



Seasonal trophic ecology of the dominant Antarctic coral *Malacobelemnion daytoni* (Octocorallia, Pennatulacea, Kophobelemnidae)



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ABSTRACT

Antarctic ecosystems present highly marked seasonal patterns in energy input, which in turn determines the biology and ecology of marine invertebrate species. This relationship is stronger at lower levels of the food web, while upper levels may be less dependent on primary production pulses. The pennatulid *Malacobelemnion daytoni*, is one of the most abundant species in Potter Cove, Antarctica. In order to assess its trophic ecology and energetic strategies, its biochemical (carbohydrates, proteins and lipids), Fatty Acid (FA) and Stable Isotope (SI) ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) compositions were studied over a year-round period. The FA and SI profiles suggest an omnivorous diet and opportunistic feeding strategy for the species. These results, together with biochemical analysis (higher lipid and carbohydrate concentration observed in July and October 2009), support the hypothesis that resuspension events may be an important source of energy, reducing the seasonality of food depletion periods in winter. The evidence presented here gives us a better insight into the success that this species has in Potter Cove and under the current environmental changes experienced by the Antarctic Peninsula.

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1. Introduction

The Southern Ocean is considered an extremely seasonal environment dominated by diatoms and flagellates (Clarke et al., 2007; Orejas et al., 2001; Sakshaug and Holm-Hansen, 1984). With the arrival of the spring-summer season, there is an increase in light intensity and a retraction of sea-ice coverage, promoting the proliferation of these microorganisms, increasing primary production in surface waters and fueling the overall system (Clarke, 1988; Holm-Hansen, 1985; Würzberg et al., 2011). Part of the organic matter (OM) produced is consumed by the zooplankton in the water column, whilst the other parts reach the bottom quickly, sometimes without significant degradation (Cripps and Clarke, 1998; Isla et al., 2009; Rossi et al., 2013; von Bodungen et al., 1988). This OM supplies the underlying complex benthic

communities with high quality material, and these are highly dependent on this seasonal energy input (Gili et al., 2006; Isla et al., 2006; Rossi et al., 2013). Knowledge of the ecology and function of the Antarctic benthos is becoming more important than previously thought, due to its role as a potential carbon sink (Peck et al., 2010; Barnes, 2015).

Seasonal trophic ecological studies are especially difficult in polar areas because samplings are quite limited in space and time. Information on the functioning of polar benthic communities and, particularly, their trophic links and the patterns of energy flow in a seasonal context is still fragmented (Norkko et al., 2007; Pasotti et al., 2015). The use of trophic biomarkers such as the biochemical balance (protein-lipid-carbohydrate), fatty acids (FAs) and stable isotopes (SIs) are useful tools to assess energy origin and fate in autecological studies. Lipids are an important chemical component of marine invertebrates. They are formed principally by FAs obtained from endogenous biosynthesis or heterotrophic feeding, associated with diet, the available food in the environment and the sexual cycle (Baptista et al., 2012; Gori et al., 2012; Pernet et al.,

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2002; Viladrich et al., 2016). They have also been long used to assess relationships between primary producers and consumers, as biomarkers of symbiotic algae and for the chemotaxonomy of octocorals (Imbs and Dautova, 2008; Imbs et al., 2007). The study of protein, carbohydrate and lipid concentrations is useful to detect oscillations in the seasonal cycle of energy storage. In addition, biochemical analysis is an excellent tool to understand the relationships between reproductive cycles, feeding and environmental factors in the water column (Bramanti et al., 2013; Gori et al., 2007; Oku et al., 2003; Pernet et al., 2002; Rossi et al., 2006a, 2008). Other biomarkers used to estimate trophic position are SIs. The analysis of isotopes has been particularly useful for studies of the trophic ecology of suspension and depositivore feeders in Antarctica (Elias-Piera et al., 2013; Gillies et al., 2013; Jacob et al., 2005; McClintic et al., 2008; Mincks, 2005; Mincks et al., 2008; Mintenbeck et al., 2007; Norkko et al., 2007; Pasotti et al., 2015; Purinton et al., 2008). Despite the extended use of SIs at different ecological and spatial levels, studies on a seasonal basis are lacking so far.

Understanding the trophic ecology of benthic suspension feeders during an annual cycle is essential not only to understand their role in benthic–pelagic coupling processes but also to improve our knowledge of ecosystem structure and function. In Antarctica, one of the dominant groups in benthic ecosystems is cnidarians (anthozoans like octocorals). Studies of trophic ecology in these organisms are scarce (Elias-Piera et al., 2013; Gili et al., 1996; Gili and Coma, 1998; Slattery et al., 1997), and most of them are based on experimental designs and gut content analysis or feeding rate studies, in experimental conditions considering only summer months (Alva et al., 1997; Gili et al., 2006; Orejas et al., 2000, 2001, 2003). Thus, a strategy of Antarctic benthic octocorals for coping with months of limited food is scarcely known.

The pennatulid *Malacobelemnion daytoni* is one of the dominant species in the shallow benthic community at Potter Cove, Antarctica (Sahade et al., 1998, 2015). Its abundance and distribution range has significantly increased during the past few years, probably favored by factors driven by climate change (increased sedimentation caused by glacier retreat and ice impact) (Sahade et al., 1998, 2015). Even though physiological tolerance to sediment and a striking reproductive strategy was recently observed (Servetto and Sahade, 2016; Servetto et al., 2013; Torre et al., 2012), the ecological success of this octocoral in Potter Cove is not yet completely understood.

We used a combined approach on the basis of biochemical (lipids, carbohydrates and proteins) and trophic marker (FAs and SIs) dynamics in a year-round period with the aim of assessing autecological strategies of *M. daytoni*. This will be an important contribution to current knowledge of coastal ecosystem responses under ongoing climate change especially in the Antarctic Peninsula (AP).

2. Material and methods

2.1. Biological sampling

M. daytoni colonies were sampled by scuba diving at 15 m at Potter Cove (62° 14' S, 58° 38' W; Isla 25 de Mayo) Antarctica (Fig. 1). Samples were collected monthly from April 2009 to February 2010; colonies were conserved at –80 °C. The same colony was divided and used for: SI, FAs and biochemical analyzes. Number of samples used was depended to the analysis.

2.2. Trophic biomarkers

FAs determination was made using samples from summer,

autumn, winter and spring (n = 6; except in spring n = 5). Approximately 10–12 mg (±0.1 mg) of each dry weight sample was studied. FA were identified and quantified with gas chromatography (GC) (Agilent Technologies 7820 A GC). Analysis was performed in splitless injection mode using a Thermo Trace GC instrument fitted with a flame ionization detector, and a DB–5 Agilent column (30 m length, 0.25 mm internal diameter and 0.25 μm phase thickness). Helium was used as a carrier gas at a constant flux of 33 cm s^{–1}. For the FAs fraction, the oven temperature was programmed to increase from 50 °C to 320 °C at 10 °C min^{–1}, and held at 320 °C for 17 min. Injector and detector temperatures were kept constant through the analysis at 300 °C and 320 °C, respectively. Methyl esters of fatty acids (FAME) were identified by comparing their retention times to those of an external standard (37 FAME compounds, Supelco® Mix C⁴–C²⁴). The reproducibility of the procedure was evaluated by injecting blanks and internal standards at different concentrations. A blank sample was analyzed in every batch of 14 samples to monitor background levels of FAME during the analysis. Fatty acids were quantified by integrating areas under the peaks in the gas chromatograph traces (Chromquest 4.1 software) with calibrations derived from internal standards (Gori et al., 2012; Viladrich et al., 2016).

SI (δ¹⁵N and δ¹³C) composition of the sea pen tissue was determined with a ThermoFinnigan delta plus mass spectrometer (GeoBio Center). Measurements were made on approximately 1–1.5 mg of dry tissue from each colony, monthly-sampled (n = 6) from April to December 2009 and January–February 2010. Isotope ratios are expressed as parts per thousand (‰; difference from a standard reference material) according to the following equation:

$$\delta^{13}\text{C} \text{ o } \delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 100$$

R = ¹³C/¹²C o ¹⁵N/¹⁴N respectively

Although lipids are depleted in ¹³C and are thought to introduce bias into stable isotope analyses that use δ¹³C, lipid extraction was not performed before SI analysis as the effect of this upon nitrogen signature is still under debate (Mintenbeck et al., 2008; Post et al., 2007). Therefore, we used two mathematical normalizations, which use the carbon-to-nitrogen (C:N) ratio of a sample to normalize δ¹³C after analysis (Post et al., 2007; Logan et al., 2008). The first model (M1, Post et al., 2007) is a lipid-correction linear equation developed for aquatic animals: δ¹³C_{lip-corr} = δ¹³C – 3.32 + 0.99 x C:N. The second model (M2, Logan et al., 2008) is a lipid-correction equation for aquatic invertebrates: δ¹³C_{lip-corr} = δ¹³C + 3.3338 – ((3.388 x 3.314)/C:N).

2.3. Organic matter content

For OM content, 10–15 mg of tissue was taken monthly from each colony, dried at 80 °C for 48 h until constant weight. The tissue was then burned for 5 h at 450 °C, and weighed again. The difference between dry and ash weight is the OM (Rossi et al., 2006a).

2.4. Biochemical analysis

Biochemical analyses (carbohydrates, proteins and lipids) consisted of colorimetric quantifications, using a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan); the results are presented in μg carbohydrate–protein–lipid mg^{–1} of OM ± standard deviation (SD), using monthly samples. The axis was separated from all the samples before measurement.

For proteins, 10–15 mg of tissue (n = 20; except: May 11; July n = 17; August n = 11; October n = 6) was weighed using a microbalance (precision: 0.01 mg), homogenized in 6 ml 1 N NaOH,

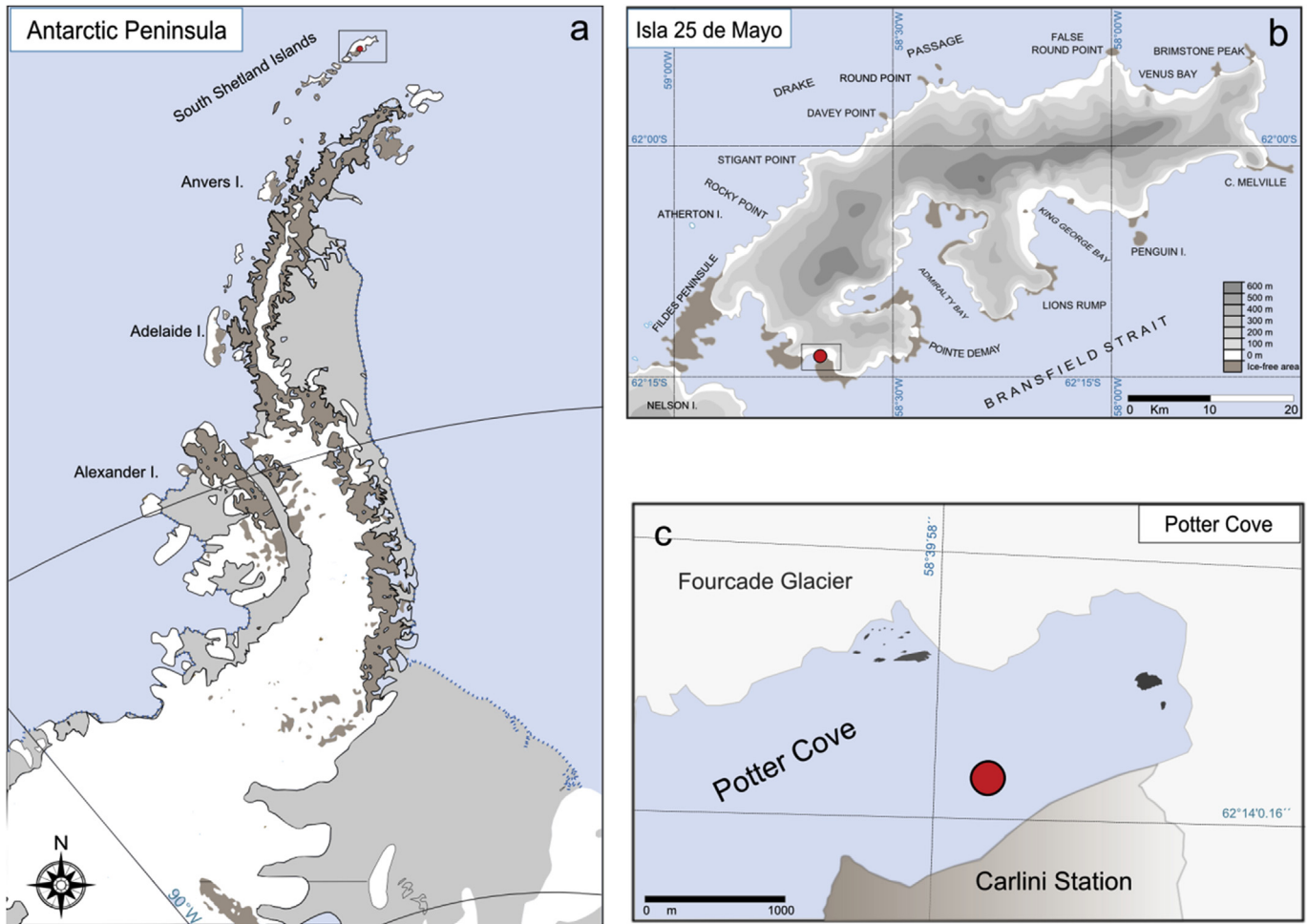


Fig. 1. a) Antarctic Peninsula. b) Location of Potter Cove in South Shetland Island and c) sampling zone in Potter Cove.

and quantified following the method of Lowry et al. (1951), using albumin as standard. For carbohydrates, 15–20 mg of tissue ($n = 20$; except: May 11; July $n = 17$; August $n = 11$; October $n = 6$) was homogenized in 6 ml of double distilled water. Carbohydrates were quantified using the method of Dubois et al. (1956), using glucose as standard. Finally, to determine lipid content ($n = 10$; except October $n = 6$), approximately 15 mg (± 0.01 mg) dry weight from each sample was homogenized in 3 ml of chloroform:methanol (2:1), and total lipids were quantified colorimetrically (Barnes and Blackstock, 1973), with cholesterol as a standard. These methodologies have been previously tried and tested with other anthozoans (Elias-Piera et al., 2013; Gori et al., 2013, 2007; Rossi et al., 2006a).

2.5. Statistical analyses

Normal distribution of residuals was tested using the Shapiro-Wilk test performed with the R-language function *shapiro.test* of the R software platform (R Development Core Team, 2014; <http://www.R-project.org>), and homoscedasticity was tested using Levene's Test. When OM, lipids, protein and carbohydrates failed to meet the assumptions, non-parametric Kruskal-Wallis (K-W) tests were used with the R-language *kruskal.test* function and subsequent post-hoc tests, after Nemenyi (multiple range tests), using a *posthoc.kruskal.nemenyi.test* function. Regression analysis was also

used to test the relationship between lipid concentration and the seasonal reproductive variables calculated in Servetto and Sahade (2016): effective relative fecundity (ERF), number of mature oocytes per month and oocyte diameter per month.

Seasonal FAs profiles were compared using non-metric ANOSIM (analysis of similarity). Similarities were calculated on the untransformed qualitative fatty acid data (%TFA) based on the Bray-Curtis similarity matrix. The ANOSIM test statistic R, based on the ratio of the between-group to within-group similarity ranking, was reported as a value between 0 and 1 (1 indicates complete dissimilarity, 0 indicates complete similarity), together with a corresponding significance value based on 9999 permutations. SIMPER (similarity percentage) was used to assess which fatty acids were primarily responsible for any differences between groups.

The Bayesian stable-isotope mixing model (SIAR; Parnell et al., 2010) was used to estimate the contribution of different potential food sources throughout the year. The lipid-corrected $\delta^{13}\text{C}$ values after M1 (Post et al., 2007) were used for *M. daytoni* samples, while the isotopic signatures of food sources were taken from the literature (Pasotti et al., 2015). Some sources were pooled, as their isotopic signatures had close values, as is the case of 'crustacea', which includes all but copepods, which stayed as a single item. Before running the model, fractionation values of $2.2 \pm 0.3\text{‰}$ and $0.5 \pm 0.13\text{‰}$, for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ respectively, were used following McCutchan et al. (2003).

3. Results

3.1. Stable isotopes

Both mathematical model (M1 and M2) corrections lead to a significant increase of $\delta^{13}\text{C}$ (ANOVA $F = 73.33$, $p < 0.0001$) with respect to raw $\delta^{13}\text{C}$ values. The average $\delta^{13}\text{C}_{\text{raw}}$ and $\delta^{15}\text{N}$ values of *M. daytoni* were -24.22 ± 0.56 and 6.33 ± 0.34 , respectively, while average $\delta^{13}\text{C}_{\text{lip-corr}}$ was -23.07 ± 0.56 (M1). The slight variation of isotopic signature was also evident in the dual-isotope plot generated from the Bayesian model (Fig. 2), in which all the samples showed a narrow range of distribution in the mixing space. Nonetheless, significant differences were detected during the year, especially for $\delta^{13}\text{C}$ (ANOVA $\delta^{13}\text{C}$: $F = 12.5$, $p = 5.79 \cdot 10^{-11}$; $\delta^{15}\text{N}$: $F = 3.18$, $p = 0.0028$) the variation of which was greater than that of $\delta^{15}\text{N}$ (Fig. 2).

All samples of *M. daytoni* fell within the mixing polygon defined by the food sources considered. The dual-isotope plot of isotopic signatures describes the mixing space defined by the potential prey, where the consumer's isotopic signature must be within a polygon bounding the signatures of the sources. Isotopic signatures of *M. daytoni* fell within the polygon, suggesting that macroalgae (detritus), sediment and copepods are likely food sources. The monthly single analysis for each source shows that the diet of *M. daytoni* seems to be mainly an omnivorous. Since copepods, phytoplankton and macroalgae seem to contribute in part of the diet; even sediment and SPM were observed (Fig. 3).

3.2. Fatty acid composition

We detected up to 29 fatty acids in *M. daytoni*, each of which was present throughout the year (Table 1). The general FA composition (%TFA), was significantly different among seasons (ANOSIM $R = 0.3456$, $p = 0.001$) (Fig. 4) with C20:4n-6, C20:3, C16:0, C20:1 and 18:0 primarily accounting for the differences (SIMPER overall average dissimilarity of 29.39%). These FAs showed a contribution of 70% to the global differences (24.66, 19.52, 12.24, 8.35, and 7.94%, respectively). Pairwise comparison determined that the summer profile of FAs significantly differed from that of winter (ANOSIM

$R = 0.7333$, $p = 0.015$) and spring (ANOSIM $R = 0.5947$, $p = 0.0498$). Pairwise comparisons among seasons are summarized in Table 2.

Total saturated fatty acids (SFAs) were not significantly different among seasons (Fig. 5) but some SFAs showed seasonal variation. Stearic acid (C18:0) slightly increased in spring (ANOVA $F = 4.26$; $p = 0.013$), while palmitic acid (C16:0) stayed steady throughout the year with negligible differences between seasons ($p > 0.05$) (Table 1). Monounsaturated fatty acids (MUFAs) varied seasonally (Fig. 4), decreasing from summer to spring (ANOVA $F = 7.80$; $p = 0.001$). The most abundant MUFAs included C18:1n-9 ($5.32 \pm 1.71\%$), C18:1n-7 ($3.44 \pm 0.73\%$) and C20:1 (4.85 ± 3.80). The variation was seasonally significant for all the three MUFAs (ANOVA C18:1n-9: $F = 7.54$, $p = 0.002$; C18:1n-7: $F = 6.85$, $p = 0.003$; C20:1: $F = 9.49$, $p = 0.001$) but showing different trends. Polyunsaturated fatty acids (PUFAs) also varied seasonally (ANOVA $F = 5.96$, $p = 0.048$) (Fig. 4). Among these, the major FAs included C20:3 ($15.52 \pm 3.40\%$) and arachidonic acid C20:4n-6 ($15.59 \pm 8.32\%$) with the C20:4n-6 being the only one that showed seasonal differences (ANOVA $F = 4.49$, $p = 0.015$).

3.3. Biochemical levels

The highest OM proportions were found in January and June 2009 (0.84 ± 0.02 ; 0.83 ± 0.02 respectively), and the lowest in July and October 2009 (0.61 ± 0.11 ; 0.60 ± 0.05 respectively). Significant differences between months were found for lipid, carbohydrate and protein concentrations (K-W; $H = 61.75$, $p < 0.0001$, $H = 99.47$, $p < 0.0001$; $H = 106.18$, $p < 0.0001$; respectively). The highest lipid concentrations were observed in July and October 2009 (164.34 ± 8.50 and $167.11 \pm 21.41 \mu\text{g lipid mg}^{-1}\text{ OM}$; respectively). The lowest values corresponded to January and February 2010 (102.28 ± 10.86 and $100.21 \pm 24.50 \mu\text{g lipid mg}^{-1}\text{ OM}$; respectively). The highest carbohydrate concentrations were observed in July and October 2009 (168.81 ± 38.90 ; $158.55 \pm 23.84 \mu\text{g carbohydrates mg}^{-1}\text{ OM}$; respectively), and the lowest in November 2009 ($104.59 \pm 15.54 \mu\text{g carbohydrates mg}^{-1}\text{ OM}$) and January 2010 ($104.09 \pm 20.05 \mu\text{g carbohydrates mg}^{-1}\text{ OM}$). Finally, the lowest protein concentration was observed in June 2009 ($508.69 \pm 49.87 \mu\text{g protein mg}^{-1}\text{ OM}$) while the highest

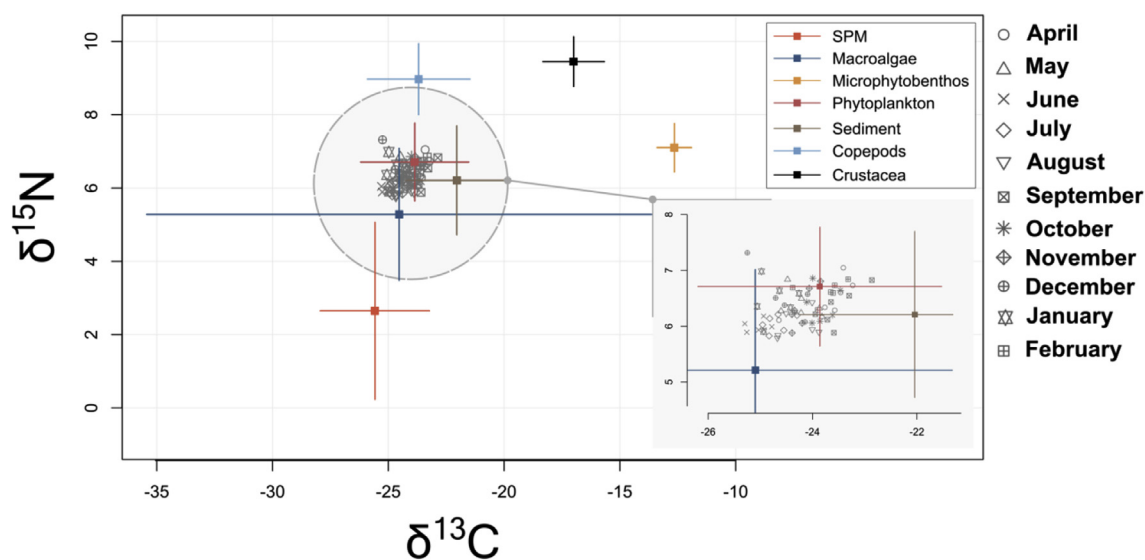


Fig. 2. Dual-stable isotope plot for potential food sources that could contribute to the diet of *Malacobolemon daytoni* throughout an eleven-month sampling period (April 2009 to February 2010). Dual-plot constructed using the Bayesian mixing model SIAR (Parnell et al., 2010). The isotopic signatures of food sources were taken from the literature for different taxa studied in Potter Cove (Pasotti et al., 2015). Error bars represent 95% confidence intervals and incorporate the error in the source isotopic signatures and in trophic enrichment factors ($-25.03 \pm 5.45\%$ $\delta^{13}\text{C}$ and $3.08 \pm 0.85\%$ $\delta^{15}\text{N}$).

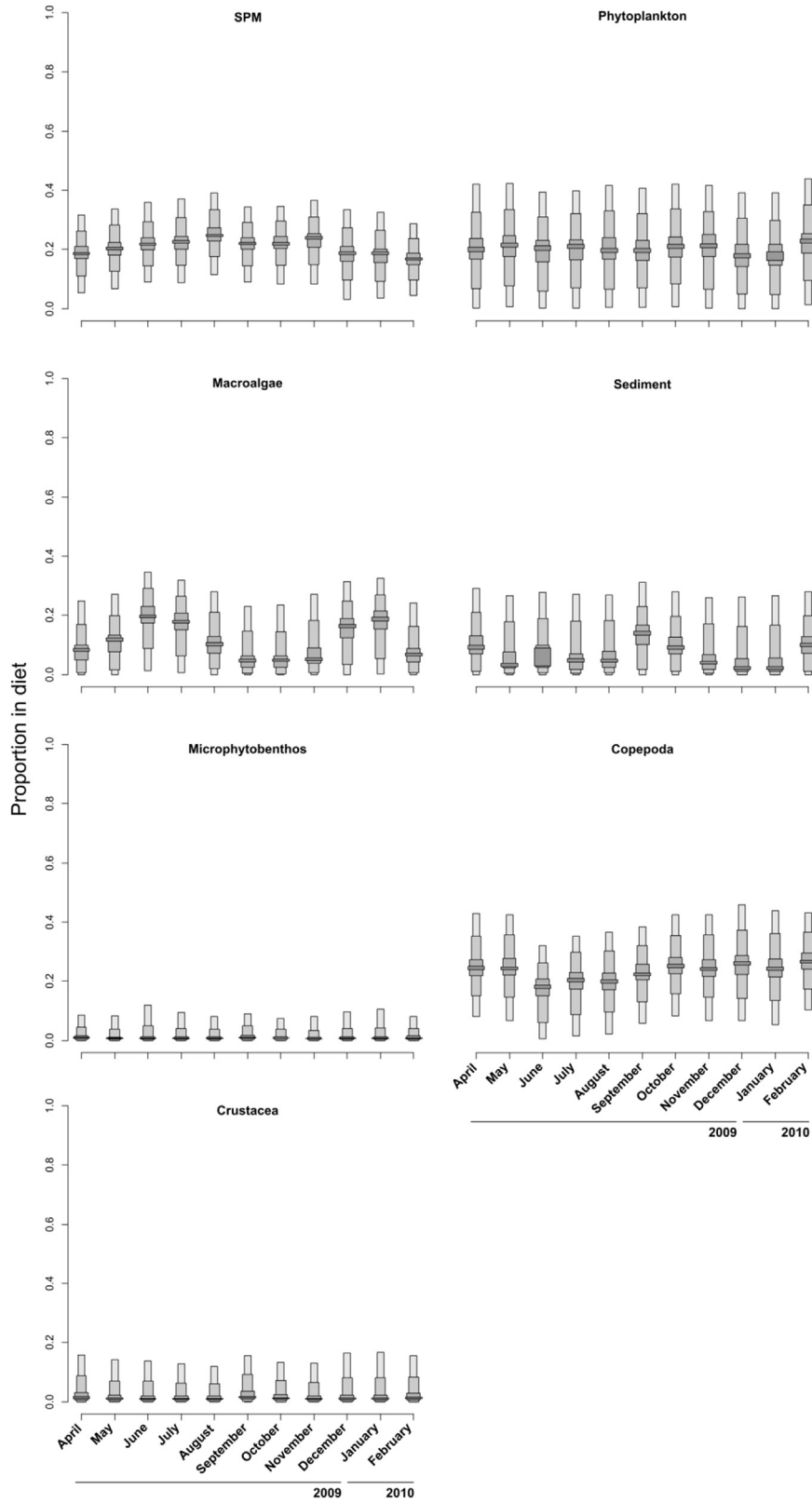


Fig. 3. Comparison of the proportions of food sources incorporated into the diet of *Malacobolemnion daytoni* from April 2009 to February 2010. Boxplots show the estimated proportional contribution of each food source to the diet of *Malacobolemnion daytoni* over the eleven sample months. The widths of the gray bars (dark to light) show the 95, 75, 50 and 25% confidence intervals.

Table 1

Proportion (%) of the different fatty acids seasonally detected in samples from *Malacobelemnion daytoni* (n = 6; spring n = 5).

	Summer	Autumn	Winter	Spring
C12:0	0.13 ± 0.07	0.10 ± 0.08	0.09 ± 0.11	0.07 ± 0.08
C13:0	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
C14:0	3.21 ± 0.65	2.61 ± 0.98	1.78 ± 0.41	1.66 ± 0.49
C15:0	0.68 ± 0.16	0.67 ± 0.24	0.58 ± 0.10	0.65 ± 0.15
C16:0	25.8 ± 3.80	20.32 ± 11.75	25.27 ± 4.22	26.39 ± 5.22
C17:0	0.82 ± 0.23	0.39 ± 0.46	0.64 ± 0.5	1.07 ± 0.23
C18:0	14.24 ± 2.93	12.2 ± 3.07	11.21 ± 2.14	17.60 ± 4.43
C20:0	1.04 ± 0.3	0.57 ± 0.15	0.57 ± 0.35	1.00 ± 0.30
C21:0	2.17 ± 0.73	3.53 ± 1.16	0.68 ± 0.56	0.77 ± 0.90
C22:0	0.24 ± 0.37	0.08 ± 0.10	0.16 ± 0.24	0.09 ± 0.13
C24:0	0.13 ± 0.03	0.08 ± 0.06	0.05 ± 0.03	0.06 ± 0.03
∑ SFA	48.5 ± 8.19	40.57 ± 6.57	41.02 ± 7.84	49.37 ± 8.89
C14:1 (n-5)	0.01 ± 0.01	0.02 ± 0.04	0.13 ± 0.19	0
C16:1 (n-7)	2.42 ± 0.65	2.69 ± 0.72	2.77 ± 0.57	1.60 ± 0.86
C18:1 (n-9)	5.56 ± 1.70	7.11 ± 1.23	5.62 ± 1.12	3 ± 1.65
C18:1 (n-7)	3.50 ± 0.36	3.57 ± 0.69	4.23 ± 0.60	2.47 ± 0.87
C20:1 (n-9)	10.29 ± 4.42	4.54 ± 3.66	2.80 ± 0.66	1.77 ± 0.34
C22:1 (n-9)	0.88 ± 0.36	0.50 ± 1.18	0.52 ± 0.21	0.65 ± 0.33
C24:1 (n-9)	0.12 ± 0.2	0.02 ± 0.04	0.11 ± 0.19	0.03 ± 0.04
∑ MUFA	22.76 ± 3.69	18.43 ± 2.67	16.18 ± 2.16	9.52 ± 1.17
C18:2 (n-6)	0.98 ± 0.27	1.14 ± 0.29	0.71 ± 0.16	0.48 ± 0.20
C18:3 (n-6)	0.31 ± 0.75	0	0	0.03 ± 0.07
C20:2 (n-6)	0.79 ± 0.23	0.06 ± 0.15	0.85 ± 0.18	0.70 ± 0.41
C20:3	10.89 ± 5.44	18.60 ± 18.47	15.14 ± 6.37	17.44 ± 5.55
C20:4 (n-6)	4.71 ± 4.49	13.63 ± 11.70	23.23 ± 4.96	20.79 ± 14.11
C20:5 (n-3)	0	3.01 ± 7.38	0	0
C22:2 (n-6)	1.14 ± 0.40	0.46 ± 0.16	0.73 ± 0.57	0.68 ± 0.41
C22:6 (n-3)	5.74 ± 5.18	3.97 ± 2.26	1.97 ± 0.54	0.89 ± 0.23
∑ PUFA	24.54 ± 3.80	40.87 ± 7.06	42.63 ± 8.84	41.01 ± 8.68

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid and PUFA: polyunsaturated fatty acids.

corresponded to July and October 2009 (772.70 ± 152.26; 821.69 ± 128.08 µg protein mg⁻¹ OM respectively) (Fig. 6).

No relationship was observed between lipids and any seasonal reproductive variables (ERF: R² = 0.23, p = 0.1648; N° of mature oocytes: R² = 0.11, p = 0.3551 and oocytes size: R² = 0.13, p = 0.3069).

Table 2

Pairwise comparison between seasons.

Pairwise comparison	R-value	p-value
Summer-Autumn	0.2593	0.0894
Summer-Winter	0.7333	0.0162
Summer-Spring	0.5947	0.0426
Autumn-Winter	0.1778	0.1710
Autumn-Spring	0.1733	0.4506
Winter-Spring	0.2773	0.1506

Bold numbers indicate statistical differences.

4. Discussion

This work is the first study in an Antarctic benthic coral analyzing trophic ecology and energetic strategies, and one of the few using such different approaches as biochemical analysis, FA and SI compositions to assess autecological features in suspension feeders in a year-round period. Knowledge of the ecological strategies used by different species is essential to obtain a better insight into the success of the species in their systems, and to also improve understanding of the functioning of these systems and their responses to environmental shifts such as those driven by the ongoing climatic change affecting the AP.

4.1. Food sources

During the study period, there was no constant dominance of any particular FA, as was also observed by (Baptista et al., 2012) in *Veretillum cynomorium*. This seasonal variation in FA profiles could be related with the different food sources used during the year. MUFA and PUFA composition in the *M. daytoni* sea pen underwent marked seasonal changes. In contrast, total SFA did not vary significantly with the seasons but some of them individually showed differences. The most abundant SFAs in *M. daytoni* were 16:0 and 18:0 year-round, and similar results have been observed in some corals (Baptista et al., 2012; Imbs et al., 2009, 2007; Pernet et al., 2002). These FAs are known to be the key starting materials for the biosynthesis of essential PUFAs (Carballeira et al., 1997), so they are always expected in high concentrations, especially in

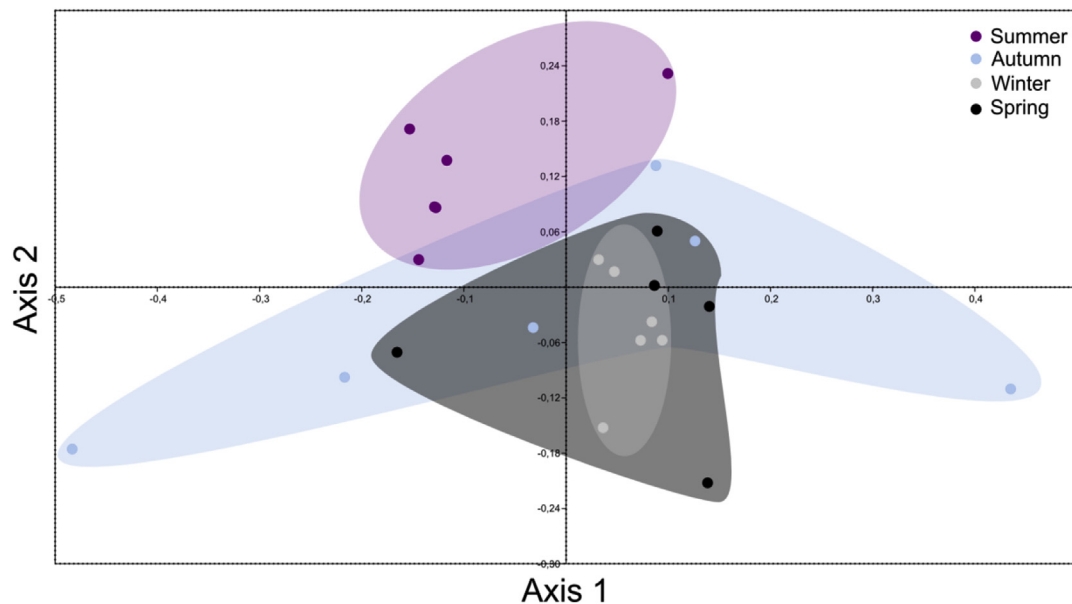


Fig. 4. Two-dimensional nMDS ordination for total fatty acid. Points refer to a colony of *Malacobelemnion daytoni*. (n = 6; spring: n = 5).

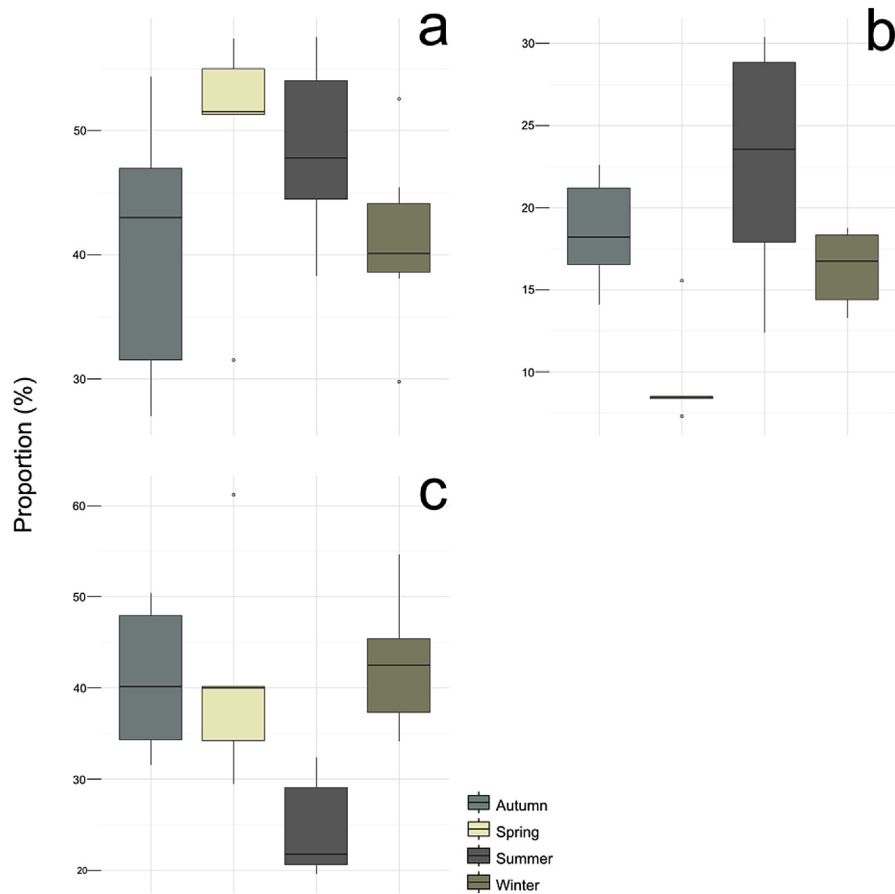


Fig. 5. Seasonal variation of a) saturated (SFA), b) monounsaturated (MUFA) and c) polyunsaturated (PUFA) fatty acids. ($n = 6$; spring: $n = 5$) in the sea pen *Malacobelemnon daytoni*. Boxplot illustrate median, second and third quartile. Whiskers are drawn to nearest value not beyond 1.5 x (inter-quartile range).

metazoans.

The most abundant MUFAs registered in *M. daytoni* were eicosanoic acid (20:1n-9) and oleic acid (18:1n-9). The high levels observed of the 18:1n-9 FA in autumn and winter points to degraded material incorporated in its diet (Graeve et al., 2001). This suggests a scavenging strategy of this species at least during that season, finding degraded material probably associated with resuspension events of the bottom. This hypothesis matches the high proportions of sediment (feeding on unselected sediment organic matter) in the diet of *M. daytoni*, mainly in autumn and late winter, observed with SI markers.

The PUFAs reflect an herbivore contribution to the diet of *M. daytoni*. There were more of these FAs in summer and autumn when phytoplankton is more abundant. This is evidenced by the FAs 22:6n-3 and 20:5n-3, which are biomarkers for diatoms and are known to accumulate in phytoplankton grazers (Graeve et al., 1994). Despite Potter Cove presenting a characteristic low pelagic primary production, during the sample year there was a particular increase in phytoplankton biomass reaching an unusual peak for this ecosystem (Schloss et al., 2014). The ratio of n-6 to n-3 FAs may reflect the ratio of the contribution of detritus to that of fresh material. A higher n-6/n-3 ratio was found in spring and winter, suggesting an increased contribution of degraded material in these seasons. It is known that n-6 PUFA originates from benthic algae and that some of the benthic invertebrates contain considerable quantities of n-6 (Fleurence et al., 1994; Khotimchenko, 1995). In *M. daytoni*, the major n-6 fatty acids observed were 20:3 and 20:4. The FA 20:4n-6, arachidonic acid (AA), occurs in marine

macroalgae, and is thought to be transferred to *M. daytoni* from macroalgal detritus (Fukuda and Naganuma, 2001; Gori et al., 2012). This was the most abundant FA observed in winter in *M. daytoni*. Similarly, with SI biomarkers, macroalgae were found as a food source mainly in winter but even in summer.

Another important component (FA 20:3) incorporated in the diet of this pennatulid was microalgae, observed principally in autumn and spring. With SIs analysis, this food source (phytoplankton) was very abundant throughout the study period. The pattern of this biomarker was similar pattern to that of FAs, but with less marked seasonal changes year-round, which is to be expected since SIs can integrate longer time signals than FAs.

The FA 20:1n-9 is considered a good indicator of copepods in the diet (Kattner and Hagen, 1995), and this was the dominant FA in summer samples of *M. daytoni*, highlighting a predatory strategy of this species when zooplankton is more available. Consistently, SIs biomarkers indicated that copepods were present as a food source throughout the year, but especially at the end of the summer and in spring. In Potter Cove, a high abundance of small copepods was recorded all year. According to Fuentes (2006), the “resident mesozooplankton” in Potter Cove is likely to consist of copepod species, such as *Oithona similis*, *Oncaea curvata* (small copepods: < 1 mm of total length), and the “sporadic mesozooplankton” to consist of *Euphausia superba*, *Salpa thompsoni* and of large copepods (> 1 mm in total length), such as *Calanoides acutus*, *Calanus propinquus* and *Rhincalanus gigas*. In the summer of 2010, colder than usual conditions prevented glacier melt, favoring the development of an exceptional micro-sized diatom bloom, which was closely followed

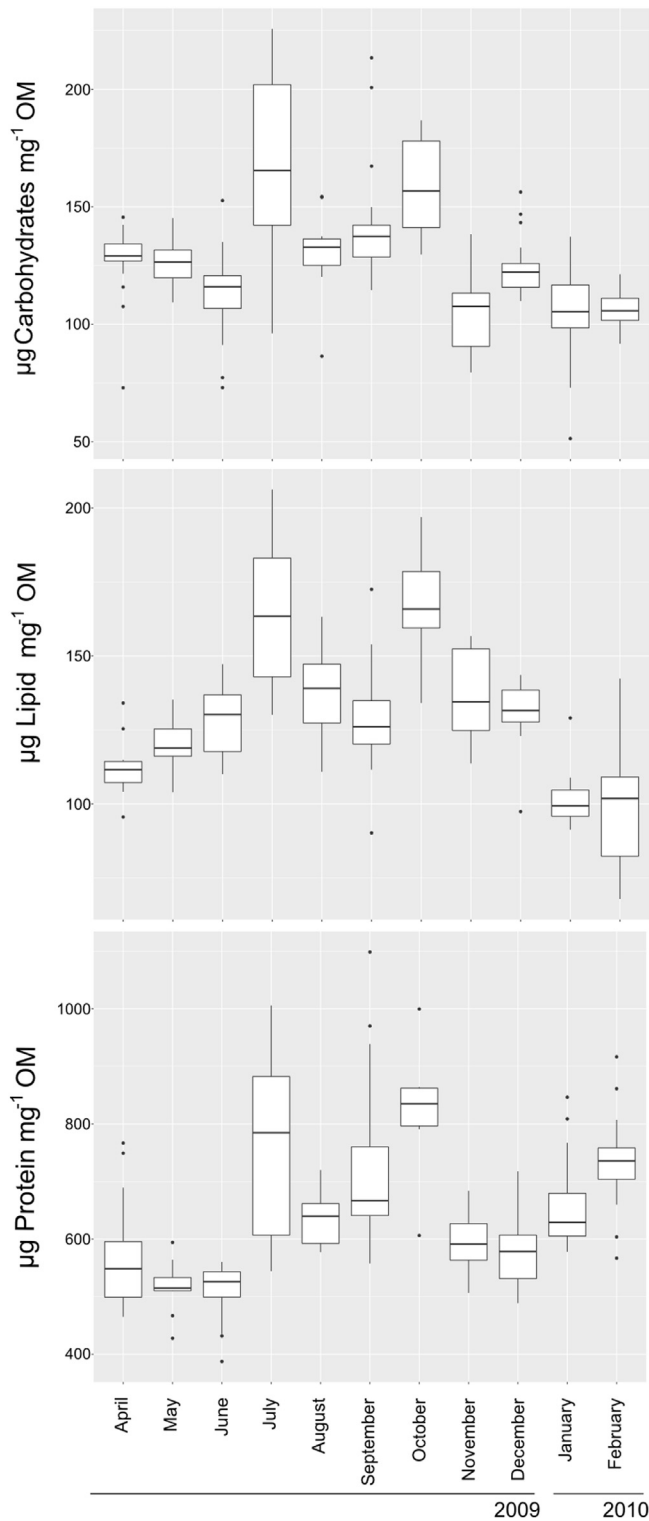


Fig. 6. Monthly concentration of proteins, lipids and carbohydrates, from April 2009 to February 2010 of *Malacobelemnon daytoni*. Boxplot illustrate median, second and third quartile. Whiskers are drawn to nearest value not beyond 1.5 × (inter-quartile range). Dots are outliers.

by a peak in copepod abundance (Garcia et al., 2016).

In the light of this, *M. daytoni* clearly has a flexible diet, shifting from phytoplankton to zooplankton and to detritus, depending on their availability in the environment, which is probably driven by

primary production pulses and resuspension events. Matching the FA profiles, the SI biomarker highlighted a wide variety of food sources in the diet of *M. daytoni*. Both biomarkers suggest that this species presents an opportunistic strategy with great flexibility in making use of diverse food sources, which helps to explain its ecological success in such a harsh environment (Sahade et al., 2015; Servetto and Sahade, 2016; Servetto et al., 2013).

4.2. FA comparison with other pennatulid species

In *M. daytoni*, the proportions of SFAs, MUFAs and PUFAs showed high seasonality. SFAs were more abundant most of the year, in contrast with several pennatulids and alcyonarians that show PUFAs > SFAs > MUFAs (Baptista et al., 2012; Pernet et al., 2002). This could be due to the high concentrations of the 16:0 and 18:0 FAs registered in *M. daytoni*, which are greater than those observed in other octocorals (Baptista et al., 2012; Imbs et al., 2009, 2007; Pernet et al., 2002; Svetashev and Vysotskii, 1998). This difference could be related to the absence of tetracosapolyenoic fatty acid (TPAs) (24:6n-3) in *M. daytoni*, which is abundant in several octocorals. However, there are few studies in pennatulids analyzing their FA profiles. Baptista et al. (2012) found that the four major FAs in the sea pen *V. cynomorium* are: the 16:0; arachidonic acid (AA, 20:4n-6); eicosapentaenoic (EPA, 20:5n-3), and a high concentration of the TPA. The first two were also present in *M. daytoni* with high proportions, but EPA, 20:5n-3, was scarce and TPA was absent in *M. daytoni*. Pernet et al. (2002) also described the absence of TPA and the seasonal patterns of FA abundance in the sea pen, *Renilla koellikeri*. Two principal PUFAs (AA and EPA) caused FA variation over the year, associated with the reproductive cycle in male and female colonies (Pernet et al., 2002). We found that the fatty acid AA decreases in *M. daytoni* during the summer, probably associated with the spawning of the species (Servetto and Sahade, 2016). While the low proportion of EPA could be related to the lack of symbionts in this species, as Bishop and Kenrick in 1980 observed, photosynthetic activity of symbiotic zooxanthellae is the source of EPA in hermatypic corals (Bishop and Kenrick, 1980).

4.3. Feeding/reproduction strategies

The lipid content registered in *M. daytoni* was low (between 100 and 164 $\mu\text{g lipid mg}^{-1}$ OM), similar to the range of those found in the aposymbiotic gorgonian, *Corallium rubrum* (Mediterranean Sea) (66–178.53 $\mu\text{g lipid mg}^{-1}$ OM), while other octocorals show higher lipid contents, such as the aposymbiotic *Paramuricea clavata* with 150–350 $\mu\text{g lipid mg}^{-1}$ OM, and *Leptogorgia sarmentosa* with 200–400 $\mu\text{g lipid mg}^{-1}$ OM (Bramanti et al., 2013; Rossi and Tsounis, 2007; Rossi et al., 2006a). The symbiotic gorgonian, *Eunicella singularis*, also had higher concentrations of lipids ranging between 180 and 400 $\mu\text{g lipid mg}^{-1}$ OM (Gori et al., 2012, 2007), depending on the season and on the trophic strategy (those that are below 40–45 m depth are heterotrophic, whilst those in shallower waters are mysotrophic). Several studies have highlighted the importance of symbiotic algae on the translocation of lipids from algae cells in the tissue of cnidarians (Patton and Burris, 1983; Tolosa et al., 2011), finding higher concentrations of lipids in shallow populations than in those of deeper areas (Gori et al., 2012). But, in this work, no clear pattern was found between lipid concentration and symbiotic/aposymbiotic species.

Whether it is advantageous to store energy or directly divert it to reproduction or growth during high food availability periods has been extensively discussed for invertebrates from different parts of the world (Ben-David-Zaslow and Benayahu, 1999; Bramanti et al., 2013; Cavaletto and Gardner, 1999; Epp et al., 1988; Gori et al., 2012, 2013, 2007; Pernet et al., 2002). Food availability is a key factor for

understanding the capability of aspecies to be successful in its contribution to the next generation colonizers, and the effect on gonad output is clear and quantifiable. In *M. daytoni*, seasonal patterns were observed in lipid levels and reproductive strategies, with low lipid concentrations in summer coinciding with a spawning event (Servetto and Sahade, 2016). This work suggests that the accumulation of lipids is not gradual, but showed rapid increments, probably associated with higher food availability at specific moments. For example, the October peak may be associated with high phytoplankton accumulation in Potter Cove at the beginning of the 2009–2010 spring–summer seasons, owing to the relatively weak wind speed observed at that time (Schloss et al., 2014). This pattern could be associated with an opportunistic strategy suggested in this pennatulid, as it uses the available energy in the environment with a quick transfer to reproduction. Male and female colonies were not analyzed separately since sex recognition is only possible under microscopy (Servetto et al., 2013). It has been shown, however, that only in a few species in which the reproductive effort is large are the differences between females and males significant prior to the spawning period (Viladrich et al., 2016). Due to the reproductive strategy of *M. daytoni*, it is possible that the energy invested in reproduction is not clearly different between males and females.

In contrast with lipid concentration, carbohydrates ranged between 104 and 168 μg carbohydrate mg^{-1} OM, which are higher values than those of the species mentioned above (*P. clavata*: 30–90 μg carbohydrate mg^{-1} OM) and *C. rubrum*: 40–50 μg carbohydrate mg^{-1} OM) (Gori et al., 2007; Rossi and Tsounis, 2007; Rossi et al., 2006a). These results are also higher than those found in seven different gorgonians in the Antarctic platform, in which the range in autumn was between 10 and 80 μg carbohydrate mg^{-1} OM (Elias-Piera et al., 2013). Carbohydrates are used as a quickly available energy molecule, while lipids are considered as energy storage, and are also a seasonal marker that explains food availability and stress in starvation periods (Rossi et al., 2006b). These results confirm lipids as the major reserves in *M. daytoni*, though carbohydrates are also an important source of energy in this species. It appears that starvation stress is more closely related with carbohydrate concentrations than with those of lipids (Rossi et al., 2006b). Carbohydrate concentration may quickly respond to starvation, having higher mobility under food deprivation (Willmer et al., 2000).

The protein concentration values observed in *M. daytoni* were similar to those found in *P. clavata* (500–750 μg protein mg^{-1} OM), *L. sarmentosa* (450–700 μg protein mg^{-1} OM) (Rossi et al., 2006a), and in the octocoral *C. rubrum* (390–437 μg protein mg^{-1} OM) (Bramanti et al., 2013; Tsounis et al., 2007). Seasonality in proteins is probably a consequence of seasonal growth in the colony. Marschal et al. (2004) observed two growth rings of different densities in the same year in red coral colonies (summer and winter). Similar observations were made in *Cladocoraca espitosa* in the Mediterranean Sea (Peirano et al., 2005, 1999). It has been shown that the OM originated from the pulses of food stimulating the formation of CaCO_3 , by constructing a primary matrix in which the inorganic part is precipitated (Houlbrèque et al., 2003). Thus, in *M. daytoni* the protein peaks may be due to increased organic matrix that is the basis for calcification. Rossi et al. (2006a) also found a higher protein content in coincidence with the period in which *P. clavata* grows.

5. Conclusion

One of the main outcomes of this work is to highlight the wide variety of food sources in the diet of *M. daytoni*, indicating the species' omnivorous diet and opportunistic feeding strategy. The

two types of trophic biomarkers (FA and SI) studied coincide in this. Both methods are complementary and proved to be important tools to detect different food sources in a year-round period.

This opportunistic feeding strategy in *M. daytoni* allows the species to successfully cope with low energy availability periods such as autumn–winter in Antarctic ecosystems. Biochemical data suggest that there is also a fast transfer of energy, as indicated by lipids and carbohydrates, to reproduction.

These ecological strategies exhibited by *M. daytoni* together with fast growth rates, early sexual maturity, reproductive seasonality and a tolerance to high sedimentation rates, can explain its ecological success in the Potter Cove ecosystem, an area highly affected by the environmental shifts caused by glacier retreat. Considering that 90% of the AP glaciers are retreating, this species could play an important role in these affected coastal ecosystems.

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References

- Alvå, V., Orejas, C., Zabala, M., 1997. Feeding ecology of Antarctic cnidarian suspension feeders (Hydrozoa, Gorgonacea, Pennatulacea). The expedition Antarktis XIII/3 (EASIZ I) of Polarstern to the eastern Weddell Sea in (1996): 14–16.
- Baptista, M., Lopes, V.M., Pimentel, M.S., Bandarra, N., Narciso, L., Marques, A., Rosa, R., 2012. Temporal fatty acid dynamics of the octocoral *Veretillum cymomorium*. Comp. Biochem. Phys. B 161, 178–187. <http://dx.doi.org/10.1016/j.cbpb.2011.11.002>.
- Barnes, D.K.A., 2015. Antarctic sea ice losses drive gains in benthic carbon draw-down. Curr. Biol. 25 (18), R789–R790.
- Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilum method for "total" lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118. [http://dx.doi.org/10.1016/0022-0981\(73\)90040-3](http://dx.doi.org/10.1016/0022-0981(73)90040-3).
- Ben-David-Zaslav, R., Benayahu, Y., 1999. Temporal variation in lipid, protein and carbohydrate content in the Red Sea soft coral *Heteroxenia fuscescens*. J. Mar. Biol. Assoc. U. K. 79, 1001–1006. <http://dx.doi.org/10.1017/S002531549900123x>.
- Bishop, D.G., Kenrick, J.R., 1980. Fatty acid composition of symbiotic zooxanthellae in relation to their hosts. Lipids 15, 799–804. <http://dx.doi.org/10.1007/BF02534368>.
- Bramanti, L., Movilla, J., Guron, M., Calvo, E., Gori, A., Dominguez-Carrió, C., Grinyó, J., Lopez-Sanz, A., Martínez-Quintana, A., Pelejero, C., Ziveri, P., Rossi, S., 2013. Detrimental effects of ocean acidification on the economically important Mediterranean red coral (*Corallium rubrum*). Glob. Chang. Biol. 19, 1897–1908. <http://dx.doi.org/10.1111/gcb.12171>.
- Carballeira, N.M., Sostre, A., Rodríguez, A.D., 1997. Phospholipid fatty acid composition of gorgonians of the genus Eunicea: further identification of tetracosapolyenoic acids. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 118, 257–260. [http://dx.doi.org/10.1016/S0305-0491\(97\)00056-4](http://dx.doi.org/10.1016/S0305-0491(97)00056-4).
- Cavaletto, J., Gardner, W., 1999. Seasonal dynamics of lipids in freshwater benthic invertebrates. Lipids Freshw. Ecosyst. 109–131.
- Clarke, A., 1988. Seasonality in the Antarctic marine environment. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 90, 461–473.
- Clarke, A., Murphy, E.J., Meredith, M.P., King, J.C., Peck, L.S., Barnes, D.K., Smith, R.C., 2007. Climate change and the marine ecosystem of the western Antarctic Peninsula. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362 (1477), 149–166.
- Cripps, G.C., Clarke, A., 1998. Seasonal variation in the biochemical composition of particulate material collected by sediment traps at Signy Island, Antarctica. Polar Biol. 20, 414–423.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. <http://dx.doi.org/10.1021/ac60111a017>.
- Elias-Piera, F., Rossi, S., Gili, J.M., Orejas, C., 2013. Trophic ecology of seven Antarctic

- gorgonian species. *Mar. Ecol. Prog. Ser.* 477, 93–106. <http://dx.doi.org/10.3354/meps10152>.
- Epp, J., Bricej, V.M., Malouf, R.E., 1988. Seasonal partitioning and utilization of energy reserves in two age classes of the bay scallop *Argopecten irradians irradians* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 121, 113–136. [http://dx.doi.org/10.1016/0022-0981\(88\)90250-X](http://dx.doi.org/10.1016/0022-0981(88)90250-X).
- Fleurence, J., Guitier, G., Mabeau, S., Leray, C., 1994. Fatty acids from 11 marine macroalgae of the French Brittany coast. *J. Appl. Phycol.* 6, 527–532. <http://dx.doi.org/10.1007/BF02182406>.
- Fuentes, V., 2006. Estudio de la Comunidad Zooplanctónica de Caleta Potter y Bahía Guardia Nacional (Isla 25 de Mayo): su Rol en la Dinámica del Carbono en Zonas Costeras Antárticas. Tesis Doctoral. Universidad de Buenos Aires, p. 249.
- Fukuda, Y., Naganuma, T., 2001. Potential dietary effects on the fatty acid composition of the common jellyfish *Aurelia aurita*. *Mar. Biol.* 138, 1029–1035. <http://dx.doi.org/10.1007/s002270000512>.
- García, M.D., Hoffmeyer, M.S., Abbate, M.C.L., Barría de Cao, M.S., Pettigrosso, R.E., Almandoz, G.O., Hernandez, M.P., Schloss, I.R., 2016. Micro- and meso-zooplankton responses during two contrasting summers in a coastal Antarctic environment. *Polar Biol.* 39, 123–137. <http://dx.doi.org/10.1007/s00300-015-1678-z>.
- Gili, J.M., Alvá, V., Pagés, F., Klöser, H., Arntz, W.E., 1996. Benthic diatoms as the major food source in the sub-antarctic marine hydroid *Silicularia rosea*. *Polar Biol.* 16, 507–512. <http://dx.doi.org/10.1007/s003000005082>.
- Gili, J.M., Arntz, W.E., Palanques, A., Orejas, C., Clarke, A., Dayton, P.K., Isla, E., Teixidó, N., Rossi, S., López-González, P.J., 2006. A unique assemblage of epibenthic sessile suspension feeders with archaic features in the high-Antarctic. *Deep. Res. Part II Top. Stud. Oceanogr.* 53, 1029–1052. <http://dx.doi.org/10.1016/j.dsr2.2005.10.021>.
- Gili, J.M., Coma, R., 1998. Benthic suspension feeders in marine food webs. *Tree* 13, 316–321.
- Gillies, C.L., Stark, J.S., Johnstone, G.J., Smith, S.D.A., 2013. Establishing a food web model for coastal Antarctic benthic communities: a case study from the Vestfold hills. *Mar. Ecol. Prog. Ser.* 478, 27–41. <http://dx.doi.org/10.3354/meps10214>.
- Gori, A., Linares, C., Rossi, S., Coma, R., Gili, J.M., 2007. Spatial variability in reproductive cycle of the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the western Mediterranean sea. *Mar. Biol.* 151, 1571–1584. <http://dx.doi.org/10.1007/s00227-006-0595-7>.
- Gori, A., Linares, C., Viladrich, N., Clavero, A., Orejas, C., Fiorillo, I., Ambrosio, S., Gili, J.M., Rossi, S., 2013. Effects of food availability on the sexual reproduction and biochemical composition of the Mediterranean gorgonian *Paramuricea clavata*. *J. Exp. Mar. Biol. Ecol.* 444, 38–45. <http://dx.doi.org/10.1016/j.jembe.2013.03.009>.
- Gori, A., Viladrich, N., Gili, J.-M., Kotta, M., Cucio, C., Magni, L., Bramanti, L., Rossi, S., 2012. Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). *Coral Reefs* 31, 823–837. <http://dx.doi.org/10.1007/s00338-012-0904-1>.
- Graeve, M., Dauby, P., Scailteur, Y., 2001. Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods. *Polar Biol.* 24, 853–862. <http://dx.doi.org/10.1007/s003000100295>.
- Graeve, M., Hagen, W., Kattner, G., 1994. Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. *Deep. Res. Part I* 41, 915–924. [http://dx.doi.org/10.1016/0967-0637\(94\)90083-3](http://dx.doi.org/10.1016/0967-0637(94)90083-3).
- Holm-Hansen, O., 1985. Nutrient cycles in Antarctic marine ecosystems. In: Siegfried, W.R., Condy, P.R., Laws, R.M. (Eds.), *Antarctic Nutrient Cycles and Food Webs*. Springer, Berlin, pp. 6–10.
- Houlbrèque, F., Tambutté, E., Ferrier-Pagès, C., 2003. Effect of zooplankton availability on the rates of photosynthesis, and tissue and skeletal growth in the scleractinian coral *Stylophora pistillata*. *J. Exp. Mar. Biol. Ecol.* 296, 145–166. [http://dx.doi.org/10.1016/S0022-0981\(03\)00259-4](http://dx.doi.org/10.1016/S0022-0981(03)00259-4).
- Imbs, A.B., Dautova, T.N., 2008. Use of lipids for chemotaxonomy of octocorals (Cnidaria: Alcyonaria). *Russ. J. Mar. Biol.* 34, 174–178. <http://dx.doi.org/10.1134/S1063074008030061>.
- Imbs, A.B., Demidkova, D.A., Dautova, T.N., Latyshev, N.A., 2009. Fatty acid biomarkers of symbionts and unusual inhibition of tetracosapolyenoic acid biosynthesis in corals (Octocorallia). *Lipids* 44, 325–335. <http://dx.doi.org/10.1007/s11745-008-3266-2>.
- Imbs, A.B., Latyshev, N.A., Zhukova, N.V., Dautova, T.N., 2007. Comparison of fatty acid compositions of azooxanthellate *Dendronephthya* and zooxanthellate soft coral species. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* 148, 314–321. <http://dx.doi.org/10.1016/j.cbpb.2007.06.009>.
- Isla, E., Rossi, S., Palanques, A., Gili, J.M., Gerdes, D., Arntz, W., 2006. Biochemical composition of marine sediment from the eastern Weddell Sea (Antarctica): high nutritive value in a high benthic-biomass environment. *J. Mar. Syst.* 60 (3), 255–267.
- Isla, E., Gerdes, D., Palanques, A., Gili, J., Arntz, W.E., König-Langlo, G., 2009. Downward particle fluxes, wind and a phytoplankton bloom over a polar continental shelf: a stormy impulse for the biological pump. *Mar. Geol.* 259, 59–72. <http://dx.doi.org/10.1016/j.margeo.2008.12.011>.
- Jacob, U., Mintenbeck, K., Brey, T., Knust, R., Beyer, K., 2005. Stable isotope food web studies: a case for standardized sample treatment. *Mar. Ecol. Prog. Ser.* 287, 251–253.
- Kattner, G., Hagen, W., 1995. Polar herbivorous copepods - different pathways in lipid biosynthesis. *ICES J. Mar. Sci.* 52, 329–335. [http://dx.doi.org/10.1016/1054-3139\(95\)80048-4](http://dx.doi.org/10.1016/1054-3139(95)80048-4).
- Khotimchenko, S.V., 1995. Uncommon 16:1 (n-5) acid from *Dictyota dichotoma* and fatty acids of some brown algae of Dictyotaceae. *Phytochemistry* 38, 1411–1415. [http://dx.doi.org/10.1016/0031-9422\(94\)00819-F](http://dx.doi.org/10.1016/0031-9422(94)00819-F).
- Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., Lutcavage, M.E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* 77, 838–846.
- Lowry, O., Rosebrough, N., Lewis, F., Randall, R., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275. [http://dx.doi.org/10.1016/0304-3894\(92\)87011-4](http://dx.doi.org/10.1016/0304-3894(92)87011-4).
- Marschal, C., Garrabou, J., Harmelin, J.G., Pichon, M., 2004. A new method for measuring growth and age in the precious red coral *Corallium rubrum* (L.). *Coral Reefs* 23, 423–432. <http://dx.doi.org/10.1007/s00338-004-0398-6>.
- McClintic, M.A., DeMaster, D.J., Thomas, C.J., Smith, C.R., 2008. Testing the FOOD-BANCS hypothesis: seasonal variations in near-bottom particle flux, bioturbation intensity, and deposit feeding based on ²³⁴Th measurements. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 2425–2437. <http://dx.doi.org/10.1016/j.dsr2.2008.06.003>.
- McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390. <http://dx.doi.org/10.1034/j.1600-0706.2003.12098.x>.
- Mincks, S., 2005. Benthic-pelagic Coupling on the Antarctic Continental Shelf: Impacts of Seasonal Phytodetritus Deposition on the Benthic Community (Doctoral thesis). University of Hawaii Library.
- Mincks, S.L., Smith, C.R., Jeffreys, R.M., Sumida, P.Y.G., 2008. Trophic structure on the West Antarctic Peninsula shelf: detritivory and benthic inertia revealed by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Deep. Res. Part II Top. Stud. Oceanogr.* 55, 2502–2514. <http://dx.doi.org/10.1016/j.dsr2.2008.06.009>.
- Mintenbeck, K., Brey, T., Jacob, U., Knust, R., Struck, U., 2008. How to account for the lipid effect on carbon stable-isotope ratio ($\delta^{13}\text{C}$): sample treatment effects and model bias. *J. Fish. Biol.* 72, 815–830.
- Mintenbeck, K., Jacob, U., Knust, R., Arntz, W.E., Brey, T., 2007. Depth-dependence in stable isotope ratio $\delta^{15}\text{N}$ of benthic POM consumers: the role of particle dynamics and organism trophic guild. *Deep. Res. Part I Oceanogr. Res. Pap.* 54, 1015–1023. <http://dx.doi.org/10.1016/j.dsr.2007.03.005>.
- Norkko, A., Thrush, S.F., Cummings, V.J., Gibbs, M.M., Andrew, N.L., Norkko, J., Schwarz, A.M., 2007. Trophic structure of coastal Antarctic food webs associated with changes in sea ice and food supply. *Ecology* 88 (11), 2810–2820.
- Oku, H., Yamashiro, H., Onaga, K., Sakai, K., Iwasaki, H., 2003. Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. *Coral Reefs* 22, 83–85. <http://dx.doi.org/10.1007/s00338-003-0279-4>.
- Orejas, C., Gili, J., Arntz, W., 2003. The role of the small planktonic communities in the diet of two Antarctic octocorals (*Primnois antarctica* and *Primnoella* sp.). *Mar. Ecol. Prog. Ser.* 250, 105–116. <http://dx.doi.org/10.3354/meps250105>.
- Orejas, C., Gili, J.M., Arntz, W.E., Ros, J.D., López, P.J., Teixidó, N., Filipe, P., 2000. Benthic suspension feeders, key players in Antarctic marine ecosystems? *Contrib. Sci.* 1, 299–311.
- Orejas, C., Gili, J.M., López-González, P.J., Arntz, W.E., 2001. Feeding strategies and diet composition of four Antarctic cnidarian species. *Polar Biol.* 24, 620–627. <http://dx.doi.org/10.1007/s003000100272>.
- Pasotti, F., Saravia, L.A., De Troch, M., Tarantelli, M.S., Sahade, R., Vanreusel, A., 2015. Benthic trophic interactions in an Antarctic shallow water ecosystem affected by recent glacial retreat. *PLoS One* 10. <http://dx.doi.org/10.1371/journal.pone.0141742>.
- Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS One* 5 (3), e9672. <http://dx.doi.org/10.1371/journal.pone.0009672>.
- Patton, J.S., Burris, J.E., 1983. Lipid synthesis and extrusion by freshly isolated zooxanthellae (symbiotic algae). *Mar. Biol.* 75, 131–136. <http://dx.doi.org/10.1007/BF00405995>.
- Peck, L.S., Barnes, D.K., Cook, A.J., Fleming, A.H., Clarke, A., 2010. Negative feedback in the cold: ice retreat produces new carbon sinks in Antarctica. *Glob. Chang. Biol.* 16 (9), 2614–2623.
- Peirano, A., Abbate, M., Cerrati, G., Difesa, V., Peroni, C., Rodolfo-Metalpa, R., 2005. Monthly variations in calyx growth, polyp tissue, and density banding of the Mediterranean scleractinian *Cladocora espitosa* (L.). *Coral Reefs* 24, 404–409. <http://dx.doi.org/10.1007/s00338-005-0020-6>.
- Peirano, A., Morri, C., Bianchi, C.N., 1999. Skeleton growth and density pattern of the temperate, zooxanthellate scleractinian *Cladocora espitosa* from the Ligurian Sea (NW Mediterranean). *Mar. Ecol. Prog. Ser.* 185, 195–201. <http://dx.doi.org/10.3354/meps185195>.
- Pernet, V., Gavino, V., Gavino, G., Anctil, M., 2002. Variations of lipid and fatty acid contents during the reproductive cycle of the anthozoan *Renilla koellikeri*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 172, 455–465. <http://dx.doi.org/10.1007/s00360-002-0268-x>.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montañá, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecol* 152, 179–189.
- Purinton, B.L., DeMaster, D.J., Thomas, C.J., Smith, C.R., 2008. ¹⁴C as a tracer of labile organic matter in Antarctic benthic food webs. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 2438–2450. <http://dx.doi.org/10.1016/j.dsr2.2008.06.004>.
- R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/>.
- Rossi, S., Gili, J.M., Coma, R., Linares, C., Gori, A., Vert, N., 2006a. Temporal variation

- in protein, carbohydrate, and lipid concentrations in *Paramuricea clavata* (Anthozoa, Octocorallia): evidence for summer-autumn feeding constraints. *Mar. Biol.* 149, 643–651. <http://dx.doi.org/10.1007/s00227-005-0229-5>.
- Rossi, S., Isla, E., Martínez-García, A., Moraleda, N., Gili, J.-M., Rosell-Melé, A., Arntz, W.E., Gerdes, D., 2013. Transfer of seston lipids during a flagellate bloom from the surface to the benthic community in the Wedd. Sea. *Sci. Mar.* 77, 397–407. <http://dx.doi.org/10.3989/scimar.03835.30A>.
- Rossi, S., Snyder, M.J., Gili, J.M., 2006b. Protein, carbohydrate, lipid concentrations and HSP 70-HSP 90 (stress protein) expression over an annual cycle: useful tools to detect feeding constraints in a benthic suspension feeder. *Helgol. Mar. Res.* 60, 7–17. <http://dx.doi.org/10.1007/s10152-005-0009-0>.
- Rossi, S., Tsounis, G., 2007. Temporal and spatial variation in protein, carbohydrate, and lipid levels in *Corallium rubrum* (Anthozoa, Octocorallia). *Mar. Biol.* 152, 429–439. <http://dx.doi.org/10.1007/s00227-007-0702-4>.
- Rossi, S., Youngbluth, M.J., Jacoby, C.A., Pagès, F., Garrofé, X., 2008. Fatty acid trophic markers and trophic links among seston, crustacean zooplankton and the siphonophore *Nanomia cara* in Georges Basin and Oceanographer Canyon (NW Atlantic). *Sci. Mar.* 72, 403–416. <http://dx.doi.org/10.3989/scimar.2008.72n2403>.
- Sahade, R., Lagger, C., Torre, L., Momo, F., Monien, P., Schloss, I., Barnes, D.K.A., Servetto, N., Tarantelli, S., Tatián, M., Zamboni, N., Abele, D., 2015. Climate change and glacier retreat drive shifts in an Antarctic benthic ecosystem. *Sci. Adv.* 1 <http://dx.doi.org/10.1126/sciadv.1500050>.
- Sahade, R., Tatián, M., Kowalke, J., Kühne, S., Esnal, G.B., 1998. Benthic faunal associations on soft substrates at Potter Cove, King George Island, Antarctica. *Polar Biol.* 19, 85–91. <http://dx.doi.org/10.1007/s003000050218>.
- Sakshaug, E., Holm-Hansen, O., 1984. Factors governing pelagic production in polar oceans. Springer-Verlag, Berlin, pp. 1–18. In: Holm-Hansen, O., Bolis, L., Gilles, R. (Eds.), *Marine Phytoplankton and Productivity. Lecture Notes on Coastal and Estuarine Studies*, vol. 8. Springer-Verlag, Berlin, pp. 1–18.
- Schloss, I.R., Wasilowska, A., Dumont, D., Almandoz, G.O., Hernando, M.P., Saravia, L., Rzepecki, M., Monien, P., Monien, D., Kopczyn, E.E., Ferreyra, G.A., 2014. On the phytoplankton bloom in coastal waters of southern King George Island (Antarctica) in January 2010: an exceptional feature? *Limnol. Oceanogr.* 59, 195–210. <http://dx.doi.org/10.1594/PANGAEA.754676>.
- Servetto, N., Sahade, R., 2016. Reproductive seasonality of the antarctic sea pen *Malacobelemnion daytoni* (Octocorallia, Pennatulacea, Kophobelemnidae). *PLoS One* 11. <http://dx.doi.org/10.1371/journal.pone.0163152> e0163152.
- Servetto, N., Torre, L., Sahade, R., 2013. Reproductive biology of the antarctic “sea pen” *Malacobelemnion daytoni* (Octocorallia, Pennatulacea). *Polar Res.* 1, 1–11.
- Slattery, M., McClintock, J.B., Bowser, S.S., 1997. Deposit feeding: a novel mode of nutrition in the Antarctic colonial soft coral *Gersemia Antarctica*. *Mar. Ecol. Prog. Ser.* 149, 299–304.
- Svetashev, V.I., Vysotskii, M.V., 1998. Fatty acids of helioporacoerulea and chemotaxonomic significance of tetracosapolyenoic acids in coelenterates. *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.* 119, 73–75. [http://dx.doi.org/10.1016/S0305-0491\(97\)00231-9](http://dx.doi.org/10.1016/S0305-0491(97)00231-9).
- Tolosa, I., Treignier, C., Grover, R., Ferrier-Pagès, C., 2011. Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. *Coral Reefs* 30, 763–774. <http://dx.doi.org/10.1007/s00338-011-0753-3>.
- Torre, L., Servetto, N., Eóry, M.L., Momo, F., Tatián, M., Abele, D., Sahade, R., 2012. Respiratory responses of three Antarctic ascidians and a sea pen to increased sediment concentrations. *Polar Biol.* 35, 1743–1748. <http://dx.doi.org/10.1007/s00300-012-1208-1>.
- Tsounis, G., Rossi, S., Gili, J.M., Arntz, W.E., 2007. Red coral fishery at the Costa Brava (NW Mediterranean): case study of an overharvested precious coral. *Ecosystems* 10, 975–986. <http://dx.doi.org/10.1007/s10021-007-9072-5>.
- Viladrich, N., Bramanti, L., Tsounis, G., Chocarro, B., Martínez-Quitana, A., Ambroso, S., Madurell, T., Rossi, S., 2016. Variation in lipid and free fatty acid content during spawning in two temperate octocorals with different reproductive strategies: surface versus internal brooder. *Coral Reefs* 35, 1033–1045. <http://dx.doi.org/10.1007/s00338-016-1440-1>.
- von Bodungen, B., Nötting, E.M., Sui, Q., 1988. New production of phytoplankton and sedimentation during summer 1985 in the South eastern Weddell sea. *Comp. Biochem. Physiol.* 90B, 475–487.
- Willmer, P., Stone, G., Johnston, I., 2000. *Environmental physiology of animals*. Blackwell, Sci. 178–211.
- Würzberg, L., Peters, J., Schüller, M., Brandt, A., 2011. Diet insights of deep-sea polychaetes derived from fatty acid analyses. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 58, 153–162. <http://dx.doi.org/10.1016/j.dsr2.2010.10.014>.