PROPOSAL

Supplemental feedings and growth rates in the captive zooxanthellate coral *Sinularia sp.*

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Purpose and Objectives

With increasing amounts of coral bleaching events around the world, the need for understanding corals symbiotic relationship with zooxanthellae is becoming increasingly important. Efforts to replenish damaged reefs have been met with varying levels of success. Aquarists on the other hand, have been highly successful in asexual reproduction of various corals, resulting in very hardy specimens capable of being transplanted into a recovering reef. The nutritional requirements of corals are well studied, however, it is not known whether corals need to be fed in captivity, or whether they can rely on their photosynthesizing zooxanthellae and the minimal amount of food captured on their own in the aquarium. Most aquarists choose not to feed their corals significant amounts of food because of the potential water fouling that may result.

The objective of this study it to determine whether captive corals grow faster when given supplemental feedings as claimed by the makers of "Coral Heaven" food for corals and invertebrates. A second objective is to determine if more feedings will result in faster growth or will infrequent feedings show sufficient growth. These objectives will be tested by dividing genetically identical coral fragments into 4 large groups and feeding them at differing weekly intervals (0x, 0.5x, 1x, 3x) for one year. Percent growth will be compared.

Background

Zooxanthellae

Many corals harbor a photosynthetic dinoflagellate in their tissues called zooxanthellae. These microscopic symbionts allow corals to thrive in nutrient poor tropical waters. In exchange for oxygen, carbon dioxide, water, and energy rich-products (carbohydrates & fats), corals provide a stable environment and protection from predation. Zooxanthellae also benefit directly from the dissolved nutrients that the polyp absorbs (mainly nitrate and phosphate). Corals also provide ammonia from metabolism.

It was thought until recently that *S. microadriaticum* was the only species to inhabit corals. It is now known that there is at least 4 orders, 7 genera, and many species, totaling about 80 strains. In fact, zooxanthellae may be species specific. However, according to Eric Borneman (2001), corals may be able to switch one species of zooxanthellae for other species that may be better adaptive to their specific conditions of lighting and location on the reef. This seems to be especially true after bleaching events, where the previous strain was ill adapted to the prevailing conditions. Currently there are three clades of zooxanthellae, termed A, B, & C. Caribbean corals harbor all three where as Pacific corals just has representatives from clade C. Clade C symbionts are often lost or bleached when exposed to unusually high temperatures or light levels (Knowlton, 1999). Clade differences may account for the more-frequent but milder bleaching events in the Atlantic, in contrast to the less-frequent but more severe bleaching events in the Pacific (Baker, 1997).

Zooxanthellae are swallowed into the gastrovascular cavity of a new coral polyp, transported into the gastrodermal tissues, and begin to reproduce until a solid blanket of dinoflagellates lines the coral's cells. The result is a plethora zooxanthellae, capable of producing between 60% and 150% of the corals metabolic carbon energy needs depending on the species of coral (Borneman, 2001). Corals can physically and chemically compensate for varying levels of light.

Mass Bleaching

Under extreme stress, corals may expel all of their symbiotic zooxanthellae in a process know as bleaching. Bleached corals appear white, because their tissues have lost their brown zooxanthellae and their normal pigmentation has been disrupted, leaving the coral's aragonite skeleton visible through the transparent tissues. The slow release of zooxanthellae at a rate of about 1-6% per day is normal. However, prolonged stress may cause a coral to bleach as a last resort to survive.

According to Borneman (2001), stressed zooxanthellae may release glycoproteins as chemical messengers of stress. In response, too much photosynthesis occurs producing large amounts of oxygen and oxygen radicals. To combat this, oxygen destroying enzymes are produced. Superoxide dismutase (SOD) removes oxygen, but produces toxic hydrogen peroxide. The peroxide is then removed by various enzymes (CAT, AsPX). These enzymes also protect against hydroxyl radicals. If the coral can't keep up, the animal would digest itself or be poisoned by the oxygen or free radicals. To prevent death, the coral will bleach and attempt to reestablish its colony of zooxanthellae when conditions become more favorable or possibly an entirely new type of better-adapted zooxanthellae may be acquired.

As of late, the media has drawn attention to mass bleaching event around the world. Despite active research, the exact reasons for bleaching are unknown, but may be attributed to ozone depletion and global warming. The conditions linked to bleaching may include increased temperature, increased exposure to ultraviolet radiation, increased light intensity, decreased light intensity, salinity changes, and chemical exposure from dumping, spills, runoff, etc.

Foods & Feeding

In spite of the large amount of available information on coral nutrition and nutrient enrichment, there is little evidence to support the need for supplemental feeding of zooxanthellate corals in captivity. Most captive reef aquariums, even if comparatively low in dissolved nitrogen and other nutrients, are many times higher in such measurements than the water surrounding a natural reef (Borneman, 2001). Anecdotal evidence has shown that many species of coral will thrive in aquariums without supplemental feedings, but comparisons in growth rates are unknown. Many aquarists argue that supplemental feedings will deteriorate water quality and the deleterious effects will far out weigh any benefit to the coral. In fact, nutrient laden water has been shown to reduce calcification and result in poor health of specimens (Delbeek & Sprung, 1994). However, even without large supplemental feedings, growth rates of corals in captivity frequently meet or exceeded those in the wild (Bingman, 1998). Even, among aquarists who do feed, the light feedings regularly offered are extremely small in comparison to the amount available on the reef. Kinsey (1991) showed zooplankton input across 1 m of reef to be on the order of 30g of carbon per day. (* *Sinularia sp*. may not consume zooplankton. Bacterioplankton may be their food of choice.)

Captive Propagation

Mass sexual spawnings among corals has occurred in established aquaria around the world and is becoming more commonplace (Nilsen, 1998). However, recruitment during spawning in captivity is currently low because of the unpredictable nature of the occurrences and the loss of gametes through heavy filtration. More significantly, asexual propagation of corals is regularly utilized on both commercial and local levels with a huge degree of success.

As reefs come under increasing environmental pressures, the need for reef replenishment has increases dramatically. Frequently, transplantation and re-introduction of various species have been utilized, sometimes including entire reef transects (Munoz, 1997). Where some efforts have been successful, others have failed miserably. The efforts of removing colonies to establish viable colonies elsewhere is labor intensive, costly and has the distinct disadvantage of removing species from one area to populate another (Carlson, 1999). Fragmentation from a natural reef usually results in death and disease (Bak and Criens, 1981). However, species grown in captivity have been overwhelmingly reported to be exceptionally durable, and may be more likely to establish themselves and survive adverse conditions as they mature (Borneman and Lowrie, 2001).

Sinluaria sp.

Sinluaria sp. is one of the largest, most predominant, and most toxic genera of all soft corals, forming low, flat, fingered, and encrusting colonies. Many *Sinularia* species are found in diverse locations and are often a major component of soft coral coverage. They are frequently the most common soft coral in the shallowest waters. *Sinularia sp* is an extremely hardy soft corals able to withstand massive pruning and growing at astonishing rates in the aquarium. Cut areas heal rapidly and attach easily to new substrate. This allows for a large population of genetically identical specimens to be broken into research groups. Research on *Sinularia sp*. will not only give insight into how to replenish reefs and alleviate the aquarium trade, but octocorals (such as *Sinularia*) produce many known and currently undescribed secondary metabolites of commercial and pharmaceutical importance (Sammarco and Coll, 1987). Although Sinularia may not be representative of all species of coral, it is extremely hardy and very common on reefs and in aquaiums.

Pilot Study

The results from a pilot study produced surprising results. For the experimental group, the percentage growth was 58% after the first week of feeding, while the control group yielded just 25 %. Growth in each group continued to rise until week 4, when the experimental group had grown 73.5%, and the control had grown 53.9 %. Measurements in week 5 showed that each group had lost volume in comparison to the previous week. From weeks 5-10, the corals in each group showed a steady rise in volume. The total percentage growth for the corals in the experimental group was 85.3%, while the control group grew 93.7%. This study showed no significant difference between the two groups. However, a long-term study (1-3 years) may indicate larger growth differences as well as differences in overall health of the animals.

Proposed Work

Materials and Methods

I. Aquarium system

A. Separate100 gallon systems for each experimental group and control, set up the same way

In each tank: four power heads, one in each corner; Berlin protein skimmer (hung on the back of each tank); Whisper filter (hung on the back of each tank); Fiji Live rock (same amount in each tank); Aragonite substrate; Two Very High Output bulbs and two actinic blue bulbs on each tank.
Additives: Buffer, iodine, strontium, Kalkwasser, "Reef Essentials"
Water quality tests: NH_3, NO_2, NO_3, pH, alkalinity

II. Preparation of Corals

A. Genetically identical *Sinularia sp*. corals, chopped into 40 pieces of volume 5 ml (having a base with a diameter of 1/4 inch, and standing about 1 inch tall) or more.

B. Each individual coral is attached to a small piece of calcareous shell of known volume. This can be done in one of two ways:

1. Super glue- Cut coral fragment from parent with a brand new razor blade. Pat dry the base (the part that needs to attach to substrate). Put a very small amount of super glue on the substrate and allow to partially cure (5 minuets). Apply coral to glue so glue covers about 10 % of the cut coral base (the part of the coral that is covered could die, so you want to use as little glue as possible). The new fragment is added to a coral dip for 7 minuets. The coral and shell are then returned to the tank.

2. No glue method (preferred)- Cut coral fragment from parent with a brand new razor blade The new fragment is added to a coral dip for 7 minuets. Place the new fragments and pieces of shell of known volume into the bottom of 1/2 pint plastic container. Place the container with fragments in the tank and be sure that the corals will not be removed from the container by the current.

Cover with window screen if necessary. Allow the corals to remain in the container for 2-3 weeks undisturbed. The corals will attach themselves to the pieces of shell.

C. Corals are divided into 4 separate groups, three experimental groups and a control group. The groups will contain the same number of equal sized corals (n=10).

D. The shells will be marked so as to distinguish between the individual corals by painting the group, and coral number on the bottom. For instance, the first coral of the experimental group would have “E 1” painted on the bottom of the shell it’s attached to. The corals from each group are placed in their respective tanks.

III. Running the Experiment

The volume of each individual coral will be measured. The experimental groups will be then be fed (0.5x, 1x, 3x) weekly. Monthly (on a non-feeding day) the volume of each individual coral will be measured, each time calculating the mean percentage growth for each group. The procedures for feeding and measuring corals are as follows:

A. Feeding

The experimental groups will be fed "Coral Heaven" food 0.5x, 1x, or 3x per week for one year. To feed, mix 1 tsp of Coral Heaven with 8 oz. of seawater. Shut off all power heads, filters, and anything generating flow in the experimental tank. With an eyedropper, gently drop food about 1 inch above the coral in the water column. Feed only the amount of food that can be consumed in 5 minutes, about one drop. After 10 minutes, the power heads and filters are turned back on.

B. Measurement

The volume of each individual coral in both the control and experimental groups will be measured at least once a month on the same day (this should be on days when the experimental groups are not being fed). A measurement will be taken before the experimental groups are fed. The procedure for measuring the volume of a coral is as follows.

1. Place 150 ml of seawater in a 250 ml beaker.
2. Place a single coral (and shell) in the beaker (the water level will rise).
3. With an eyedropper, remove water from the beaker and place it in a 25 ml graduated cylinder until the water in the beaker is once again at the 150 ml mark.

4. The amount of water in the graduated cylinder represents the volume of water displaced when a coral was placed in the beaker; therefore, **it is the volume of the coral and its substrate**.
5. Record this volume. From this volume, subtract the volume of that coral's substrate (shell) to get the total volume of the coral. Record the volume of that coral.
6. Repeat steps 1-5 with each individual coral in both the experimental and control groups.
7. From the volume of the corals, determine the mean percentage growth from the start of the experiment

IV. Analysis

A. Our hypothesis states that the mean growth of corals being fed (experimental groups) will be greater than the mean growth of those not being fed (control). Our alternative hypothesis is that the small feedings will cause reduced or equal growth rates to the unfed control.

H_o: $U_C = U_E$
H_1: $U_E > U_C$
H_2: $U_E < U_C$

B. Each experimental group will be compared with the control using a t-test for two unpaired groups.

C. To determine if more food results in faster growth, the three experimental groups will be compared with an ANOVA.

D. The data used in the analysis will be the overall mean percentage growth from all of the corals in each group.

E. Another useful analysis would be a plot of the mean percentage growth of coral over time to determine if there is a certain period when percentage growth is highest in the experimental corals. This may be useful to direct further research. Another possiblility is to film the corals to determine how often each group actively forages. Well fed corals may not keep there polpys out as often.

Impact

This research study will benefit the future coral farming industry. Not only will reefs need to be replenished, but also aquarists are seeking hardy "tank raised" corals and there is the potential for of commercial and pharmaceutical use. As in fish farming, a huge cost of raising their animals is feeding them and coping with the resulting degraded water quality. This research will shed some light onto the question as to weather these coral farmers and aquarist need to feed their corals at all.

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