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Polyp expansion of passive suspension feeders: a red coral case study

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Polyp expansion of passive suspension feeders: a red coral case study

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ABSTRACT

Polyp activity in passive suspension feeders has been linked to several environmental factors such as hydrodynamics, water temperature and food concentration. To better elucidate the driving forces controlling polyp expansion in these organisms and the potential role of particle concentration, the octocoral *Corallium rubrum* was investigated in accordance with two approaches: 1) High-frequency *in-situ* observations testing various environmental and biological variables affecting the water column, and 2) Video-recorded flume-controlled laboratory experiments performed under a range of environmental and biological conditions in terms of water temperature, flow speed, chemical signals and zooplankton. In the field, *C. rubrum* polyp activity correlated positively with particle (seston and zooplankton) concentration, which was related to current speed. This observation was confirmed by the flume video records of the laboratory experiments, which showed differences in polyp activity due to changes in temperature and current speed, but especially in response to nutritional stimuli and the presence of zooplankton. Zooplankton and water movement appeared to be the main factors controlling polyp expansion.

These results suggest that the energy budget of passive suspension feeders (and probably the benthic community as a whole) may rely on their ability to maximise prey capture during food pulses. The latter, which may be described as discontinuous organic matter (dead or alive) input, may be the key to a better understanding of benthic-pelagic coupling processes and trophic impacts on animal forests composed of sessile suspension feeders.
Keywords: Octocorals, passive suspension feeders, *Corallium rubrum*, activity rhythms, benthic-pelagic coupling, trophic ecology, optimal foraging theory.
INTRODUCTION

Passive suspension feeders play an important role in energy transfer from the water column to the benthos (Gili & Coma, 1998). These organisms are important biomass contributors in benthic communities, being an essential part of the ‘animal forest’, in which the main three-dimensional builders are clonal or individual organisms of animal origin (Rossi et al., 2017a). Seston availability, which depends on its abundance, composition and renewal rate, is one of the most important parameters affecting the distribution, energy fluxes and biological constraints of suspension feeders (Grémare et al., 1997; Coma et al., 2001). As such, it is key to expanding our knowledge of community dynamics and the potential regression, substitution and/or mortality of suspension feeder populations, processes that have led to profound transformations over the last few decades (Rossi, 2013).

An immediate response of passive suspension-feeders to changes in seston availability and hydrodynamism is to change their feeding activity, which can also be affected by other short-term and seasonal environmental and biological changes (Coma et al., 1994; Rossi & Gili, 2007; Previati et al., 2010). In gorgonians, alcyonarians and zoanthids for example, feeding activity is reflected in polyp extension (Dai & Lin, 1993). It has been demonstrated to vary seasonally (Coma et al., 1994; Garrabou, 1999; Rossi, 2002), with the frequency of food inputs a potential factor driving polyp expansion (Tsounis et al., 2006; Rossi & Gili, 2007, Rossi et al., 2017b).

The more variable the water column environmental factors, the more diversified the mechanisms to enhance prey capture and feeding optimisation, as organisms adopt a range of strategies to take advantage of every potential source of food (Coma et al.,
In laboratory experiments, capture rates and polyp expansion among passive suspension feeders have been shown to be related to nutritional stimuli, particle concentration and flow speed (Leversee, 1976; Dai & Lin, 1993; Anthony, 1999), but there is almost no information on how environmental factors affect in situ polyp activity during short-term cycles (Rossi & Gili, 2007). Epibenthic water masses and associated plankton and seston concentrations can change rapidly, with particulate organic matter concentration tripling or quadrupling in less than a day (Grémare et al., 2003; Rossi & Gili, 2007; Rossi et al., 2013). These non-continuous food pulses have never been fully studied in relation to the activities of passive suspension feeders, and may be a key factor for understanding the overall energy budget of sessile organisms in animal forests.

The aim of this study is to achieve a better understanding of which factors drive polyp activity in passive suspension feeders, seeking to determine whether environmental and biological factors act synergistically in polyp expansion, using red coral (*Corallium rubrum*) as a model organism. To achieve this objective, two different methodological approaches were used: 1) High-resolution polyp observations in the field (Rossi & Gili, 2007), under recorded environmental conditions (i.e. current speed, zooplankton concentration, chlorophyll *a* and protein content of epibenthic seston). This will help to better understand whether there is any pulse-like energy input (Palardy et al., 2006; Tsounis et al., 2006). 2) Ex-situ high-resolution flume experiments to test *C. rubrum* polyp activity in relation to a range of environmental factors (i.e. temperature, current speed, nutritional stimuli and presence of zooplankton). The main aim is to understand
how intermittent food supply may influence the energy budgets of passive suspension feeders.

MATERIALS AND METHODS

Field survey

The field survey was conducted in the Medes Islands, NW Mediterranean (40° 02' 55''N, 3° 13' 30''E). Sampling and observations were carried out at 18–20 m depth among a coralligenous community located in a channel. The channel was alternately influenced by northerly and southerly currents, which may reach high speeds (from 2 up to 30 cm s⁻¹. Rossi & Gili, 2007).

*C. rubrum* polyp expansion was monitored at a high frequency (i.e. once every 6 hours) from June 24 to 29, 1997. This period was chosen because pelagic primary production and the frequency of seston pulses is high (Rossi & Gili, 2007). Expansion is defined as the maximum aperture of polyps (Sebens, 1987). Polyp activity was observed in ten groups of ten colonies each time by scuba divers.

Several environmental and biological parameters were concomitantly monitored: (1) hydrodynamics (using an Aandera SDP® Doppler current meter, moored in the same place as the observed passive suspension feeders, recording currents 0.5 m above the benthic surface), (2) seston concentration and quality, the latter determined by assessment of chlorophyll *a* and protein concentrations (see Rossi & Gili, 2007), and (3) zooplankton concentration, determined by analysing two samples collected by a scuba diver towing a plankton net (22 cm in diameter with a mesh size of 100 μm) a distance of 40 m (Coma et al., 1994; Rossi et al., 2004). Wind, wave height and tidal oscillation
were recorded every day by the Estartit Meteorological Station in accordance with the protocols of Cebrián et al. (1996).

Experimental observations on polyp activity

On March 15, 2007, several small colonies of *C. rubrum* were collected at depths of 28-30 m (water temperature 14ºC) using an axe, and were immediately transferred to the Observatoire Océanologique de Banyuls (France). Colonies were placed in small cylinders, 4 cm in diameter (two colonies per cylinder), attaching them with non-toxic rubber, and kept at 15ºC in running seawater for two weeks prior to the start of the experiments. Corals were fed three times a week with copepods, ground mussels and *Artemia* nauplii. Running seawater supplied to the tanks was filtered through a 4 µm filter, allowing only pico- and nano-plankton to be present in the aquaria, which is a negligible part of the diet of these species (Tsounis et al., 2006; Picciano & Ferrier-Pagés, 2007).

The flume used for laboratory experiments was a closed transparent plastic ellipsoid channel placed in a temperature-controlled chamber (Fig. 1). The flume was 10 cm wide × 20 cm high with a total length of 450 cm, resulting in an overall maximum water capacity of 85 litres. For each experiment, 65 litres of filtered (2 µm) seawater were used. Four colonies (two cylinders) were tested in each experiment. The pump used to generate water flow could move up to 10 litres min⁻¹, and was therefore able to generate regulated water speeds of 1 to 6 cm s⁻¹. A Minilab SD12 ultrasonic current meter (Sensordata, Bergen, Norway) (resolution 1 mm s⁻¹, bandwidth 35 Hz and effective acoustic path length 29 mm) was used to measure water flow.
during each experiment. The acquisition frequency was 1 Hz and values were averaged
over 10s. Water temperature was recorded to the nearest 0.5°C.

The polyps of all colonies were closed at the beginning of each experiment. Colonies
were placed in the flume and acclimated for 30 min, with experiments conducted from
24 to 72 hours.

For assessment of the effects of zooplankton and nutritional stimuli, natural
Mediterranean zooplankton was used.

Zooplankton was collected from epibenthic waters using 200 µm mesh nets near the
coast on the 6 and 14 April 2007. The samples were transported in a cooler to the main
lab. The zooplankton was centrifuged (3000 rpm), and stored at -20ºC pending
experimental set-up. An aliquot of 1 ml in 5 replicates from each sampling was fixed
with 6% formalin in order to count the number of items added to the flume water in each
experiment (Coma et al., 1994).

For the chemical signal experiments, the selected volume of zooplankton (40 or 120 ml
in 65 litres) was gently ground with a glass homogeniser. The homogenised
zooplankton was filtered through a 10 µm mesh and stored 60-90 minutes before the
experiment. For the “zooplankton” experiments, the samples were not previously
filtered. The selected volume (120 ml in 65 litres, final concentration 1500 ± 252 ind. m⁻³)
was used directly in the experiment. In both cases, the zooplankton (filtrate or
particles) was placed directly in the flume once the water was running. All the
experiments were conducted at a flow speed of 3 cm s⁻¹.

The flume was illuminated using LEDs (Lunartec 48 LED white bulbs). A mirror oriented
45º with respect to the flume’s main axis was placed downstream from the monitored
colonies so that they could be observed through cameras without major disturbance to the water current. A video system with a colour camera (JAI S3200® fitted with a 50 mm objective) was placed next to the flume and used to monitor the colonies via the reflection of their images in the mirror. The sensitivity of the camera was increased by a factor of 4 (using the Jai camera’s internal settings) and image acquisition was set to 0.05 Hz (0.1 Hz during both experiments). The signal was received using a Falcon Plus® video grabber and transferred to a PC, where the images were recorded in JPEG format (80% compression). Real image size was 104 x 78 mm (Duchêne et al., 2000; Maire et al., 2007) corresponding to 736 X 568 pixels and thus to a resolution of 140 µm pixel⁻¹. The frame capture rate was set at 3 images/min.

Maximum polyp expansion was recorded and calculated. The coral’s white polyps and tentacle crowns contrast with the black background and red coenenchima, allowing image analysis. The JPEG images were assembled into AVI films (SEM VIDEO 1). Image processing was then performed on the films using CVAB software (J.C. Duchêne). Image analysis allowed computation of the surface area of open polyps on the coral branches. The surface area of open polyps on each branch was separated and accounted for in every image of each film. Segmentation of the images allowed the open polyps to be separated from the coenenchima in distinct pixel patches corresponding to existing regions of the coral. The labelled regions were tracked across images in the films, providing information on the activity of the polyps forming the colonies. Two types of information were derived from the observations: (1) the total surface area of the open polyps, and (2) the polyp activity index, obtained from subsequent image differentiation. The total surface area was obtained from the 2-
dimensional projection of the open or opening polyps on the lens plane. The polyp activity index showed the changes occurring in each region at any given time, including opening and closing polyps and moving tentacles. If a polyp moved its tentacles the recorded surface area is expected to remain the same, with a null difference between the two successive apparent surface areas. However, such movements may add noise to the recording. These variables were recorded in the CVAB software in a compressed but modifiable format. While measuring colony size, it also allowed for measurement of events characterised by low dynamism, such as slow feeding movements, and examination of mesoglea inflation before polyp opening.

Statistical analysis

The differences in i) *C. rubrum* polyp expansion, ii) seston composition and iii) zooplankton composition were assessed at various temporal scales by multivariate analyses and correlated with the environmental variables recorded during the sampling. By means of two laboratory experiments, we assessed the differences in *C. rubrum* polyp expansion in response to a range of temperatures, current speeds and nutritional stimuli.

In situ, to assess differences in *C. rubrum* polyp expansion, the design incorporated two factors: Cycle (Cy, as a fixed factor with 5 levels, each 24 h) and Time (Ti, as a random factor with 4 levels, nested in Cycle, each 6 h), with n = 3. Multivariate analyses of variance (PERMANOVA, Anderson, 2001) considered Euclidean distances based on untransformed polyp expansion data and previously normalised seston data, using 9,999 random permutations of the appropriate units (Anderson & Braak, 2003).
To assess differences in zooplankton composition we performed permutational analyses of variance (PERMANOVA, Anderson, 2001) considering Bray Curtis dissimilarities based on transformed data (fourth root), using 9,999 random permutations of the appropriate units (Anderson & Braak, 2003), adopting a design with one factor, i.e. Cycle (Cy, as a fixed factor with 5 levels, n = 4). In order to detect which taxa contributed most to dissimilarity among the cycles, a similarity percentage (SIMPER) analysis was performed (Clarke, 1993). To examine the generality of patterns in polyp expansion, seston composition and zooplankton composition, we generated MDS plots.

In addition, we performed two laboratory experiments in order to evaluate C. rubrum polyp expansion under a range of physical conditions and nutritional stimuli. To assess the effect of temperature and current speed on polyp activity, we performed permutational analyses of variance (PERMANOVA, Anderson, 2001) considering Euclidean distances based on untransformed data, using 9,999 random permutations of the appropriate units (Anderson & Braak, 2003), adopting a design with two factors: Temperature (Te, as a fixed factor with 3 levels) and Current (Cu, as a fixed factor with 3 levels) with n = 12. Moreover we performed a further experiment in order to evaluate the effects of nutrient levels, following a design with one factor, i.e. Nutritional Stimuli (Nu, as a fixed factor with 4 levels) with n = 8.

When significant differences were encountered (p < 0.05), post-hoc pairwise tests were carried out in order to ascertain the consistency of the differences across the different conditions tested. Because of the restricted number of unique permutations in the pairwise tests, p values were obtained from Monte Carlo samplings. The analyses were performed using PRIMER v. 6 (Clarke & Gorley, 2006).
Results

Polyp activity in high time-resolution field monitoring

The activity rhythms (polyp expansion) of *C. rubrum* between the 24th of June (15:00) and the 29th of June (9:00) 1997 in relation to the environmental variables tested are shown in Figure 2 (A to D). The activity of the 100 colonies varied between 0% (polyps fully closed) and 100% (polyps fully open), the change occurring in only 6 hours in some cases (see for example the transition between 26 of June at 9:00 and 26 of June at 15:00). Current speed ranged from 0.2 cm s$^{-1}$ to 30 cm s$^{-1}$. The mean current speed during the activity rhythm observations was 9.3±9.4 SD cm s$^{-1}$, with the highest speeds recorded towards the middle and end of the experimental period (Fig. 2A). Zooplankton concentration (mainly copepods and nauplii) varied from 298 individuals m$^{-3}$ to 8437 individuals m$^{-3}$. The mean concentration was 2122±2412 SD individuals m$^{-3}$. Zooplankton had higher concentrations in the later cycles (Fig. 2B). Chlorophyll *a* concentration varied from 0.28 µg L$^{-1}$ to 0.70 µg L$^{-1}$, with a mean of 0.4±0.1 SD µg L$^{-1}$, with the highest values recorded at the beginning of the experimental period (Fig. 2C). Protein concentration followed a different tendency (Fig. 2D), ranging from 135 µg L$^{-1}$ to 243 µg L$^{-1}$. The mean concentration was 176±32 SD µg L$^{-1}$, with the highest concentrations found towards the middle and end of the experimental period. No significant relationships were directly observed between the tested environmental variables and red coral polyp expansion.
The results of the PERMANOVA and pairwise analyses reveal that *C. rubrum* polyp expansion varied significantly within cycles and among sampling times (Tables 1 and 2) at all temporal scales.

Following the same experimental design, PERMANOVA and pairwise analyses on seston composition exhibited significant differences at the investigated temporal scales (Tables 1 and 2).

MSD plots of seston composition in relation to each sampling time show separation of cycles and sampling times (Fig. 3).

Significant differences among cycles are also seen for zooplankton composition (Table 3).

The SIMPER analysis revealed the highest dissimilarity in the zooplankton assemblages, reaching 30.74% for C1 vs. C5, followed by C2 vs. C5 (30.12%), C1 vs. C4 (27.02%), C1 vs. C3 (25.09%) and C2 vs. C4 (25.06%) (Supp. Table 1), highlighting variation between the first and last cycles. The MSD plots confirm the separation of cycles (Fig. 4).

**Laboratory experiments**

In the experiments, a clear rhythm appears in the opening events of the coral branches (Fig. 1 SEM). Records of individual polyp activity showed that when the colony’s polyps are fully extended, movements increase until the colony suddenly closes (Fig. 2 SEM).

**Temperature and Current**
The results of the PERMANOVA showed a significant Te x Cu term (Table 4, Fig. 5), demonstrating that *C. rubrum* polyp expansion varied significantly among the tested temperatures and currents. Pairwise analyses clarified the significance of individual comparisons (Table 5).

**Nutritional stimuli**

The results of the PERMANOVA (Table 6) and pairwise analyses (Table 7) reveal that *C. rubrum* polyp expansion varied significantly among the different nutritional stimuli tested, except for N1 vs. N2 (Fig. 6).

**DISCUSSION**

The present study provides new insights into the relationship between environmental-biological conditions and the capacity of passive suspension feeders to intercept pulse-like energy inputs. High-resolution observations of polyp activity in the field highlighted the complex combination of environmental variables linked to seawater movement even on the small scale. Ex-situ high-resolution flume experiments showed that polyp expansion accelerates with current speed. In addition, the presence of nutritional stimuli, especially zooplankton, induces a clear response in *C. rubrum* polyp activity, confirming its capacity to detect food availability.

The obtained outcomes also contribute to our understanding of the biology and ecology of red coral. *Corallium rubrum* polyp expansion seems to be most affected by water temperature, as observed by Picciano & Ferrier-Pages (2007), and by seawater movement. Passive suspension feeding depends on current flow. Nevertheless, given
constant seston concentrations, increasing current speed enhances filtration up to a maximum beyond which filtration no longer increased (Wildish, & Kristmanson, 2005). Our in situ results suggest complex hydrodynamic conditions act in a complementary way to shape polyp activity. While the assessment of the effect of drag forces on polyp retention ability is beyond the scope of the current study, we found a clear relationship between polyp efficiency and current speed, similar to what has been seen in studies of other gorgonian species (Dai & Lin, 1993). The rigid structure of the CaCO$_3$ skeleton of C. rubrum creates a highly inflexible structure that has a limited range of movement, unlike other highly flexible gorgonian passive suspension feeders used in other studies (Dai & Lin, 1993). Highly flexible organisms may be able to minimise drag forces by altering colony shape and reducing projected colony area when exposed to increased flow (Vogel, 1996). Previous studies of scleractinian corals suggest that current velocity within colonies has an upper limit, or saturation velocity, which is dependent on colony morphology (Chamberlain & Graus, 1975). The current speeds used in our study have no dramatic effect on polyp shape and are considered optimal for polyp particle capture (Leversee, 1976; Sponaugle, 1991). An increase in current speed (from 0 to 6 cm s$^{-1}$) therefore increases particle delivery to the polyps. Dai and Lin (1993) showed that Acanthogorgia vega had a broader spectrum of polyp efficiency (as capture rates) at flow speeds ranging from 0 to 15 cm s$^{-1}$ than the other two species tested, probably due to its bushy shape. In fact, the effect of flow on particle capture by polyps is probably a general phenomenon among octocorals (Robbins & Shick, 1980; Patterson, 1991; Dai & Lin, 1993), polyp capture efficiency falling as the Reynolds number increases. In the present study, C.
rubrum polyp activity tends to increase with current speed, even in the absence of increased abundance of food.

Similar to previous studies of C. rubrum polyp activity, the current study found a significant relationship between temperature and polyp activity, with polyp activity lower at high temperatures. Previati et al. (2010) showed a significant relationship between oxygen consumption and activity (open polyps) at 18-20ºC and a current of 1 cm s\(^{-1}\) (approx.): oxygen consumption and activity increased, but above this temperature oxygen consumption decreased. Studies of other octocorals follow the same trend of closing their polyps at higher temperatures and decreasing oxygen consumption to maintain a decreased metabolic rate in a quasi-dormant stage (Coma et al., 2002; Previati et al., 2010). Although the relationship between temperature and polyp activity might be the result of endogenous rhythms related to an internal clock (Previati et al., 2010), it seems that lack of water flow (decreased current speed) is a key factor in the spontaneous opening of polyps in the absence of external stimuli. In the present study, colonies also decreased their maximum opening frequency as temperature increased. One hypothesis that might explain this is that polyp expansion is needed for gas exchange and excretion, even if the majority of colonies tend to remain closed as much as possible as temperature increases. When food availability in the water column is low, the increase in temperature and flow seems to increase the response of C. rubrum (maximum opening frequency), indicating a balance between the need for opening and the current stimulus. Differences between the still-water and current-flow experiments in terms of maximum opening frequency suggest current stimulus has a greater influence
than temperature constraints. Food acquisition thus appears to have priority over gas exchange in this gorgonian.

Of all the variables used to test for a response in polyp activity in the present study, addition of zooplankton elicited the fastest response in *C. rubrum* colonies. *C. rubrum* is considered a passive suspension feeder, capturing particulate organic matter (POM) from the surrounding environment (Tsounis et al., 2006). Other Mediterranean and tropical asymbiotic octocorals (gorgonians, soft corals) are able to capture POM (Ribes et al., 2003), small zooplankton (Coma et al., 1994; Rossi et al., 2004) and phytoplankton (Widding and Schlichter 2001). *C. rubrum* is also able to feed on bacterioplankton (pico- and nanoplankton) (Picciano & Ferrier-Pages, 2007). A chemical or chemical/physical (zooplankton) stimulus caused a rapid response in terms of polyp activity, in some cases within a few seconds. The rapid response of polyp activity increases with temperature, but at higher food concentrations the response becomes even more rapid (Grémare et al., 2004). Relying completely on heterotrophic inputs from seston, the detection of chemical signals and/or food particles may be more important than other variables (i.e., temperature or current). Although there may be inter-individual variability in the response (Duchêne et al., 2000; Duchêne, 2017), there is clearly less variability in polyp activity when zooplankton stimuli are combined with flow speed than with flow speed alone. This result is not surprising, as it has been demonstrated in previous studies that the addition of food to the water column can elicit a response in other taxa (Duchêne & Rosenberg, 2001; Maire et al., 2007; Duchêne, 2017). The response to a chemical signal indicating increased zooplankton concentrations has not previously been experimentally tested in octocorals, but has
been tested in other important tropical taxa, with Anthony (1999) showing that particle concentration elicited a response in scleractinian corals. It is clear that the synergistic effects of higher current speed and the presence of natural prey (or its chemical signal) provide a stimulus for the expansion of the polyps of this gorgonian. Therefore, we hypothesise that if food and current stimulate polyp activity, a recurring hydrodynamic parameter (food pulses due to high seston concentration) may cause current speed and particle concentration (dead or alive) to act synergistically.

The high-frequency *in situ* monitoring used in this study is currently the only known method for detecting the response in terms of polyp activity to changes in zooplankton and particulate organic matter availability and current flow speeds. In the space of just a few hours, epibenthic seston concentrations may fluctuate dramatically, with large increases and decreases in the concentrations of available zooplankton or seston (Rossi & Gili, 2007; Rossi et al., 2013). A greater frequency of high-speed current episodes may have a synergistic effect on the entire coralligenous community, by both increasing currents and resuspending particulate organic matter, thereby creating optimal conditions for nutrient cycling and capture of crustacean zooplankton, and increasing prey capture rates among benthic suspension feeders in general. The relationship between food pulses and feeding activity has also been studied in other tide-dominated environments (Naylor, 1976; Naylor, 2005).

We hypothesise that in the Mediterranean Sea (and in other benthic systems), food availability is non-continuous for benthic suspension feeders. Increasing the frequency of high current-speed events and hence the quantity of available epibenthic seston may be a driver of pulse-like temporal changes in the particulate organic matter available in
the water column for the energy budgets of coralligenous (and other) benthic communities. Many authors have shown the relationship between prey capture rates and concentrations of plankton (Sebens & De Reimer, 1977; Coma et al., 1994; Palardy et al., 2006). In intertidal systems, there is a clear relationship between benthic suspension feeding activity and tidal fluxes (Sebens, 1987). In *C. rubrum*, short periods of high seston and zooplankton abundance could be the key to understanding energy input, high-current episodes creating high prey concentrations, leading to maximum particle capture rates.

Optimal foraging theory (Hughes, 1980) posits the need to take advantage of favourable feeding pulses as an individual colony but also as a population within a community. Palardy et al. (2006) suggested that the energy budget of passive suspension feeders may be dependent on non-continuous zooplankton availability, and Robbins & Shick (1980) related the activity of *Metridium senile* to tidal flux. It is clear that even if polyp seston capture is an important source of nutrition (being a more constant food source, Ribes et al., 1999) the detected seasonal concentrations may not be sufficient, given the energy constraints of most passive suspension feeders. In the complex coralligenous community, a broad spectrum of energy constraints is shown by the diverse range of activities and behaviours observed during our study period. Many organisms take advantage of the food pulses related to tidal patterns of water movement, resuspension and nutrient recirculation (Robbins & Shick, 1980; Gibson, 2003). We hypothesise that the foraging strategy of *C. rubrum* (but also other benthic organisms and communities) is influenced by the frequency of high current-speed events. The presence of food pulses is key to understanding global energy inputs and
the energy budgets of these organisms, and these synergistic effects (current speed with particle concentration) bring energy pulses to the benthic community which may change our view of the energy budgets of benthic communities.

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FIGURE CAPTIONS

Figure 1 The flume used for laboratory experiments. It was a closed transparent plastic ellipsoid channel placed in a temperature-controlled chamber.

Figure 2 The activity rhythms (polyp expansion) of *C. rubrum* in relation to the environmental variables. (A) Current speed (B) Zooplankton concentration (C) Chlorophyll a concentration (D) Protein concentration.

Figure 3 MDS plots for seston composition, with Euclidean distances based on normalised data.

Figure 4 MDS plots for the zooplankton community, with Euclidean distances based on normalised data.

Figure 5 The influence of temperature and current on *C. rubrum* polyp expansion under a range of experimental conditions. Data are reported as mean values ± S.E.

Figure 6 The influence of nutritional stimuli on *C. rubrum* polyp expansion under a range of experimental conditions. Data are reported as mean values ± S.E.
Figure 1 SEM: Periodograms from three different colonies. Example of three periodograms from three different colonies (peaks represent polyp expansion), showing endogenous rhythms at 18°C and still-water conditions. On the left the recorded normalised activities (i.e. the number of pixels divided by the maximum polyp expansion for that experiment); on the right the Lomb periodogram with frequencies on the X axis and number of occurrences on the Y axis. Figures close to the peaks indicate the periods. The 3 dashed lines represent the significativity of the peaks, 0.1, 0.01 and 0.001, the smallest value corresponding to the highest significativity.

Figure 2 SEM: Records of individual polyp activity. (A) The area below the peaks for a given experiment. (B) The derivative of this curve with absolute values (increase or decrease in polyp expansion). These records usually show a steeper descent after opening.

VIDEO RECORDING: Corallium rubrum polyp activity at 18°C and 3 cm s⁻¹ current speed.
Figure 1

The flume used for laboratory experiments.

**The flume used for laboratory experiments.** It was a closed transparent plastic ellipsoid channel placed in a temperature-controlled chamber.
The activity rhythms (polyp expansion) of C. rubrum in relation to the environmental variables. (A) Current speed (B) Zooplankton concentration (C) Chlorophyll a concentration (D) Protein concentration
Figure 3

MDS plots for seston composition, with Euclidean distances based on normalised data.
Figure 4

MDS plots for the zooplankton community, with Euclidean distances based on normalised data
Figure 5

The influence of temperature and current on *C. rubrum* polyp expansion under a range of experimental conditions.

The influence of temperature and current on *C. rubrum* polyp expansion under a range of experimental conditions. Data are reported as mean values ± S.E.
Figure 6

The influence of nutritional stimuli on *C. rubrum* polyp expansion under a range of experimental conditions.

The influence of nutritional stimuli on *C. rubrum* polyp expansion under a range of experimental conditions. Data are reported as mean values ± S.E.
Table 1. Results of multivariate permutational analyses (PERMANOVA) of variation in *C. rubrum* polyp expansion and seston composition among cycles and sampling times.
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df = degree of freedom; MS = mean sum of squares; F = F value by permutation. *** = $P<0.001$.

| Source     | df | MS   | F     | P(perm) | MS   | F     | P(perm) |
|------------|----|------|-------|---------|------|-------|---------|
| Polyp Expansion |    |      |       |         |      |       |         |
| Cy         | 4  | 92.929 | 18.296 |         | 32.743 | 16.945 |         |
| Ti(Cy)     | 14 | 81.599 | 16.065 | ***     | 13.4 | 6.9347 | ***     |
| Res        | 38 | 5.0792 |       |         | 1.9323 |       |         |
| Total      | 56 |      |       |         |      |       |         |
Table 2. Results of pairwise tests contrasting different factor levels.

Table 2. Results of pairwise tests contrasting different factor levels. df = degree of freedom; MS = mean sum of squares; T = T value; P = probability level. ns = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. 
**Table 2.** Results of pairwise tests contrasting different factor levels. df = degree of freedom; MS = mean sum of squares; T = T value; P = probability level. ns = not significant; * = P<0.05; ** = P < 0.01; *** = P< 0.001.

|        | Polyp Expansion |        |        |        |        |        |        |        |
|--------|-----------------|--------|--------|--------|--------|--------|--------|--------|
|        | t               | P(MC)  | T      | P(MC)  | t      | P(MC)  | t      | P(MC)  |
| C1     |                 |        |        |        |        |        |        |        |
| C2     | 5.3712          | **     | 1.4124 | ns     | 5.1708 | **     | 1      | ns     |
| C3     | 9.9367          | ***    | 1      | ns     | 3      | ns     | 9.367  | ***    |
| C4     | 2.5328          | *      | 2.3306 | ns     | 1.5695 | ns     | 10.332 | ***    |
| C5     | 4.8647          | *      | 2.5371 | ns     | 2.8007 | *      | 2.661  | *      |
|        | 2.9011          | *      | 3.0772 | *      | 6.2224 | **     | 0.8854 | ns     |
|        | 90.254          | ***    | 0.53199| ns     | 1.642  | ns     | 1      | ns     |
|        | 6.9985          | **     | 4.1082 | **     | 1.6991 | ns     | 6.3454 | **     |
|        | 6.6688          | **     | 10.119 | ***    | 3.7404 | **     | 3.7495 | *      |

|        | Seston Composition |        |        |        |        |        |        |        |
|--------|--------------------|--------|--------|--------|--------|--------|--------|--------|
|        | t                 | P(MC)  | T      | P(MC)  | t      | P(MC)  | t      | P(MC)  |
| C1     | 5.9206            | **     | 2.0901 | ns     | 2.3717 | *      | 2.3458 | ns     |
| C2     | 3.3612            | **     | 1.6031 | ns     | 2.4643 | *      | 3.0539 | *      |
| C3     | 4.2089            | **     | 1.7729 | ns     | 2.0148 | ns     | 2.2335 | *      |
| C4     | 4.8647            | **     | 2.5371 | *      | 2.8007 | *      | 2.661  | *      |
| C5     | 4.5115            | **     | 1.9588 | ns     | 1.6917 | ns     | 1.5277 | ns     |
|        | 2.7498            | *      | 1.0386 | ns     |       |       | 2.7262 | *      |
|        |                    |        |        |        |        |        |        | 3.0224 | *      |
Table 3. Results of multivariate permutational analyses (PERMANOVA) of variation in the zooplankton community among cycles and sampling times.

\textbf{Table 3.} Results of multivariate permutational analyses (PERMANOVA) of variation in the zooplankton community among cycles and sampling times. df = degree of freedom; MS = mean sum of squares; Pseudo-F = F value by permutation. \(*\ast\ast\ast = P< 0.001.\)
Table 3. Results of multivariate permutational analyses (PERMANOVA) of variation in the zooplankton community among cycles and sampling times. df = degree of freedom; MS = mean sum of squares; Pseudo-F = F value by permutation. *** = P < 0.001.

| Source | df  | MS    | F     | P(perm) |
|--------|-----|-------|-------|---------|
| Cy     | 4   | 703.58| 3.945 | ***     |
| Res    | 14  | 178.19|       |         |
| Total  | 18  | 18.19 |       |         |
Table 4. Results of the multivariate permutational analyses (PERMANOVA) of variation in *C. rubrum* polyp expansion under different temperature and current conditions.

*Table 4.* Results of the multivariate permutational analyses (PERMANOVA) of variation in *C. rubrum* polyp expansion under different temperature and current conditions. df = degree of freedom; MS = mean sum of squares; F = F value by permutation. * = P < 0.01; *** = P < 0.001.
Table 4. Results of the multivariate permutational analyses (PERMANOVA) of variation in *C. rubrum* polyp expansion under different temperature and current conditions. 

df = degree of freedom; MS = mean sum of squares; F = F value by permutation. * = P < 0.01; *** = P < 0.001.

| Source | Df | MS     | F       | P(perm) |
|--------|----|--------|---------|---------|
| Te     | 2  | 1.57E+05 | 3.5749 |         |
| Cu     | 2  | 9.34E+05 | 21.251 |         |
| TexCu  | 4  | 2.55E+05 | 5.7999 | ***     |
| Res    | 99 | 43973   |         |         |
| Total  | 107|         |         |         |
Table 5. Results of pairwise tests contrasting different levels of the two factors tested.

Table 5. Results of pairwise tests contrasting different levels of the two factors tested. df = degree of freedom; MS = mean sum of squares; T = T value; P(MC) = probability level after Monte Carlo simulations. ns = not significant; * = P<0.05; ** = P < 0.01; *** = P< 0.001.
Table 5. Results of pairwise tests contrasting different levels of the two factors tested. df = degree of freedom; MS = mean sum of squares; T = T value; P(MC) = probability level after Monte Carlo simulations. ns = not significant; * = P<0.05; ** = P < 0.01; *** = P < 0.001.

| Groups     | T      | P(perm) | t   | P(perm) | t   | P(perm) |
|------------|--------|---------|-----|---------|-----|---------|
| C0         |        |         |     |         |     |         |
| C1         |        |         |     |         |     |         |
| C2         |        |         |     |         |     |         |
| T1 vs T2   | 1.0294 | ns      | 3.6746 | **      | 2.2583 | *       |
| T1 vs T3   | 1.4163 | ns      | 1.758 | ns      | 5.1054 | ***     |
| T2 vs T3   | 0.86483 | ns   | 3.7428 | ***      | 0.72957 | ns     |
Table 6. Results of the multivariate permutational analyses (PERMANOVA) of variation in the polyp expansion of *C. rubrum* exposed to different nutritional stimuli.

**Table 6.** Results of the multivariate permutational analyses (PERMANOVA) of variation in the polyp expansion of *C. rubrum* exposed to different nutritional stimuli. df = degree of freedom; MS = mean sum of squares; F = F value by permutation. *** = P < 0.001.
Table 6. Results of the multivariate permutational analyses (PERMANOVA) of variation in the polyp expansion of *C. rubrum* exposed to different nutritional stimuli. df = degree of freedom; MS = mean sum of squares; F = F value by permutation. *** = P < 0.001.

| Source | df | MS     | F       | P(perm) |
|--------|----|--------|---------|---------|
| Nu     | 3  | 9.7956 | 17.149  | ***     |
| Res    | 28 | 0.5712 |         |         |
| Total  | 31 |        |         |         |
Table 7. Results of the pairwise tests contrasting the different temperatures and currents tested in the laboratory experiments.

Table 7. Results of the pairwise tests contrasting the different temperatures and currents tested in the laboratory experiments. df = degree of freedom; MS = mean sum of squares; T = T value; P(MC) = probability level after Monte Carlo simulations. ns = not significant; ** = P < 0.01; *** = P < 0.001.
Table 7. Results of the pairwise tests contrasting the different temperatures and currents tested in the laboratory experiments. df = degree of freedom; MS = mean sum of squares; T = T value; P(MC) = probability level after Monte Carlo simulations. ns = not significant; ** = P < 0.01; *** = P < 0.001.

| Groups     | t     | P(MC) |
|------------|-------|-------|
| N0 vs N1   | 2.9936| **    |
| N0 vs N2   | 4.5724| ***   |
| N0 vs N3   | 5.5413| ***   |
| N1 vs N2   | 1.948 | ns    |
| N1 vs N3   | 3.5632| **    |
| N2 vs N3   | 3.97  | **    |