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Feeding biology of a habitat-forming antipatharian in the Azores

Archipelago

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Abstract

Benthic suspension feeders have developed a variety of feeding strategies and food availability has often proven to be a key factor explaining their occurrence and distribution. The feeding biology of coral species has been the target of an increasing number of studies, however most of them focus on Scleractinia and Octocorallia, while information for Antipatharia is very scarce. The present study focused on *Antipathella wollastoni*, a common habitat-forming antipatharian in the Azores Archipelago, forming dense black coral forests between 20 and 150 meters. The objective of the study was to investigate the food preferences of the target species upon availability of different isotopically enriched food substrates and determine its ability to capture zooplankton prey under different flow speeds. The species was able to utilize different food sources including live phytoplankton, live zooplankton and Dissolved Organic Matter (DOM), indicating the ability to exploit seasonally available food sources. However ingestion of zooplankton enhanced Carbon (C) and Nitrogen (N) incorporation in coral tissue and metabolic activity, highlighting the importance of zooplankton prey for vital physiological processes such as growth and reproduction. Maximum zooplankton capture rates occurred under 4 cm^{-1} , however the species displayed high capacity to capture zooplankton prey over different flow rates highlighting the ability of *A. wollastoni* to exploit high quantities of shortly available prey.

Key words

Benthic suspension feeders, capture rates, flow, Atlantic, black corals, ecophysiology

Introduction

Corals are key components of sublittoral ecosystems not only in tropical (Spalding et al. 2001) but also in temperate and cold ecosystems (Bo et al. 2014; Buhl-Mortensen et al. 2018; Orejas and Jimenez 2017). They can be encountered over a wide bathymetric range including the euphotic, mesophotic and aphotic zone, where they build communities that have been recently termed as marine animal forests (Rossi et al. 2017). While there is an increasing number of studies on the classes of Scleractinia and Octocorallia, inhabiting various depth zones, Antipatharia have received relatively less attention (Bo et al. 2012). Antipatharians occur in all oceans over a wide bathymetric range and are generally found in environments with low light and strong currents (Wagner et al. 2012). In temperate regions, antipatharians have been recognized as key species in mesophotic and deep waters (e.g. Bo et al. 2014; Ingrassia et al. 2016; Massi et al. 2018) where they form dense populations that provide habitat to a variety of associated species (Gaino et al. 2013; Bo and Bavestrello 2019).

Antipathella wollastoni is a common habitat forming species in the Macaronesia, including Cape Verde, Madeira, Canaries and the Azores (Brito and Ocaña 2004). In the temperate and subtropical islands of the Macaronesia, including the Azores Archipelago, the species is encountered on island slopes at 15-520m (Brito and Ocaña 2004), however, when encountered in shallow depths it is mostly restricted to dark sites such as cavern environments and rock crevices (Brito and Ocaña 2004).

One of the main drivers of the occurrence and distribution of benthic suspension feeders is food availability, controlled by several factors including seasonal productivity cycles and flow (Richârd and Larsen 2015; Huguet 2017). Up to date, information on antipatharian feeding biology is limited to aquaria or field observations of a few tropical

species (e.g. Grigg 1965; Goenaga 1977; Lewis 1978; Tazioli et al. 2007) and studies of isotope ratios (e.g. Sherwood et al. 2008; Coppari et al. 2020). According to these studies, antipatharians seem to be able to feed on various food sources including zooplankton, phytoplankton and detritus similarly to other suspension feeders (Carlier et al. 2009; Williams and Grottoli 2010). While shallow-water tropical (Goenaga 1977; Lewis 1978; Terrana et al. 2019) and mesophotic temperate antipatharian species (Coppari et al. 2020) have been shown to feed mainly on zooplankton, deeper species feed mainly on suspended particulate organic matter (Sherwood et al. 2008; Williams and Grottoli 2010). However, up to date no experimental approach has been attempted with Antipatharia in order to unravel their ability to capture and ingest different food sources. Moreover, although current knowledge suggests that antipatharia require strong currents to feed (Wagner et al. 2012), there is no information on how different current speeds can influence their food capture.

Due to the important role of *A. wollastoni* as a habitat-forming species, the present study aimed at determining the ability of the species to (1) utilize different food sources, including live zooplankton, live phytoplankton and dissolved organic matter (DOM) and (2) capture small live zooplankton prey under different flow velocities. To our knowledge, this is the first attempt to provide a detailed experimental insight to the feeding biology of an antipatharian species.

Materials and Methods

Study Area & Sampling

Fragments of *A. wollastoni* were collected in October 2017 at the reef Baixa da Feteira and the bay of São Caetano (Fig. 1) in the natural parks of Faial and Pico island

respectively (Regional Legislative Orders no. 46/2008/A and 20/2008/A). Baixa da Feteira is a rock formation rising from 40 m with its top located at 12 m. The coral thicket is located at 16-38m on a long crevice of a steep wall facing southeast. While the area is known by local divers for the strong tidal currents, the coral thicket is located in a relatively protected position. São Caetano is a bay facing southeast, comprised of a steep rocky drop off on its eastern part and lava fingers positioned on a smoother sandy drop-off on the south. The coral thicket is located between lava fingers at 40-45 m where it is also relatively protected from tidal currents.

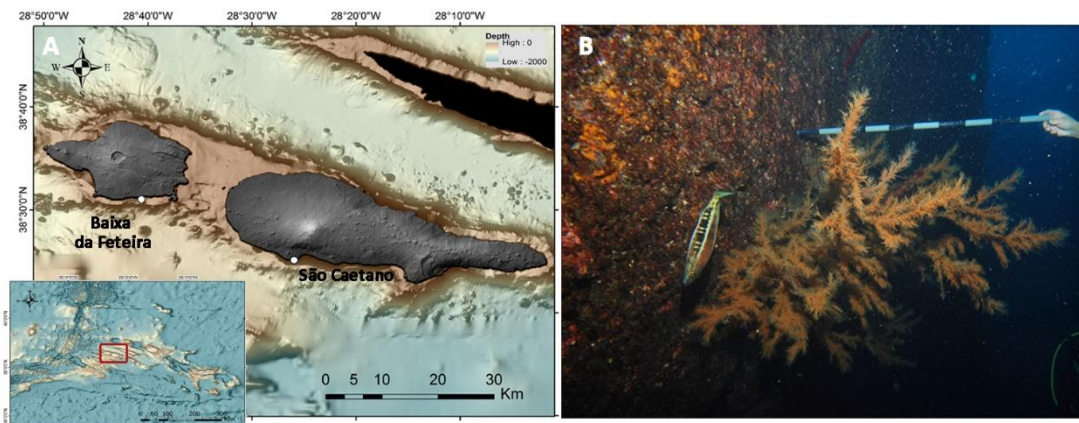


Figure 1: (A) Collection sites of fragments of the species *Antipathella wollastoni*. Baixa da Feteira: 38° 31.06 N 28° 40.70 W, São Caetano: 38° 25.25 N 28° 25.98 W (Bathymetry: EMODnet, 2018), (B) Colony of *A. wollastoni* in the vertical wall of Baixa da Feteira (photo: J. Monteiro)

A total of 15 colonies (higher than 40 cm) were selected, 8 at Baixa da Feteira and 7 at São Caetano, and four fragments between 8 and 10 cm were collected by scuba divers from each colony, at depths between 20-40 m. Fragments (n=60) were kept in coolers and immediately transferred to aquaria in the DeepSeaLab facilities at IMAR/DOP where they were fixed in epoxy bases. All fragments were kept in four 30L aquaria in continuous flow-through open systems supplied with sand-filtered water pumped from

5 m depth (salinity 36 ppm) and treated by UV-light (Vecton 600, TMC™). Aquaria were equipped with pumps (Iwaki, 4.5 W, 280 L h⁻¹) to ensure water circulation. They were placed in a water bath keeping temperature at 19 ± 0.6 °C by means of heaters (EHEIM 100w), matching the temperature at the collection site, and were kept in conditions of dim blue light that followed the natural photoperiod.

Field measurements

Water sampling was performed to obtain estimates of suspended Particulate Organic Carbon (sPOC) at the field and use it as reference to create realistic conditions of feeding quantity during the feeding experiments. Water samples (5 L, n= 3 for São Caetano and n= 5 for Baixa da Feteira) were collected at the two sampling sites, above the coral thickets, in June 2018 by scuba diving. Water samples were transferred to the laboratory facilities in coolers and were immediately vacuum-filtered through 0.7 µm GF/F filters (WHATMAN) and stored at -20°C. Filters were dried at 70° C to constant dry weight (DW), weighted with an analytical balance (Mettler-Toledo MS204S, accuracy 0.1 mg) and burned in a muffle at 550 °C for four hours to obtain estimates of ash dry weight (ADW). The quantity of suspended Particulate Organic Matter (sPOM) was calculated by subtracting the ADW from DW and sPOC was approximated by assuming an organic carbon (OC) content of 30% (Clegg and Whitfield, 1990; Sarmiento and Gruber, 2006).

Feeding on different food substrates

The ability of *A. wollastoni* to utilize different food substrates was investigated by providing different isotopically enriched food substrates and assessing the incorporation of tracers (¹³C, ¹⁵N) in coral tissue. Three food substrates were used: the diatom *Chaetoceros calcitrans* (PHYTO), Dissolved Organic Material (DOM) in the

form of an algae derived product of dissolved amino-acids (Cambridge Isotopes, U 13C 97–99 %, U 15N 97–99 %, CNLM-452-0.5) and the rotifer *Branchionus plicatilis* (ZOO). A control treatment was also considered by letting corals unfed (STAR), leading to a total of four food treatments. Selection of food sources was made taking into account the natural communities of plankton species in the study sites and the small polyp (average transverse diameter: 0.67 ± 0.09 mm) and polyp mouth size (diameter 0.31 ± 0.06 mm) of *A. wollastoni*.

Previous studies on the phytoplankton communities in coastal waters in the Azores showed that diatoms of the genus *Chaetoceros* are commonly found during spring bloom in the Azores (Silva et al. 2013; Santos et al. 2013). Continuous monocultures of the diatom *C. calcitrans* (diameter: 2.5 - 6 μ m) were established at 19 °C under a 12h-12h dark-light cycle using artificial SW and an F/2 culture medium. Cultures were artificially enriched with stable isotopes ¹³C and ¹⁵N using 100% ¹³C-bicarbonate (NaHCO₄, Cambridge Isotopes) and 50 % ¹⁵N-sodium nitrate (NaNO₃, Cambridge Isotopes). Subsequently, cultures were harvested by light filtering with membrane filters (0.2 μ m), rinsed with filtered SW and re-suspended in artificial SW.

The small-sized (140-330 μ m) rotifer *B. plicatilis* was selected as live zooplankton food source. Although rotifers have not been observed as a natural component of local zooplankton communities (Carmo et al. 2013), *B. plicatilis* is similar in size to several species of copepod nauplii that are common in the area (Carmo et al. 2013), but is easier to keep and label in laboratory cultures. This prey has also been previously used for maintenance of tropical antipatharian species (Montgomery and Crow 1998). To prepare ¹³C and ¹⁵N enriched cultures of *B. plicatilis*, starter cultures (concentration: 45 rotifers ml⁻¹) were kept under continuous presence of enriched *Nannochloropsis gaditana*, cultured as *C. calcitrans*, for six days. Rotifer cultures were left without food

for 4-6 hours before they were used to ensure complete assimilation of the provided food. Subsequently they were harvested by filtering (nylon, 40 μm), rinsed and re-suspended in artificial SW.

In order to use live food substrates, cell concentration of enriched cultures was monitored and cultures were prepared to reach the desired concentrations on the day they would be provided to the aquaria. Subsamples of known concentration of labelled cultures of *C. calcitrans* and *B. plicatilis* were harvested, freeze-dried and analyzed for C and N content by elemental analysis (see sample processing). Mean DW and C content per cell were calculated and used to standardize the amount of C provided in each experimental aquaria. During the experiments, cell concentration was used as a proxy of the provided C quantity and special care was given during harvesting to ensure it did not cause a significant decrease in cell concentration.

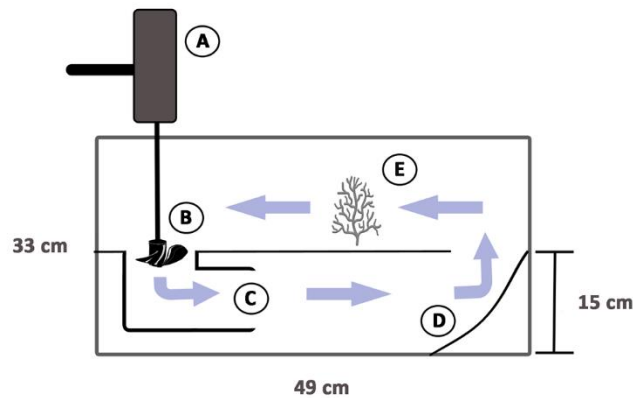


Figure 2: Experimental aquaria (33L) for the creation of laminar flows (1.5-6 cm s⁻¹). A: heavy duty 313 rpm motor, B: 8 cm plastic propeller, C: 9 cm PVC tube to increase flow creation, D: PVC ramp to decrease eddies, E: coral fragment.

The experiments were performed in four 33 L aquaria that were adapted in order to create conditions of laminar flow (Fig. 2). A series of 8 cm marine propellers

(Graupner) were attached to heavy duty gear motors (313 rpm, 12V, Servocity) creating a system able to recreate different flow velocities, from 1.5 s^{-1} to 6 cm s^{-1} . An electromagnetic current meter (JFE Advantech AEM1-DA) was used to determine and control the flow velocities created in the aquaria.

Due to the low number of available experimental aquaria, experiments for each treatment did not take place simultaneously, but in three consecutive experimental runs (Fig. 3). In each experimental run, three aquaria were used to recreate one of the three food treatments including a food substrate (PHYTO, DOM, ZOO) and one aquarium was used to recreate the STAR treatment (Fig. 3). One fragment from each colony was assigned to each food treatment, adding to a total of 15 fragments per treatment. Five fragments from each treatment were randomly distributed to each aquaria leading to a total of three aquaria per treatment (Fig. 3). While distributing fragments, we ensured that all treatments and aquaria contained coral fragments with comparable size range. Fragment characteristics are provided on Table 1.

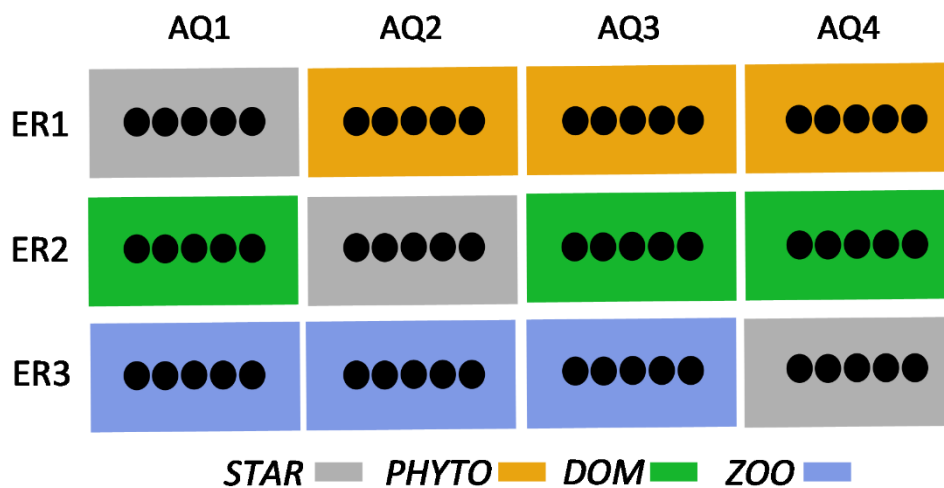


Figure 3: Experimental design for feeding experiments with *Antipathella wollastoni*. The set-up consisted of four experimental aquaria (AQ1-4) which were randomly

distributed to each treatment in each experimental run (ER1-3). Each aquaria contained five coral fragments (black circles)

Each experimental run lasted for a total of five days. On the first day corals were positioned in aquaria and a continuous flow of 4 cm s^{-1} was established. This flow was adequate to keep prey in suspension without overheating the aquaria motors. Corals were left to acclimatize to the created flow for approximately one hour. After closing water circulation, a predetermined amount of C was provided, to reach a target daily concentration of $17 \mu\text{mol C L}^{-1}$, which was the maximum concentration that could be logistically achieved considering the laboratory capacity to culture live prey. The food was left in the aquaria for 12 hours. After this period, all aquaria were cleaned, water circulation was restored and coral fragments were left without food for another 12 hours under low flow speed ($< 2 \text{ cm s}^{-1}$). This procedure was repeated once every day for a total of five days. A power analysis was conducted to assess the statistical power of the implemented experimental design, revealing that it can identify differences in tracer incorporation equal or higher than 30% of the average, with an estimated power of 79% (Fig S2, supplementary material).

Table 1: Average dry weight (DW), organic carbon (C) content, organic nitrogen (N) content and C to N ratio (C:N) \pm SD for fragments of *Antipathella wollastoni* in the four different food treatments: PHYTO: *Chaetoceros calcitrans*; ZOO: *Branchionus plicatilis*; DOM: Dissolved Organic Material; STAR: Starved.

Food Substrate	DW (g)	Organic C content (%)	Organic N (%)	C:N
PHYTO	0.23 \pm 0.085	28.12 \pm 1.35	5.98 \pm 0.43	5.49 \pm 0.25
ZOO	0.23 \pm 0.090	28.86 \pm 1.14	6.19 \pm 0.46	5.45 \pm 0.25

DOM	0.21±0.086	29.84±0.92	6.72±0.49	5.20±0.27
STAR	0.20±0.060	29.87±2.7	6.5± 0.83	5.35±0.36

Oxygen consumption was used as an additional response variable of the total metabolic activity of coral fragments under different food treatments. Respiration measurements were taken for each food treatment before providing food on day 1 (T0) and immediately after the last food provision on day 5 (T1). Estimates at T0 were used to assess the initial metabolic activity of the fragments and detect potential effects of long-term maintenance in the aquaria. A total of six fragments from each treatment were selected and placed in 450 ml glass respiration chambers filled with filtered (0.2 µm) natural SW. In each incubation, two additional chambers were left empty and served as control chambers. All chambers contained glass-coated magnetic stirrers and were placed in a single water bath maintaining temperature between 18.8 -19.4 °C with the aid of automatic aquaria heaters (EHEIM 100w). Incubations took place in the dark and lasted for a maximum of four hours or upon reaching oxygen saturation of 80 %. Oxygen concentration was measured at the beginning and end of each respiration incubation from seven out of the eight chambers, while the chamber containing the largest fragment was continuously connected to the O₂ electrode (Presens Precision Sensing GmbH, Germany) to monitor oxygen saturation. Oxygen consumption was calculated as the decrease in O₂ during the incubation and standardized to coral organic carbon (mmol OC, see sample processing).

Upon completion of each experimental run all coral fragments were preserved at -80° C, freeze-dried, weighed and grinded by mortar and pestle. A subsample of ground material was analyzed for C and N content and isotope ratio ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) using an elemental analyzer (Thermo Electron Flash 1112) coupled to an isotope ratio mass

spectrometer (EA-IRMS, DELTA-V, THERMO Electron Corporation). A second subsample was acidified stepwise with drops of HCl to completely remove the inorganic C before analysis on the EA-IRMS for organic C content and isotope ratio.

Tissue C and N content were standardized to DW and expressed as mmol C or N (g DW^{-1}). The incorporation of C and N derived from the substrates into the coral tissue is referred to as tracer C and N incorporation, and was derived from the coral $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as described in Maier et al. (2019). The $^{13}\text{C}:^{12}\text{C}$ ratio of each coral fragment (R_{sample}) was calculated as $R_{\text{sample}} = ([\delta^{13}\text{C}_{\text{sample}}/1000] + 1) \times R_{\text{ref}}$, where $R_{\text{ref}}(\text{C}) = 0.0111802$. Fractional abundance of ^{13}C , i.e. $F_{13} = ^{13}\text{C}/[^{12}\text{C} + ^{13}\text{C}]$ was calculated as $F_{13} = R_{\text{sample}} / (R_{\text{sample}} + 1)$. The fractional enrichment of each enriched coral fragment, F_{13} , (F_E) was calculated by subtracting the F_{13} of that sample with the F_{13} of the respective unfed fragment of the same colony. Subsequently ^{13}C incorporation was estimated by multiplying its fractional enrichment with its organic C content ($\mu\text{mol } ^{13}\text{C fragment}^{-1}$). Final tracer C incorporation, i.e. the total amount of C (^{13}C plus ^{12}C) incorporated into coral tissue from the enriched food substrate, was calculated by dividing the ^{13}C incorporation by the fractional abundance (F_{13}) of the respective food substrate. Tracer N incorporation was calculated accordingly by using $R_{\text{ref}}(\text{N}) = 0.0036782$.

Prey capture under different flow velocities

Zooplankton prey capture was further assessed by providing *B. plicatilis* as prey under three different flow velocities: 1.5 cm s^{-1} , 4 cm s^{-1} and 6 cm s^{-1} , in three consecutive experimental runs. In each run, one of the three flow velocities was established in four aquaria placed in a common water bath (19°C , Orejas et al. 2019) which was kept in the dark. Five coral fragments were placed in each aquaria and left to acclimatize to the

corresponding flow for approximately 15 minutes. Prey was added at concentrations of 2000 prey L⁻¹, the same C concentration that was used in the experiment for food preferences. Four replicate water samples were taken from each aquaria with 10 ml glass pipettes approximately 30 seconds after placing prey and subsequently every 15 minutes for the next hour and every hour until completion of 5 hours. Prey concentration was determined under a dissecting microscope (Zeiss StemiDV4). Control runs (CTR) were performed by repeating the experiments using the skeleton of dead coral fragments, to determine the aquaria effect on rotifer concentration.

After completion of all feeding trials, coral fragments were dried at 70 °C for 72 hours and weighted with an analytical balance. Dry weight was used to standardize the capture of each feeding trial (standardized coral capture) since polyp number was difficult to determine due to the small polyp size and high density of polyps.

Statistical analysis

For all statistical analyses, we followed the procedure described by Crawley (2007). Data exploration of each dataset was done according to Zuur et al. (2010). To study the effect of the independent variables, including random and fixed effects, these were added progressively in the respective model and the Akaike Information Criterion (AIC) along with maximum likelihood ratio (MLR) tests were used to select the most appropriate model. Model diagnostics were inspected to ensure that there were no violations of the model assumptions. Detailed results of the MLR tests are presented in Table 2, whereas model coefficients of the most appropriate model for each tested dependent variable are presented in Table 3. All the variables in the text are given as average \pm standard deviation. Whenever needed, variance structure components were added to account for violation of homogeneity of variances, i.e. whenever residual

spread was observed to vary differently across levels of an independent variable (Zuur et al. 2009). Statistical analysis was performed in R 3.5.0 (R Core Team 2018). Linear and Non-Linear Mixed Effects Models (LMEs and NLMEs) were build using the packages LME4 (Bates et al. 2015) and nlme (Pinheiro et al. 2019). Detailed analysis of each dataset is presented below.

For the study of food selectivity, we had to account for (1) the distribution of fragments from the same colonies in different treatments and (2) the existence of multiple fragments within each experimental aquaria (Fig. 3). Therefore, we used Linear Mixed Effect Models (LMEs) with colony and experimental aquaria as random effect variables to deal with (1) and (2) respectively (Crawley 2007). Food treatment was considered as the fixed effect variable in question, examining its effect on the following dependent variables: tracer C and N incorporation and O₂ consumption.

For the study on optimum flow rate, the analysis had to account for the repetitive water sampling in regular time intervals and therefore LMEs and NLMEs (Pinheiro et al. Bates 2019) were used, with experimental run nested in time as random factors. Firstly the potential decrease of prey concentration due to the aquaria design was assessed by focusing on the experimental runs without live corals (CTR). Subsequently, the decrease in prey concentration in experimental runs with coral presence (COR) was analyzed in each flow velocity separately, applying a logarithmic LME to account for non-linearity. To compare prey capture among flow rates, prey capture was standardized by the total coral DW in each flume, accounting for the multiple fragments in each aquaria. Standardized prey capture was further divided by the initial prey concentration to estimate cumulative consumption (%). An NLME with asymptotic exponential (formula 1; Crawley, 2007) was chosen as appropriate given the typical asymptotic pattern of prey removal.

$$Con_{st} = a e^{bF} \quad (1)$$

Where Con_{st} the cumulative consumption, expressed as a percentage (%) of the initial concentration, a the first term of the asymptotic exponential expressing the maximum consumption, b the second term of the asymptotic exponential expressing a coefficient of consumption rate and F the flow speed.

Results

Field measurements

Suspended particulate organic matter (sPOM) concentration was $149 \pm 43.58 \mu\text{mol L}^{-1}$ at São Caetano and $180.5 \pm 52.3 \mu\text{mol L}^{-1}$ at Baixa da Feteira, corresponding to sPOC quantities of $44.7 \pm 13.07 \mu\text{mol C L}^{-1}$ and $54 \pm 15.69 \mu\text{mol C L}^{-1}$ respectively

Table 2: Akaike Information Criteria (AIC) and Likelihood Ratio Test results (df: degrees of freedom, L ratio: Likelihood Ratio, p-value) used for the selection of best models for each respective dependent variable.

	Model Type	Dependent variable	Independent variable	AIC	df	L.Ratio	p-value
Feeding preferences	LME	Organic Carbon (%)		243.17	5		
			Food substrate	239.15	8	16.290	0.001
	LME	Organic Nitrogen (%)		119.1	6		
			Food substrate	104.58	9	20.520	0.000
	LME	Carbon/Nitrogen ratio		12.02	6		
			Food substrate	2.05	9	15.970	0.001
	LME	¹³ C tracer incorporation		497.03	5		
			Food substrate	428.01	7	73.010	<0.0001
	LME	¹⁵ N tracer incorporation		355.89	5		
			Food substrate	282.64	7	77.260	<0.0001
	GLS	Oxygen Consumption _{T0}		17.2	5		
			Food substrate	20.28	8	2.960	0.3975
	GLS	Oxygen Consumption _{T0}		17.24	5		
			Incubation date	18.9	6	0.310	0.577
	GLS	Oxygen Consumption _{STAR}		22.4	2		
Incubation time			21.16	3	3.260	0.0706	
GLS	Oxygen Consumption _{CH}		6.35	2			
		Incubation time	-7.5	3	15.860	<0.0001	
GLS	Oxygen Consumption _{DOM}		2.47	2			
		Incubation time	-0.73	3	5.200	0.0226	
GLS	Oxygen Consumption _{ROT}		6.34	2			
		Incubation time	8.15	3	0.190	0.6592	
GLS	Oxygen Consumption _{T1}		8.7	5			
		Food substrate	2.29	8	12.4	<0.0001	
Flow speed	LME	Prey Concentration _{time=0}		56.88	4		
			Flow speed	59.96	6	0.917	0.632
	LME	Prey Concentration _{time=0;Treatment=COR}		37.27	4		
			Flow speed	37.11	6	4.160	0.125
	LME	Prey Concentration		146.62	5		
			Time	147.69	6	0.929	0.335
			Flow speed	148.13	7	1.560	0.211
			Time*Flow speed	149.99	8	0.143	0.705
	LME	Prey Concentration _{flow=2.5cm/sec}		112.08	4		
	LME	Prey Concentration _{flow=4cm/sec}		98.52	5	15.550	0.000
log(Time)			37.16	4			
LME	Prey Concentration _{flow=6cm/sec}		11.58	5	27.570	0.001	
		log(Time)	105.48	4			
LME	Prey Concentration _{flow=6cm/sec}		91.18	5	16.300	0.000	

Table 3: Summaries of best models for the analysis of each independent variable

Model Type	Model	Coefficient	Estimate	Std. Error	p-value	Variance function(var)	Random Effects						
							r_{colony}^2	Residual					
LME	OC=b x FS + varFS + V_{FS} + ϵ	b_{PHYTO}	28.120	0.347	0.000	0.497							
		b_{DOM}	29.832	0.420	0.000	0.340							
		b_{ZOO}	28.863	0.455	0.109	0.423							
		b_{STAR}	29.867	0.779	0.029	1.000							
		b_{PHYTO}	5.980	0.105	0.000	0.685							
		b_{DOM}	6.708	0.139	0.000	1.000							
		b_{ZOO}	6.183	0.125	0.113	0.851							
		b_{STAR}	6.529	0.225	0.019	1.837							
		b_{PHYTO}	5.491	0.058	0.000	0.344							
		b_{DOM}	5.200	0.070	0.000	1.000							
LME	CN=b x FS + varFS + r_{colony}^2 + ϵ	b_{ZOO}	5.455	0.047	0.432	0.605	0.2012	0.249					
		b_{STAR}	5.354	0.057	0.021	0.784							
		b_{PHYTO}	0.565	0.212	0.013	1.413							
		b_{DOM}	3.705	0.203	0.000	1.000							
		b_{ZOO}	58.005	7.994	0.000	68.075							
LME	$T_c = b \times FS + varFS + r_{colony}^2 + \epsilon$	b_{PHYTO}	0.100	0.042	0.022	56.650	0.497	0.439					
		b_{DOM}	0.848	0.038	0.000	1.000							
		b_{ZOO}	10.910	1.465	0.000	1.100							
		b_{PHYTO}	0.270	0.170	0.149	1.000							
		b_{DOM}	0.380	0.180	0.434	2.230							
GLS	$O_2C_{12} = b \times FS + varFS + \epsilon$	b_{ZOO}	0.840	0.240	0.145	4.320							
		b_{STAR}	0.520	0.160	0.007	6.750							
Model Type	Flow speed	Model	Coefficient	Estimate	Std. Error	p-value	Time	Trial in Time	Residual				
LME	2	$Con = a + b1 \times \log(TIME) + r_{trialTime}^2 + \epsilon$	a	2.310	0.210	0.000	0.202	0.063	0.355				
			b_1	-0.318	0.040	0.006							
			a	2.020	0.090	0.000	0.077			0.054	0.230		
			b_1	-0.330	0.021	0.001							
			a	2.530	0.230	0.000	0.219					0.216	0.307
			b_1	-0.390	0.050	0.000							
			a	90.950	6.410	0.000							
			b	0.009	0.001	0.880							
			a	92.040	3.050	0.738							
			b	0.021	0.003	0.000							
a	93.520	4.200	0.003										
b	0.013	0.002	0.108										
NILME	4	$Con_{st} = a \cdot e^{bF}$	a	93.520	4.200	0.003							
			b	0.013	0.002	0.108							
NILME	6	$Con_{st} = a \cdot e^{bF}$	a	93.520	4.200	0.003							
			b	0.013	0.002	0.108							

OC: Organic carbon (%); NC: Nitrogen/Carbon ratio; T_c: ¹³C Tracer incorporation; T_N: ¹⁵N Tracer incorporation; FS: food substrate; O₂C: Oxygen consumption; r_{colony}²: random factor; ϵ : residual error; PHYTO: phytoplankton *Chaetoceros californicus*; DOM: Dissolved Organic Matter; ZOO: zooplankton, *Branthionus plicatilis*; STAR: starved

Feeding on different food substrates

All food substrates were significantly enriched above background (Table 4). Target carbon concentrations ($17 \mu\text{mol C L}^{-1}$) were successfully recreated in the case of DOM but were 63% lower than the target concentration in the case of the PHYTO treatment and 31% higher in the case of the ZOO treatment (Table 4).

All coral fragments incorporated tracer C and N from all food substrates. Tracer C and N incorporation were significantly higher in fragments fed with zooplankton (ZOO), followed by fragments fed with DOM (Fig. 4, Table 3).

Table 4: Average recreated carbon concentration, total provided carbon, tracer fractional abundance (F13, F15) and C:N ratio of food substrates \pm SD : phytoplankton *Chaetoceros calcitrans* and *Nannochloropsis gaditana*, zooplankton *Branchionus plicatilis* and Dissolved Organic Matter (DOM) used in the feeding experiments with *Antipathella wollastoni*.

	<i>C. calcitrans</i>	<i>N. gaditana</i>	DOM	<i>B. plicatilis</i>
C concentration ($\mu\text{mol L}^{-1}$)	6.39 ± 0.69	-	17.72 ± 0	23.26 ± 0.57
Total C provided ($\mu\text{mol fragment}^{-1}$)	210 ± 22.72	-	585.06 ± 0	767.67 ± 18.91
F13	0.47	0.55	0.96	0.36
F15	0.60	0.70	0.87	0.39
C:N	10.4	15.21	4.41	5.5

The addition of experimental aquaria as a random effect was not supported by the MLR tests and therefore the existence of multiple fragments in each experimental aquaria did not have any influence on tracer incorporation. Tracer C incorporation was higher than tracer N incorporation in all treatments (Fig. 4). However, C and N from the ZOO and PHYTO treatments were incorporated at higher tracer C:N ratios than in the DOM

treatment: tracer C:N was 5.72 ± 0.57 under the PHYTO treatment, 4.35 ± 0.16 under the DOM treatment and 5.27 ± 0.12 under the ZOO treatment.

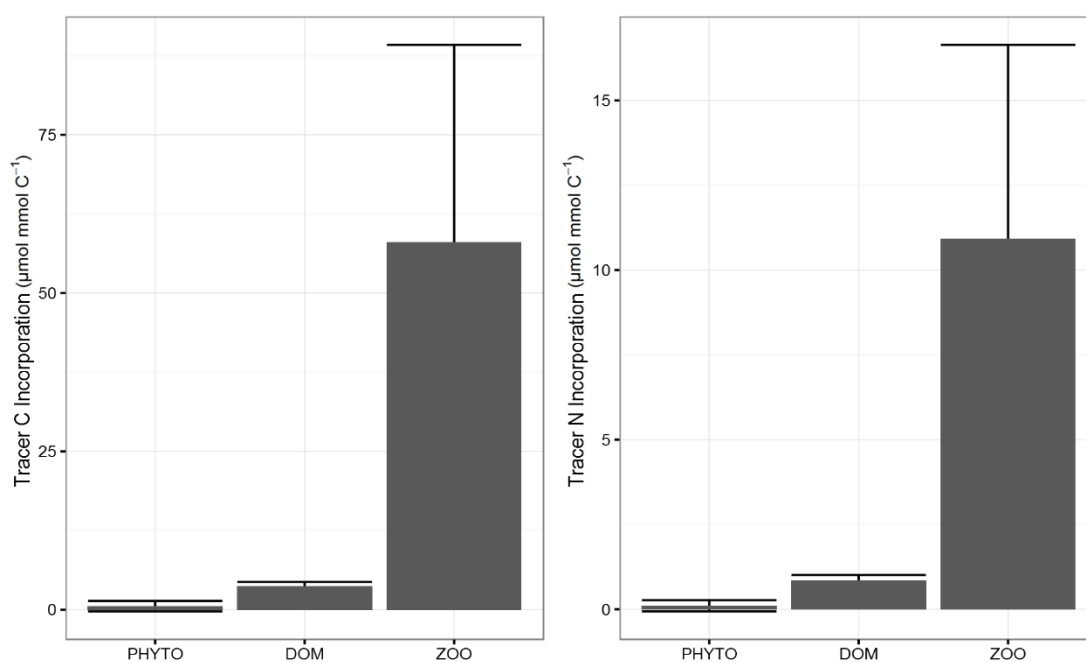


Figure 4: ¹³C and ¹⁵N tracer incorporation (average ± SD) of fragments of *Antipathella wollastoni* under three food substrates (PHYTO: diatom *Chaetoceros calcitrans*; DOM: Dissolved Organic Material; ZOO: rotifer *Branchionus plicatilis*).

O₂ consumption rates before the feeding experiments (T₀) was not statistically different among treatments (Table 2). Moreover, incubation date did not have a significant effect on O₂ consumption (Table 2) suggesting that there was no bias due to the fact that treatments were not run simultaneously. Oxygen consumption rates at the end of the feeding experiments (T₁) did not differ significantly from initial rates (T₀) for coral fragments fed with zooplankton (ZOO) and unfed fragments (STAR) (Figure 5, Table 2). In contrast, a significant decrease in O₂ consumption between T₀ and T₁ was observed in the case of the PHYTO and DOM treatments, showing a decrease in metabolic rate during the course of the experimental trials under these two food

treatments. Moreover, fragments fed with zooplankton (ZOO treatment) showed a significantly higher O₂ consumption at T1 compared to fragments in the rest of the treatments (Fig. 5, Table 3).

Coral fragments under the ZOO treatment incorporated the highest proportion of the provided C quantity (39.88 ± 21.28 %), followed by the DOM (3.45 ± 0.58 %) and PHYTO treatments (1.37 ± 1.98 %). This corresponded to incorporation of 1.16 ± 0.62 % of their biomass per day under the ZOO treatment, compared to 0.07 ± 0.01 % under the DOM treatment and 0.01 ± 0.01 % under the PHYTO treatment. In the case of the ZOO treatment and considering the results on standardized prey capture under 4 cm s^{-1} (capture of 95.6 ± 2.4 % of the provided prey), C incorporation was estimated at 41.72 ± 22.2 % of captured prey.

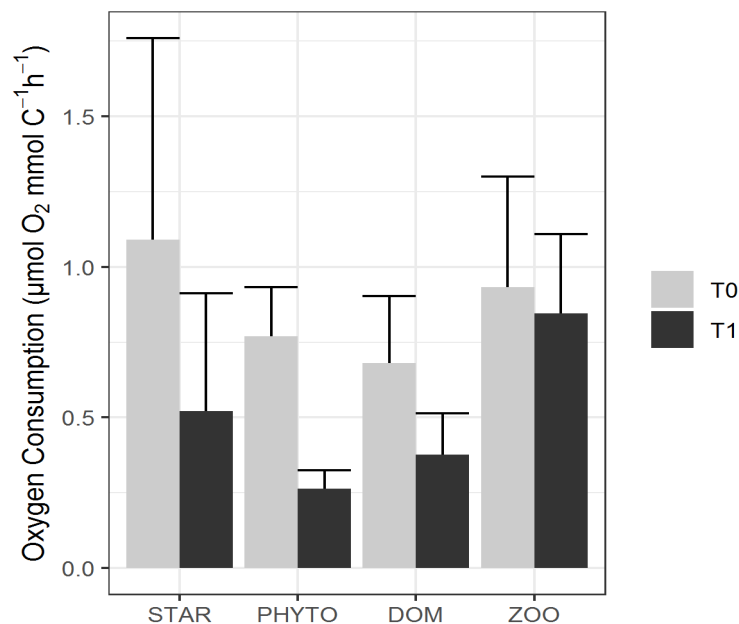


Figure 5: Oxygen consumption (average \pm SD) of fragments of *Antipathella wollastoni* under three different food substrates (STAR: unfed; PHYTO: diatom *Chaetoceros calcitrans*; DOM: Dissolved Inorganic Material; ZOO: rotifer *Branchionus plicatilis*)

Prey capture under different flow velocities

No significant change in zooplankton prey concentration (rotifer *B. plicatilis*) was observed in the CTR treatment (Fig. 6), independently of flow velocity (Table 2), indicating no loss of prey due to the flume design. Within the COR treatment, initial prey concentrations were slightly lower but not significantly different under 4 cm s⁻¹ when compared to 1.5 cm s⁻¹ (1870 ± 87 prey L⁻¹ compared to 2041 ± 38 prey L⁻¹, respectively) (Table 3).

Coral presence caused a significant decrease in prey concentration under all flow velocities which followed asymptotic curves (Fig. 6). Prey concentration reached extremely low values at the end of all the experimental runs. The LME models estimated a lower intercept for experimental runs with coral presence and flow velocity of 4 cm s⁻¹ (Table 3, Fig. 6), highlighting the differences in initial prey concentration mentioned above. Standardized prey capture estimates reached final values of 3.02 ± 0.41 captured prey g coral DW⁻¹ under 1.5 cm s⁻¹, 2.81 ± 0.21 captured prey g coral

DW⁻¹ under 4 cm s⁻¹ and 3.67 ± 0.77 captured prey g coral DW⁻¹ under 6 cm s⁻¹.

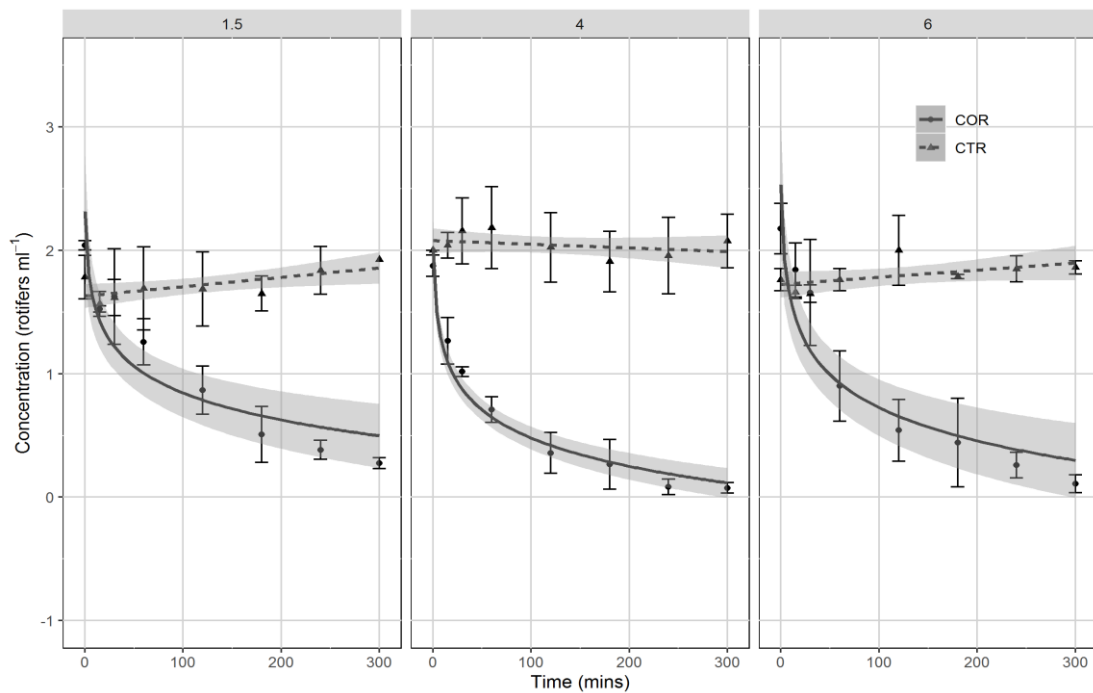


Figure 6: Prey concentration (average ± SD) during experiments under the CTR treatment including only coral skeletons (left) and the COR treatment (right) with presence of coral fragments in three experimental flows: 1.5 cm s⁻¹, 4 cm s⁻¹, 6 cm s⁻¹

Coral fragments in the first hours of the experimental runs removed prey significantly faster under 4 cm s⁻¹, compared to the other flow rates (Fig. 7), as highlighted by the higher coefficient *b* of the NLS model under this flow (Table 3). Final capture levels were above 85% of the offered prey and were not statistically significant under all tested flow velocities (86.5 ± 2.4 %, 95.6 ± 2.4 % and 95.2 ± 3.1 % under 1.5 cm s⁻¹, 4 cm s⁻¹ and 6 cm s⁻¹ respectively; coefficient *a*, Table 3).

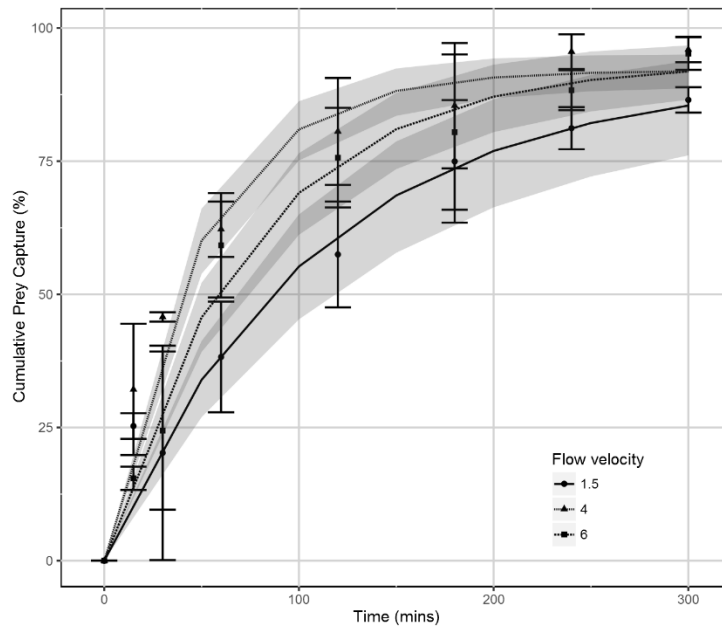


Figure 7: Standardized prey capture (%) and cumulative prey capture (%) of fragments of *Antipathella wollastoni* including predicted values (lines) and confidence intervals (shaded area) of the NLS model with quadratic asymptotic, under three experimental flows (1.5 cm s⁻¹, 4 cm s⁻¹, 6 cm s⁻¹)

Discussion

Antipathella wollastoni was able to utilize different food substrates, from DOM to phytoplankton and zooplankton, suggesting high flexibility to exploit seasonally available food sources. While phytoplankton ingestion by antipatharians has been inferred before through studies using N stable isotopes (Carlier et al. 2009; Wagner et al. 2012), to our knowledge this is the first time it is actually documented. However, even if recreated C concentrations under the ZOO treatment were 3.6x and 2.5x higher compared to the phytoplankton and DOM treatments respectively, tracer C incorporation was almost 10x higher under the zooplankton treatment. This corroborates with previous studies which have reported that antipatharian diet is mainly consisted of zooplankton (Carlier et al. 2009; Wagner et al. 2012, Coppari et al. 2020).

Furthermore, the fact that a higher proportion of zooplankton derived C and N was utilized compared to the other food treatments, suggests a higher importance of this food source for tissue growth and maintenance.

The higher O₂ consumption of fragments fed with zooplankton indicates higher metabolic activity under zooplankton feeding, further supporting zooplankton as a vital source sustaining key physiological processes of the target species. Similar results have been reported for tropical (Ferrier-Pagès et al. 2003) and cold-water (Naumann et al. 2011) scleractinian species with higher growth, O₂ consumption and calcification when fed with zooplankton, but this is the first time a metabolic increase upon feeding with zooplankton is reported for an antipatharian. Although antipatharians do not calcify, they possess a hard organic skeleton with high concentration of proteins (Goldberg et al. 1994). The formation of such a skeleton is expected to be energetically demanding (Nowak et al. 2009). Thus, it is plausible that the high protein content of the provided zooplankton prey (low C: N ratio, Table 4) might have allowed the target species to incorporate part of the provided C and N into coral skeleton and may explain the simultaneous high O₂ consumption and high C and N incorporation. Reproduction is another process that requires high energy input. *Antipathella wollastoni* in the Azores displays annual reproductive cycles lasting from spring to late summer (Rakka et al. 2017) when zooplankton biomass and abundance are higher (Carmo et al. 2013). It is therefore possible that the seasonal availability of zooplankton prey has a fundamental role in gametogenesis and reproductive timing.

Seasonality in food availability and the existence of seasonal energetic constraints for marine invertebrates have been highlighted as key factors in communities of many suspension feeders (Coma et al. 2000; Coma and Ribes 2003; Orejas et al. 2003). Seasonally variable feeding behaviour has been observed in benthic suspension feeders

such as the gorgonians *Paramuricea clavata* (Ribes et al. 1999; Rossi et al. 2006) and *Leptogorgia sarmentosa* (Ribes et al. 2003; Rossi et al., 2004), as well as the antipatharian *Antipathella subpinnata* (Coppari et al. 2020) in the Mediterranean. These feeding strategies are likely related to adaptations to site-specific and temporal variations in food availability which are very common in oligotrophic, temperate ecosystems such as the Mediterranean (Lazzari et al. 2012) and the Azores Archipelago (Carmo et al. 2013; Silva et al. 2013). *Antipathella wollastoni* persists in regions with temperate, subtropical and tropical climates, however in the studied region it is subject to pronounced seasonality including temperature and food availability (Carmo et al. 2013; Santos et al. 2013). Therefore, the fact that *A. wollastoni* was able to utilize phytoplankton and DOM, upon which it displayed lower metabolic activity, might suggest a possible decrease in metabolic activity in periods when these are the main available food sources and an increase in metabolically demanding processes such as growth and reproduction in periods when zooplankton is more abundant.

Different flow velocities may favor the capture of different food types based on their size, buoyancy, swimming speed and general behaviour (Shimeta and Koehl 1997; Sebens et al. 2017). This phenomenon has been reported by Orejas et al. (2016) for the cold-water coral *L. pertusa* which displayed higher capture of zooplankton in low velocities (2 cm s^{-1}) and higher capture of phytoplankton in higher velocities (5 cm s^{-1}). While this food preference study was performed only under one flow velocity, *A. wollastoni* was able to capture similar quantities of zooplankton under all flow velocities tested.

Cumulative prey capture suggested that the species has the capacity to consume higher amounts of zooplankton prey once it becomes available under intermediate flow velocities (4 cm s^{-1}) but capture rates were lower under 1.5 cm s^{-1} , possibly due to lower

prey encounter rates or more successful escape of the zooplankton prey (Sebens et al. 1997). Capture rates were also lower under 6 cm s^{-1} compared to 4 cm s^{-1} which is likely due to higher drag and tentacle bending and prey loss (Shimeta and Koehl 1997; Riisgård and Larsen 2015). Standardized capture rates were lower under 4 cm s^{-1} , however this might be due to the lower initial concentrations observed in these experimental runs. Prey concentration is a known factor influencing feeding rates (Purser et al. 2010), but in this case the difference between the created initial concentrations was very small (8-13 %) and is not expected to affect coral behaviour.

The high variation in prey capture observed among experimental runs under the same flow velocity (Fig. 7) highlights differences in the ability of fragments to capture prey. This can be attributed to a variety of factors, such as polyp number, polyp and fragment position, the use and position of sweeper tentacles (Goldberg et al. 1990) and satiation (Lin et al. 2002). Moreover, due to the high polyp density and small polyp size of *A. wollastoni*, a balanced distribution of fragments with similar polyp number among experimental runs is very difficult, compared to similar experiments with Scleractinia where polyp number in each experimental run can be easier manipulated *a priori* (e.g. Purser et al. 2010; Orejas et al. 2016).

Capture rates might have been higher under intermediate flows, however final prey capture was very high (85-95% of available prey) under all tested flow speeds (Fig. 7). This is an expected outcome, considering that the target species is encountered on island slopes where C concentrations are higher compared to the recreated C concentrations in the experiments, as revealed by the water samples collected at the sampling sites. Due to the presence of multiple fragments within each aquaria it is not clear if satiation occurred or if coral fragments fed continuously until concentration diminished, leading to such high final capture levels. Coral fragments were not overlapping but were closely

positioned in the aquaria. While the number of fragments is likely to affect net capture (Sebens et al. 1997) and create conditions of competition, densely positioned branches are very common in populations of *A. wollastoni* in the field, therefore we consider that the situation simulated in aquaria closely represented natural *in situ* conditions.

The importance of zooplankton for vital physiological processes and high efficiency of the species to capture it under different flow velocities highlights the complex dynamics between pelagic and benthic communities in the studied ecosystem. Such knowledge is extremely useful in order to understand potential impacts of upcoming environmental changes. Global warming and acidification are likely to impact seasonal cycles of food availability and zooplankton dynamics (Rossoll et al. 2012; Cripps et al. 2014). Therefore, such disturbances might affect coral physiology directly (Hennige et al. 2014; Gori et al. 2016) or indirectly by altering the availability of energy sources which are very important for the health and function of entire coral populations and their associated biodiversity.

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Conflict of interest statement and compliance with Ethical Standards

On behalf of all authors, the corresponding author states that there is no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67
- Bo M, Bavestrello G (2019) Mediterranean Black Coral Communities. In *Mediterranean Cold-Water Corals: Past, Present and Future*. Springer, Cham, pp 249–251
- Bo M, Canese S, Bavestrello G (2014) Discovering Mediterranean black coral forests: *Parantipathes larix* (Anthozoa: Hexacorallia) in the Tuscan Archipelago, Italy. *Ital. J. Zool.* 81:112–125
- Bo M, Canese S, Spaggiari C, Pusceddu A, Bertolino M, Angiolillo M, Giusti M, Loreto MF, Salvati E, Greco S, Bavestrello G (2012) Deep Coral Oases in the South Tyrrhenian Sea. *PLoS One* 7:e49870
- Brito A, Ocaña O (2004) Corales de las Islas Canarias: antozoos con esqueleto de los fondos litorales y profundos.
- Buhl-Mortensen L, Buhl-Mortensen P, Rungruangsak-Torrissen K, Schwach V, Hjort J, Jakobsen T, Ozhigin VK, Bergh Ø, Hamre J, Torgersen T, Toresen R (2018) Cold Temperate Coral Habitats. *Corals in a Changing World*, p 9
- Carlier A, Le Guilloux E, Olu K, Sarrazin J, Mastrototaro F, Taviani M, Clavier J (2009) Trophic relationships in a deep Mediterranean cold-water coral bank (Santa Maria di Leuca, Ionian Sea). *Mar. Ecol. Prog. Ser.* 397:125–137
- Carmo V, Santos M, Menezes GM, Loureiro CM, Lambardi P, Martins A (2013) Variability of zooplankton communities at Condor seamount and surrounding areas, Azores (NE Atlantic). *Deep Sea Res. Part II Top. Stud. Oceanogr.* 98:63–74
- Clegg SL, Whitfield M (1990) A generalized model for the scavenging of trace metals in the open ocean—I. Particle cycling. *Deep Sea Res. Part A. Oceanogr. Res. Pap.* 37: 809–832. Coma R, Ribes M (2003) Seasonal energetic constraints in

Mediterranean benthic suspension feeders: effects at different levels of ecological organization. *Oikos* 101:205–215

- Coma R, Ribes M, Gili J-M, Zabala M (2000) Seasonality in coastal benthic ecosystems. *Trends Ecol. Evol.* 15:448–453
- Coppari M, Ferrier-Pagès C, Castellano M, Massa F, Olivari E, Bavestrello G, Povero P, Bo M (2020) Seasonal variation of the stable C and N isotopic composition of the mesophotic black coral *Antipathella subpinnata* (Ellis & Solander, 1786). *Estuarine, Coastal and Shelf Science* 5 (233):106520
- Crawley M (2007) *The R Book*. West Sussex, England
- Cripps G, Lindeque P, Flynn KJ (2014) Have we been underestimating the effects of ocean acidification in zooplankton? *Glob. Chang. Biol.* 20:3377–3385
- EMODnet Bathymetry Consortium (2018): EMODnet Digital Bathymetry (DTM).
<http://doi.org/10.12770/18ff0d48-b203-4a65-94a9-5fd8b0ec35f6>
- Ferrier-Pagès C, Witting J, Tambutté E, Sebens KP (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. *Coral Reefs* 22 (3): 229-40
- Gaino E, Bavestrello G, Boyer M, Scoccia F, Bo M (2013) Biological and ecological relevance of black corals (Antipatharia) in the benthic environment. In: Liñán-Cabello MA (ed) *Corals: classification, habitat and ecological significance*. NOVA Science Publishers, New York, pp 37–74
- Goenaga C (1977) Two new species of Stichopathes (Zoantharia; Antipatharia) with observations on aspects of their biology. University of Puerto Rico
- Goldberg WM, Grange KR, Taylor GT, Zuniga AL (1990) The Structure of Sweeper Tentacles in the Black Coral *Antipathes fiordensis*. *Biol. Bull.* 179:96–104
- Goldberg WM, Hopkins TL, Holl SM, Schaefer J, Kramer KJ, Morgan TD, Kim K (1994) Chemical composition of the sclerotized black coral skeleton (Coelenterata: Antipatharia): a comparison of two species. *Comp. Biochem. Physiol. Part B Comp. Biochem.* 107:633–643
- Gori A, Ferrier-Pagès C, Hennige SJ, Murray F, Rottier C, Wicks LC, Roberts JM (2016) Physiological response of the cold-water coral *Desmophyllum dianthus* to thermal stress and ocean acidification. *PeerJ* 4:e1606
- Grigg RW (1965) Ecological Studies of Black Coral in Hawaii. *Pac Sci* 19(2): 244-260
- Hennige SJ, Wicks LC, Kamenos NA, Perna G, Findlay HS, Roberts JM (2014) Hidden impacts of ocean acidification to live and dead coral framework. *Proc. R. Soc. B Biol. Sci.* 282:20150990

- Huguet C (2017) Seston Quality and Available Food: Importance in the Benthic Biogeochemical Cycles. *Marine Animal Forests*. Springer International Publishing, Cham, pp 733–759
- Ingrassia M, Macelloni L, Bosman A, Chiocci FL, Cerrano C, Martorelli E (2016) Black coral (Anthozoa, Antipatharia) forest near the western Pontine Islands (Tyrrhenian Sea). *Mar. Biodivers.* 46:285–290
- Lazzari P, Solidoro C, Ibello V, Salon S, Teruzzi A, Béranger K, Colella S, Crise A (2012). Seasonal and inter-annual variability of plankton chlorophyll and primary production in the Mediterranean Sea: a modelling approach. *Biogeosciences* 9(1): 1965-1985
- Lewis J (1978) Feeding mechanisms in black corals (Antipatharia). *J. Zool. London* 186:393–396
- Lin M, Liao C, Dai C (2002) Modeling the Effects of Satiation on the Feeding Rate of a Colonial Suspension Feeder, *Acanthogorgia vegae*, in a Circulating System under Lab Conditions. *Zool. Stud.* 41:355–365
- Maier SR, Kutti T, Bannister RJ, van Breugel P, van Rijswijk P, van Oevelen D (2019) Survival under conditions of variable food availability: Resource utilization and storage in the cold-water coral *Lophelia pertusa*. *Limnol. Oceanogr.*
- Massi D, Vitale S, Titone A, Milisenda G, Gristina M, Fiorentino F (2018) Spatial distribution of the black coral *Leiopathes glaberrima* (Esper, 1788) (Antipatharia: Leiopathidae) in the Mediterranean: a prerequisite for protection of Vulnerable Marine Ecosystems (VMEs). *Eur. Zool. J.* 85:169–178
- Montgomery AD, Crow GL (1998) Collection and husbandry techniques for black coral at the Waikiki Aquarium. 103–108
- Naumann MS, Orejas C, Wild C, Ferrier-Pagès C (2011) First evidence for zooplankton feeding sustaining key physiological processes in a scleractinian cold-water coral. *J. Exp. Biol.* 214:3570–3576
- Nowak D, Florek M, Nowak J, Kwiatek W, Lekki J, Chevallier P, Hacura A, Wrzalik R, Ben-Nissan B, Van Grieken R, Kuczumow A (2009) Morphology and the chemical make-up of the inorganic components of black corals. *Mater. Sci. Eng. C* 29:1029–1038
- Orejas C, Gili J, Arntz W (2003) The role of the small planktonic communities in the diet of two Antarctic octocorals (*Primnoisis antarctica* and *Primnoella* sp.). *Mar. Ecol. Prog. Ser.* 250:105–116
- Orejas C, Gori A, Rad-Menéndez C, Last KS, Davies AJ, Beveridge CM, Sadd D, Kiriakoulakis K, Witte U, Roberts JM (2016) The effect of flow speed and food size on the capture efficiency and feeding behaviour of the cold-water coral *Lophelia pertusa*. *J. Exp. Mar. Bio. Ecol.* 481:34–40

- Orejas C, Jiménez C (2017) The Builders of the Oceans – Part I: Coral Architecture from the Tropics to the Poles, from the Shallow to the Deep. In: Rossi S., Bramanti L., Gori A., Orejas C. (eds) Marine Animal Forests. Springer, Cham
- Orejas C, Taviani M, Ambroso S, Andreou V, Bilan M, Bo M, Brooke S, Buhl-Mortensen P, Cordes E, Dominguez-Carrió C, Ferrier-Pagès C, Godinho A, Gori A, Grinyó J, Gutiérrez-Zárate C, Hennige S, Jiménez C, Larsson AI, Lartaud F, Lunden J, Maier C, Maier SR, Movilla J, Murray F, Peru E, Purser A, Rakka M, Reynaud S, Roberts JM, Siles P, Strömberg SM, Thomsen L, van Oevelen D, Veiga A, Carreiro-Silva M (2019) Cold-Water Coral in Aquaria: Advances and Challenges. A Focus on the Mediterranean. Springer, Cham, pp 435–471
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2019) nlme: Linear and Nonlinear mixed effects models. R package version 3.1-140.
- Purser A, Larsson AI, Thomsen L, van Oevelen D (2010) The influence of flow velocity and food concentration on *Lophelia pertusa* (Scleractinia) zooplankton capture rates. J. Exp. Mar. Bio. Ecol. 395:55–62
- Rakka M, Orejas C, Sampaio I, Monteiro J, Parra H, Carreiro-Silva M (2017) Reproductive biology of the black coral *Antipathella wollastoni* (Cnidaria: Antipatharia) in the Azores (NE Atlantic). Deep Sea Res. Part II Top. Stud. Oceanogr. 145:131–141
- Ribes M, Coma R, Rossi S (2003) Natural feeding of the temperate asymbiotic octocoral-gorgonian *Leptogorgia sarmentosa* (Cnidaria: Octocorallia). Mar. Ecol. Prog. Ser. 254:141–150
- Ribes M, Coma R, Gili JM (1999) Heterogeneous feeding in benthic suspension feeders: The natural diet and grazing rate of the temperate gorgonian *Paramuricea clavata* (Cnidaria: Octocorallia) over a year cycle. Mar. Ecol. Prog. Ser. 183:125–137
- Riisgård HU, Larsen PS (2015) Filter-Feeding Zoobenthos and Hydrodynamics. Marine Animal Forests. Springer International Publishing, Cham, pp 1–25
- Rossi S, Gili JM, Coma R, Linares C, Gori A, Vert N (2006) Temporal variation in protein, carbohydrate, and lipid concentrations in *Paramuricea clavata* (Anthozoa, Octocorallia): Evidence for summer-autumn feeding constraints. Mar. Biol. 149:643–651
- Rossi S, Bramanti L, Gori A, Orejas C (2017) An Overview of the animal forests of the world. In: Rossi S., Bramanti L., Gori A., Orejas C. (eds) Marine Animal Forests. Springer, Cham
- Rossoll D, Bermúdez R, Hauss H, Schulz KG, Riebesell U, Sommer U, Winder M (2012) Ocean Acidification-Induced Food Quality Deterioration Constrains Trophic Transfer. PLoS One 7:e34737

- Santos M, Moita MT, Bashmachnikov I, Menezes GM, Carmo V, Loureiro CM, Mendonça A, Silva AF, Martins A (2013) Phytoplankton variability and oceanographic conditions at Condor seamount, Azores (NE Atlantic). *Deep Sea Res. Part II Top. Stud. Oceanogr.* 98:52–62
- Sarmiento J, Gruber N (2006) *Ocean biogeochemical dynamics*. Princeton University Press.
- Sebens KP, Witting J, Helmuth B (1997) Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). *J. Exp. Mar. Bio. Ecol.* 211:1–28
- Sebens K, Sarà G, Nishizaki M (2017) Energetics, particle capture, and growth dynamics of benthic suspension feeders. In: Rossi S., Bramanti L., Gori A., Orejas C. (eds) *Marine Animal Forests*. Springer, Cham, pp 813–854
- Sherwood OA, Jamieson RE, Edinger EN, Wareham VE (2008) Stable C and N isotopic composition of cold-water corals from the Newfoundland and Labrador continental slope: Examination of trophic, depth and spatial effects. *Deep Sea Research Part I: Oceanographic Research Papers* 55(10):1392-402
- Shimeta J, Koehl MAR (1997) Mechanisms of particle selection by tentaculate suspension feeders during encounter, retention, and handling. *J. Exp. Mar. Bio. Ecol.* 209:47–73
- Silva A, Brotas V, Valente A, Sá C, Diniz T, Patarra RF, Álvaro NV, Neto AI (2013) Cocolithophore species as indicators of surface oceanographic conditions in the vicinity of Azores islands. *Estuar. Coast. Shelf Sci.* 118:50–59
- Spalding MD, Green EP, Ravilious C (2001) *World Atlas of Coral Reefs*. Univ of California Press.
- Tazioli S, Bo M, Boyer M, Rotinsulu H, Bavestrello G (2007) Ecological Observations of Some Common Antipatharian Corals in the Marine Park of Bunaken (North Sulawesi, Indonesia). *Zool. Stud.* 46:227–241
- Terrana L, Lepoint G, Eeckhaut I. (2019) Assessing trophic relationships between shallow-water black corals (Antipatharia) and their symbionts using stable isotopes. *Belgian Journal of Zoology* 149 (1)
- Wagner D, Luck DG, Toonen RJ (2012) The biology and ecology of black corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia). *Adv. Mar. Biol.* 63:67–132
- Williams B, Grotoli AG (2010) Stable nitrogen and carbon isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) variability in shallow tropical Pacific soft coral and black coral taxa and implications for paleoceanographic reconstructions. *Geochim. Cosmochim. Acta* 74:5280–5288
- Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer New York, New York, NY

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1:3–14

Supplementary Material

Power analysis

A power analysis was conducted to determine the optimum number of aquaria and fragments to be used in each food treatment of the food preference experiments. Six realistic scenarios were created (Supplementary material, Table 1), depending on the potential experimental designs and including different number of colonies and fragments per colony. Another two scenarios were created, in respect to the potential effect of food treatments on tracer incorporation: it was assumed that in one of the treatments, fragments would display 20% and 30% higher tracer incorporation in the two scenarios respectively. One dataset was created for each experimental design scenario, using average tracer incorporation levels from (Mueller et al., 2014), variance levels close to 30% of the average in order to reflect high variability among colonies, and a colony effect, allowing one of the colonies to display lower tracer incorporation than the rest of the colonies. The package SimR (Green and MacLeod, 2016) was used to calculate the power of each of the created scenarios, using 1000 simulations for each power estimate. The scenario with the highest power was selected and implemented in both the flow rate and feeding preference experiments.

References

- Green P, MacLeod CJ (2016) SIMR : an R package for power analysis of generalized linear mixed models by simulation. *Methods Ecol. Evol.* 7:493–498
- Mueller CE, Larsson AI, Veuger B, Middelburg JJ, van Oevelen D (2014) Opportunistic feeding on various organic food sources by the cold-water coral *Lophelia pertusa*. *Biogeosciences* 11:123–133

Table s1: Description of the scenarios used for the performance of a power analysis for the experimental design of food preference experiments with *Antipathella wollastoni*.

Experimental design scenario	Number of flumes	Total number of colonies	Fragment per aquaria
1	4	16	1
2	4	8	2
3	4	12	3
4	3	9	3
5	3	15	5

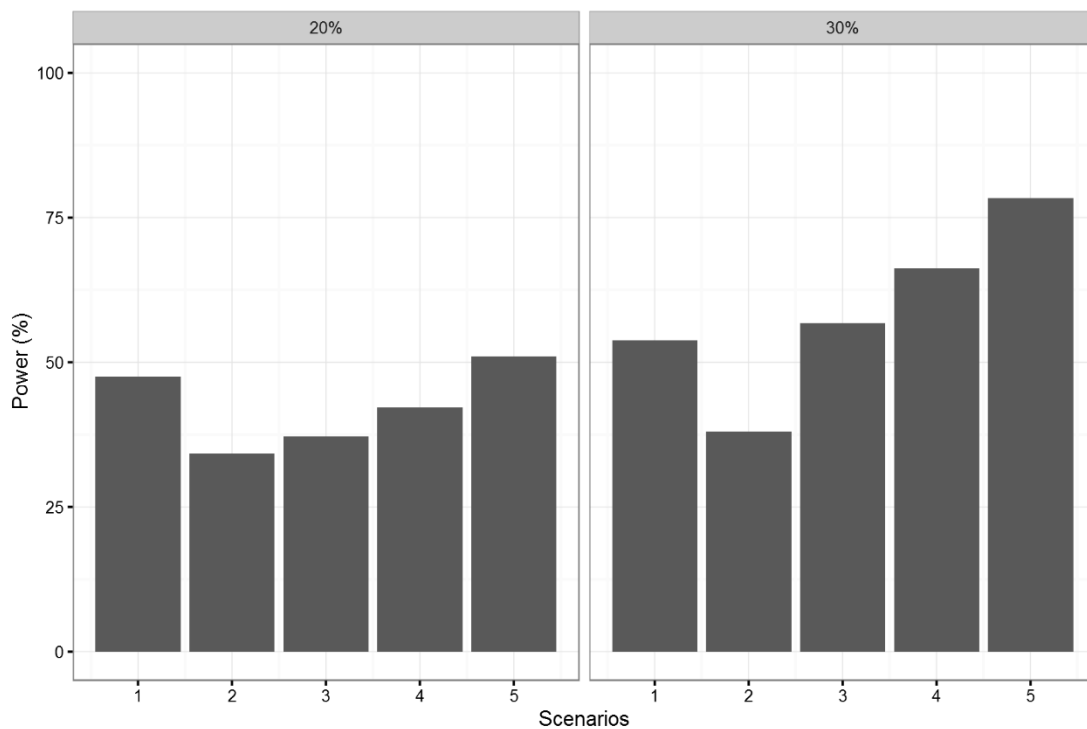


Figure S2: Power analysis for five experimental design scenarios under 20% and 30% increase in tracer incorporation of fragments under one of the food treatments.

Scenario 1: One fragment per aquaria, 4 aquaria, 16 colonies; Scenario 2: Two fragments per aquaria, 4 aquaria, 8 colonies; Scenario 3: Three fragments per aquaria, 4 aquaria, 12 colonies; Scenario 4: Three fragments per aquaria, 3 aquaria, 9 colonies; Scenario 5: Five fragments per aquaria, 3 aquaria, 15 colonies.