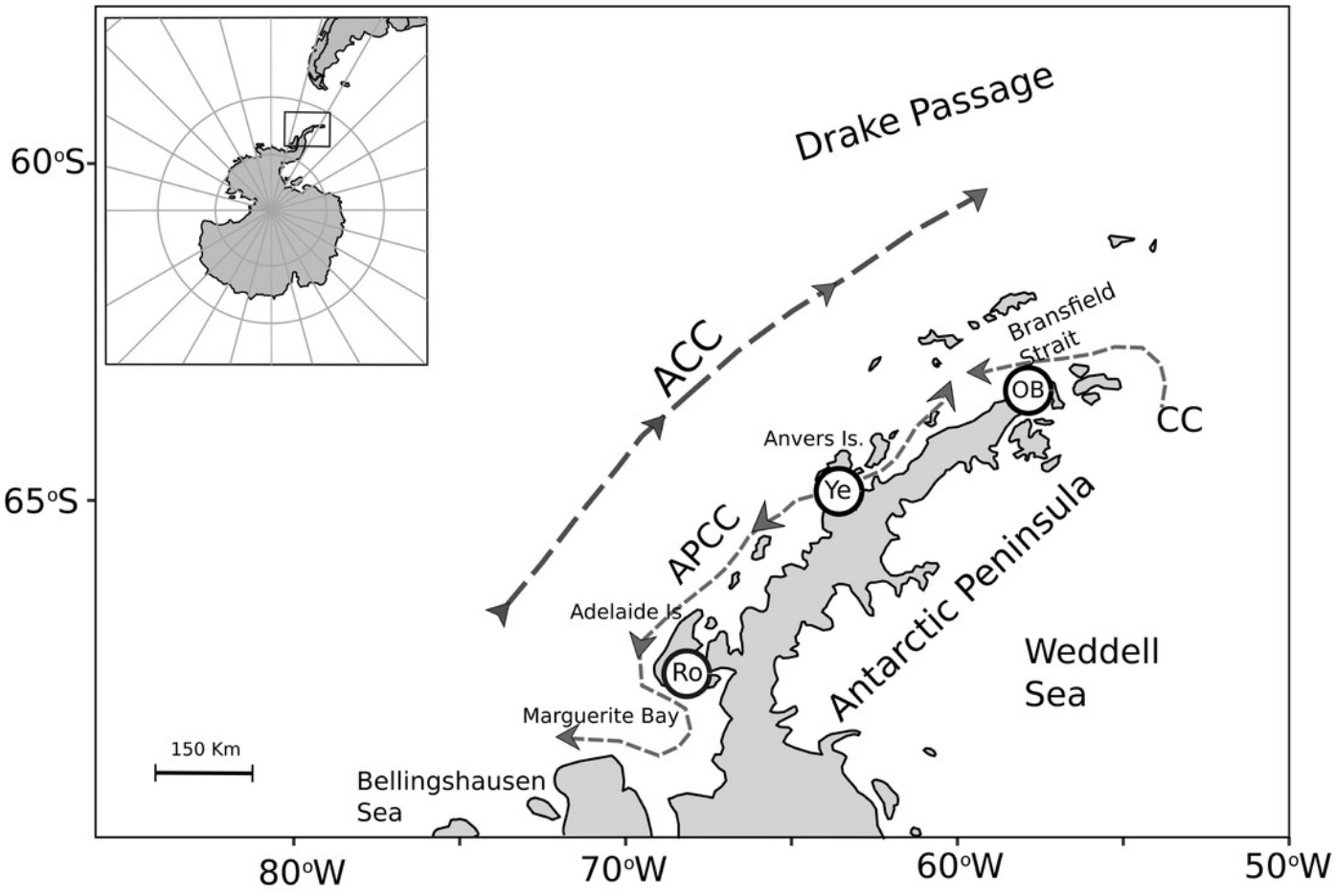


Figure 2

Boxplot of (a) shell length (mm ind.$^{-1}$) and (b) tissue dry mass (mg ind.$^{-1}$) of adult individuals of *A. eightsii* collected at three different localities of the WAP.

Different letters on box indicate significant differences among sites after a multiple range test with a Bonferroni correction. Values represent mean ± SD (n = 58).

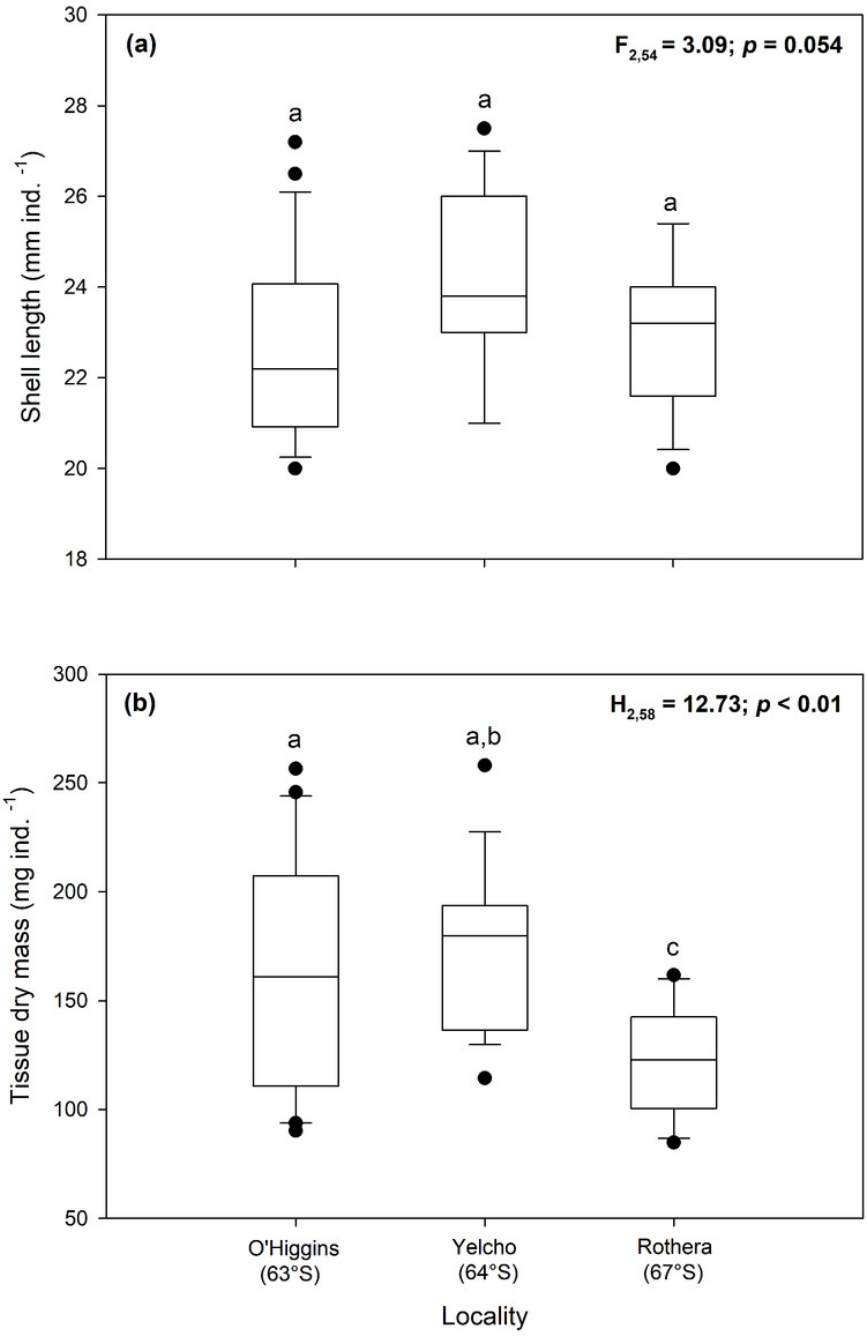

(a)
$F_{2,54}$ = 3.09; p = 0.054
Shell length (mm ind. $^{-1}$)
30
28
26
24
22
20
18
a
a
a
(b)
$H_{2,58}$ = 12.73; p < 0.01
Tissue dry mass (mg ind. $^{-1}$)
300
250
200
150
100
50
a
a,b
c
O'Higgins (63°S)
Yelcho (64°S)
Rothera (67°S)
Locality

Figure 3

Boxplot of (a) lipid content (mg 20 mg^{-1}), (b) lipid content (% DM), (c) protein content (mg 20 mg^{-1}), (d) protein content (% DM) in adult individuals of *A. eightsii* collected at three different localities of the WAP.

Different letters on box indicate significant differences among sites after a multiple range test with a Bonferroni correction. Values represent mean ± SD (n = 58).

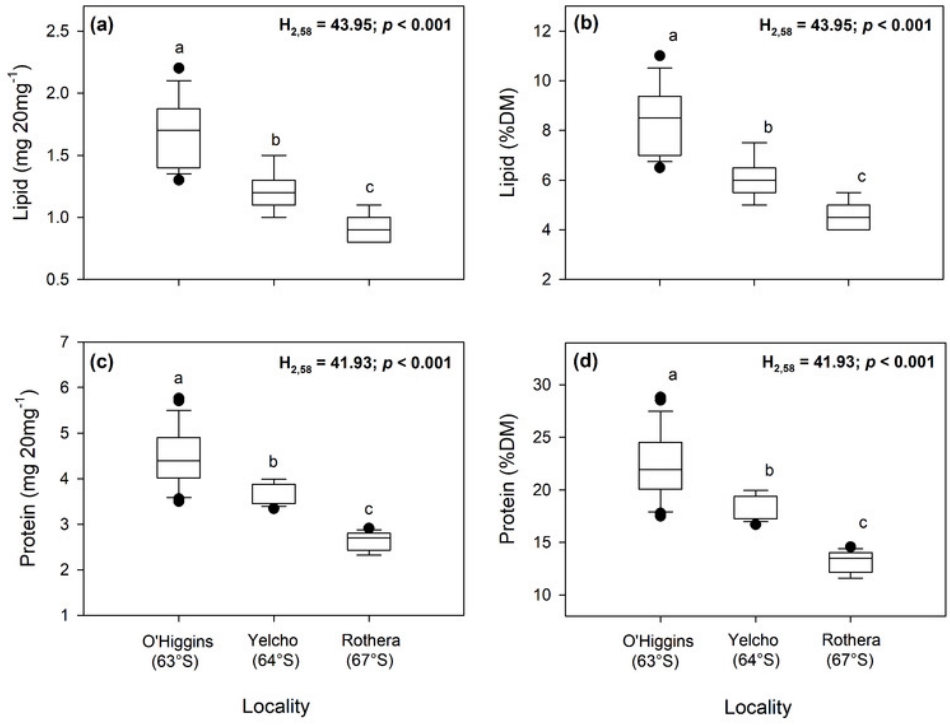
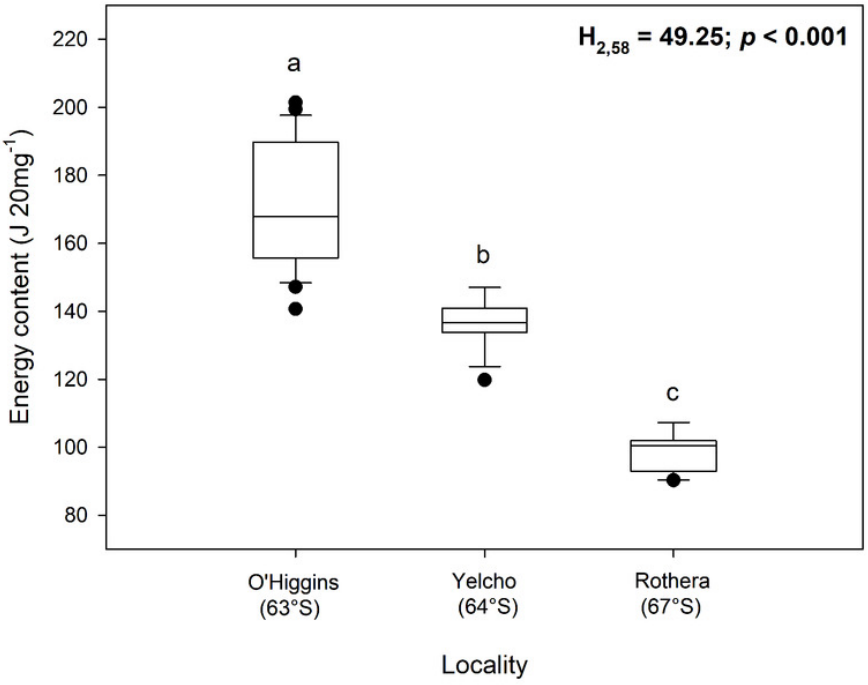


Figure 4

Boxplot of the energy content (J 20 mg^{-1}) in adult individuals of *A. eightsii* collected at three different localities of the WAP.

Different letters on box indicate significant differences among sites after a multiple range test with a Bonferroni correction. Values represent mean ± SD (n = 58).


$H_{2,58}$ = 49.25; p < 0.001
a
b
c
220
200
180
160
140
120
100
80
Energy content (J 20mg^{-1})
O'Higgins (63°S)
Yelcho (64°S)
Rothera (67°S)
Locality