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Feeding corals in captivity: uptake of four *Artemia*-based feeds by *Galaxea fascicularis*.

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This study evaluates the capture efficiency of the scleractinian coral *Galaxea fascicularis* for four *Artemia*-based feeds: (1) live, non-enriched *Artemia* nauplii; (2) Instant Baby Brine Shrimp (IBBS, pasteurized *Artemia* nauplii); (3) live, SELCO-enriched *Artemia* nauplii, and (4) live *Artemia* nauplii enriched with SELCO that was supplemented with the antibiotics Sulfamethoxazole and Trimethoprim. All prey types were rapidly consumed by the corals (6-11 nauplii per polyp per hour), showing that (I), IBBS can be a cost-effective alternative to feeding freshly hatched nauplii, and (II), corals can be provided with specific additives via their food. The corals preferred live *Artemia* over IBBS, indicating that IBBS can be further optimised for use in coral reef aquaria. SELCO-enriched *Artemia* and medicated *Artemia* were also consumed at lower rates (40% and 30% lower, respectively) than non-enriched *Artemia*. However, these results are a useful first step towards (I), development of coral-specific feed-enrichments, and (II), oral treatment of diseased corals in captivity.

Key words: *Galaxea fascicularis*, feeding, *Artemia* nauplii

Introduction

The keeping of scleractinian (stony) corals in aquaria has become increasingly popular over the last few decades (Wabnitz et al. 2003). Numerous innovations in aquarium technology and improved methods for coral husbandry have made it relatively easy to keep captive corals alive (see overviews by Borneman 2001; Delbeek and Sprung 2005). The present study deals with two topics in coral husbandry that up till now have received relatively little attention: heterotrophic feeding and treatment of diseases.

Scleractinian corals are known to possess two major mechanisms for fulfilling their nutritional needs; photosynthesis through symbiotic zooxanthellae and heterotrophy. They ingest a wide variety of food, such as dissolved and particulate organic matter, sediment, bacteria and zooplankton (Sorokin 1973; Titlyanov and Titlyanova 2002). The importance of heterotrophy relative to autotrophy varies with species and habitat. According to Titlyanov and Titlyanova (2002), the relative contribution of photoassimilation to the total nutritional requirements of zooxanthellate corals ranges from 30 to 90%. Under high-light conditions, such as in shallow, tropical waters, the metabolic energy provided by photosynthesis alone exceeds the coral's daily energetic needs (Edmunds and Davies 1986 and references therein). Deep living corals, or corals living in turbid environments, are more dependent on heterotrophy (Falkowski et al. 1984; Anthony 2000).

Most aquarium corals thrive well in well-illuminated tanks. Therefore, much literature on culturing corals in aquaria focuses on technology for lighting, whereas quantitative studies on heterotrophic nutrition remain scarce. There is consensus among aquarists that at least a minimal amount of food is needed to supplement the resources provided by photosynthesis. Evidence is growing that enhanced feeding can stimulate coral growth considerably (Buongiorni et al. 2003; Ferrier-Pages et al. 2003; Houlbreque et al. 2004), thus emphasizing the importance of improving feeding methods for aquarium corals.

Artemia salina nauplii are widely used for feeding corals in aquaria and are commercially available in the form of dried cysts. Cysts hatch after 18-24 hours and the young nauplii can be fed immediately. Even though hatched nauplii can be

stored up to 48 hours (2-4 °C) without losing any nutritional value (Léger et al. 1983), methods to store them longer, in large quantities and especially without having to hatch them, could be more economically attractive in terms of labour and time management. For this reason, INVE® (Dendermonde, Belgium), a company specialized in food products for aquarium animals, has developed Instant Baby Brine Shrimp (IBBS): dead, pasteurized *Artemia* nauplii that can be stored for one year and fed until 6 weeks after opening. This product would be extremely useful for feeding corals in public and private aquaria. Therefore, the product was tested by comparing the rate at which the scleractinian coral *Galaxea fascicularis* captures IBBS to the rate at which it captures freshly hatched, live *Artemia* nauplii.

A second possibility to improve coral breeding and husbandry is provided by the technology to enrich *Artemia* nauplii with specific components. SELCO (Self Emulsified Lipid Concentrate) is one of the most commonly used enrichments in aquaculture. Specific components such as pro-biotics, antibiotics and food supplements can be coupled to SELCO so that they accumulate in the SELCO-fed nauplii. This technology has been successfully applied by Chair et al (1996) to treat diseases in aquacultured fish and shrimp by using *Artemia* nauplii as a vector for oral addition of antibiotics. This principle may also be applied to corals. During the last decades, extensive research has been done on coral diseases, as large outbreaks seem to become more common on reefs in the wild (Richardson 1998; Weil et al. 2006). Moreover, a disease outbreak can be disastrous in an aquarium. Recently, pathogens have been identified that cause, or are at least associated with coral diseases (Luna et al. 2007; Rosenberg et al. 2007). These findings will enable targeted treatment of coral diseases using specific antibiotics. In this study, a first step is made towards development of treatments of coral diseases through oral delivery of antibiotics: it was tested whether corals capture medicated *Artemia* nauplii. The uptake efficiency of eight colonies of *Galaxea fascicularis* was compared to their uptake efficiency for non-enriched nauplii.

Materials and Methods

Coral materials

Experiments were carried out in the laboratory of the aquarium in Burgers' Zoo, Arnhem, The Netherlands. The coral species used, *Galaxea fascicularis*, was grown in the coral nursery of Burgers' Zoo, registered under number ARKS 611377. One mother colony was fragmented into nine smaller colonies, which were attached to small PVC plates of 5 X 5 cm. Hence, all colonies were genetically identical. Eight colonies of 8 x 8 cm were used for the experiment. The number of polyps of each colony were counted as an estimate for coral biomass.

Methods

Feeding experiments were conducted in cylindrical Perspex incubation chambers with a volume of 1.4 l. The water used was taken directly from the tank in which the corals were kept in between experiments, to reduce ambient stress for the corals as much as possible. During experiments, conditions in the incubation chambers were kept the same as in the tank (Table 1). Water temperature was controlled by a TECO TC20 water cooler (TECO, Ravenna, Italy) and kept constant at 26°C. Light was provided by a 70 Watt HQI lamp, type BLV, 10.000 Kelvin. Water flow was controlled by an IKA® Big-Squid Ocean magnetic stirring plate and kept constant at 2.4 notches; at this speed (resulting in a water velocity of approximately 10 cm/s), the *Artemia* nauplii remained in suspension. Colonies were incubated one at a time for measurements of capture rates.

Table 1: Water conditions in the maintenance tank and in the incubation chamber.

Temperature	26°C
Salinity	34 ppt
Calcium	400-410 mg/l
Alkalinity	2.5-3.0 mEq/l
Nitrate	< 0.02 mg NO_3-N/l
Phosphate	< 0.01 mg PO_4/l

All feeding experiments were carried out by adding an initial concentration of approximately 2000 nauplii / l. Colonies were allowed to feed for 15 minutes. A pilot study had revealed that at these experimental settings, neither saturation of the corals, nor depletion of the food resource occurred, thus making it possible to compare capture rates for different feeds.

Before the start of each experiment, the concentration of nauplii in a concentrated batch was calculated by counting the number of nauplii in 15 replicate samples of 200 µl taken from that batch. From this, the volume to be added to the incubation chamber was calculated and added. Coral colonies were allowed to acclimatise in the respiration chamber for 20 minutes before introduction of the food. A sample of 50 ml was taken from the

respiration chamber with a syringe two minutes after adding the nauplii and the number of nauplii in the sample was counted under a binocular microscope. A second sample was taken 15 minutes after adding the nauplii. Capture rates were expressed as the number of nauplii cleared from the water in the respiration chamber in this 13 minute interval per coral polyp.

Experiments
All colonies were subjected to four feeding treatments:
Treatment 1: Non-enriched nauplii. *Artemia* nauplii from Great Salt Lake (Aquafeed, Utah, USA) were hatched by the Zoo's aquarists at temperatures of 25-26°C and used when 26 hours old.
Treatment 2: Ocean Nutrition Instant Baby Brine Shrimp (IBBS, dead *Artemia* nauplii, manufactured and supplied by INVE® (Dendermonde, Belgium).
Treatment 3: Enriched nauplii. 0.17 ml Easy DHA SELCO enrichment per litre was added once to 30 hour old *Artemia* nauplii. 19 hours after this, the batch was put on air and stored in a refrigerator during the day to ensure that the *Artemia* used at the end of the day had the same SELCO content as the ones used at the start of the day (Léger et al. 1983).
Treatment 4: *Artemia* were enriched with medicated Easy DHA SELCO, consisting of an emulsion with 5% Sulfamethoxazole/Trimethoprim (5:1), prepared by INVE®. Storage was done as described in Treatment 3.
Every treatment was repeated 3 times for each replicate colony (i.e. 24 experiments per treatment) before switching to the next treatment. The colonies were not fed by the aquarium staff on the days of the experiments. All colonies were given at least one day to recuperate from the experimental treatment in the maintenance tank, during which they were fed by the aquarium staff.

Blank controls
For every treatment, 8 blank control tests were conducted to check for the accuracy of the start concentration of added nauplii and for possible effects of the chamber on nauplii concentration. Blank tests were conducted following the protocol of the incubations with corals. A limestone coral skeleton in the shape of those of the experimental colonies was placed in the chamber to create a similar flow pattern.

Carbon content
The carbon content of the live *Artemia*-based products was measured to enable expression of uptake rate in organic carbon equivalents. Enriched *Artemia* are fed as nauplii of the Instar II type, while freshly hatched *Artemia* are fed as the smaller-sized Instar I type. Organic carbon was determined by the Wet Oxidation Method, using an OIC 700 Total Organic Carbon analyzer.

Data analysis
Statistical analysis of the data was done with SPSS 12.01. Data were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Homogeneity of variances was tested by a Levene's Test for Equal Variances. Paired-Samples T-Tests and Mann-Whitney tests were used for determining differences in samples from the blank controls taken at t = 0 and t = 15 minutes, respectively. To determine differences in uptake rates between the different treatments, Paired-Samples T-Tests were used ($\alpha = 0.05$).

Results
It was observed that all coral colonies responded immediately to all treatments by capturing prey from the moment it was added to the chamber. No significant reduction in nauplii numbers was observed in the blank controls, except for IBBS. The IBBS were observed to stick to dead surfaces easily (both in blank controls and in incubations with corals). Due to this stickiness, the numbers of IBBS in the blank controls decreased by 14% within the 13 minute experimental interval. This was taken into account by subtracting this difference from the number of consumed IBBS in the experiments with corals.

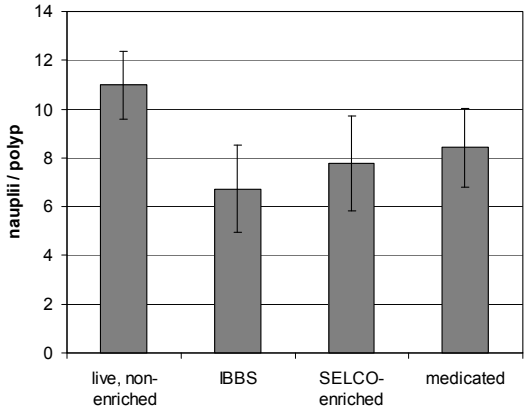

Figure 1. Average number of nauplii captured per polyp within the 13 minute incubation interval. Error bars indicate standard deviations (n = 24).

The average capture rates (expressed as nauplii captured per polyp within the 13 minute time

interval) for the different prey types were 10.98 (freshly hatched), 6.73 (IBBS), 7.77 (SELCO-enriched) and 8.42 (medicated) (Fig. 1). The uptake of freshly hatched, non-enriched nauplii was significantly higher than the uptake of the other prey types ($p < 0.001$). The reduction in capture rate was 38% for LBBS, 29% for SELCO-enriched nauplii and 23% for SELCO-enriched nauplii supplemented with antibiotics.

When normalized to organic carbon (Table 2), uptake per polyp was highest for medicated *Artemia*, which had a carbon content that was slightly higher than that of freshly hatched, non-enriched nauplii. Surprisingly, SELCO-enriched Instar II nauplii had a lower carbon content than freshly hatched, non-enriched nauplii. The measured carbon contents were comparable to literature data: Ohman (1987) reported a value of 0.5 µg C per nauplius, Evjemo and Olsen (1999) reported a value of 0.77-0.95 µg C per nauplius.

Table 2. Organic carbon content per individual for live *Artemia* products and calculated mean organic carbon uptake per polyp within the 13 minute incubation interval.

Treatment	Organic carbon content (µgC)	Organic carbon uptake (µgC/polyp)
Live, non-enriched	0.30	3.29
SELCO-enriched	0.25	1.94
Medicated	0.42	3.54

Discussion

Live Artemia *nauplii versus Instant Baby Brine Shrimp*

Our study showed some limitations of Instant Baby Brine Shrimp for feeding corals in captivity. The scleractinian coral *Galaxea fascicularis* preferred live, non-enriched *Artemia* nauplii over IBBS. Besides, IBBS tend to precipitate and stick to other surfaces. We conducted a similar experiment on another coral species, *Seriatopora caliendrum*, which also significantly preferred freshly hatched live nauplii over IBBS, IBBS uptake rates being on average 80% lower. However, the fact that IBBS are captured by both coral species demonstrates that the product is in principle suitable for coral culture.

In order to improve the IBBS product for feeding corals, it is important to understand why live nauplii are preferred. Live zooplankters are able to swim and have been reported to show an active escape response when approaching a predator tentacle (Trager et al. 1994; Heidelberg et al. 1997). From this point of view, one would expect that IBBS are captured more easily by the corals than live nauplii. But, apparently, IBBS does not trigger the feeding response of the corals to the same extent as live nauplii do. Corals kill their prey by nematocyst discharge, a process that is controlled by both chemical and mechanical factors (Thorington and Hessinger 1988). With respect to the latter, Heidelberg et al (1997) suggested that prey items approaching tentacles with a higher velocity would be captured more easily. When feeding corals with IBBS, it may therefore be beneficial to increase the water flow in the aquarium. This may also reduce the loss of IBBS due to precipitation and stickiness.

The possibility that IBBS lack a chemical trigger can also not be ruled out. Live zooplankters excrete small amounts of amino acids, which have been reported to trigger the feeding response in coral polyps (Mariscal et al. 1968). Lehman and Porter (1973) showed that addition of proline, glutamic acid, aspartic acid and arginine stimulates corals to ingest pieces of filter paper. In this light, there are possibilities to improve Instant Baby Brine Shrimp.

Enriched and medicated nauplii

Although captured with a lower efficiency than non-enriched nauplii, the SELCO-enriched and medicated nauplii were readily consumed by the corals, showing that *Artemia* nauplii can effectively be used as a vector to supply the corals with specific components such as antibiotics or probiotics.

This result provides a useful first step towards development of validated procedures to treat diseased corals with medicated *Artemia*, in analogy with Chair et al (1996), who conducted successful experiments with these antibiotics in fish and shrimp. However, successful application of this technique to corals will depend on several factors that remain to be studied, such as the digestion rate of the antibiotics in the gut of the polyps and the localization of the antibiotics' target. Oral administration of antibiotics may not be the most optimal way to treat diseases that primarily affect the outer cell layer of the coral tissue. In addition, diseased corals may cease their feeding activity.

Nevertheless, the possibilities for coral-specific enrichments are not limited to the addition of antibiotics. Corals are known to be dependent on heterotrophic feeding in order to obtain specific components needed for growth. For example, Allemand et al (1998) suggested that in *Stylophora pistillata*, heterotrophic feeding was the main source for aspartic acid, which is a main constituent

of the skeletal organic matrix of this species. Specific enrichment of *Artemia* nauplii with aspartic acid may reduce the amount of food needed to breed these corals.

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