

**ATTEMPTS TO CULTURE AND MONITOR GROWTH RATES IN DEEPWATER ANTIPATHARIANS  
AND GORGONIANS<sup>1</sup>**

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**ABSTRACT**

The objective of this study was to quantify vertical and radial skeletal growth, and calibrate the isotopic signature of tissue and skeleton of hexacoral and octocoral specimens maintained under laboratory conditions. *Lophelia pertusa*, antipatharian and gorgonian specimens were collected from the Gulf of Mexico in 2005. Specimens were stained with Alizarin Red and Calcein skeletal dyes in the cold room aboard the R/V Seward Johnson II, and were maintained in tanks at 10°C aboard the R/V Seward Johnson II for the duration of the cruise. All specimens were then transported to Ohio State University and maintained in a climate controlled dark chamber for several weeks. Unfortunately, all of the specimens died either during transportation or during the first two weeks of the experiment. The experiment was terminated. Thus, our ability to quantify growth was not successful due to the premature death of the experimental specimens. In addition, the slow death of the specimens most likely resulted in abnormal respiration by the animals and isotopic fractionation. Consequently, the isotopic measurements of the dead specimens would not reflect the natural isotopic variability of these organisms and our ability to quantify the natural variation in the isotopic composition of these organisms was also unsuccessful.

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<sup>1</sup> Due to technical problems at sea, the objectives of this component of the overall program could not be completed sufficiently to yield substantive results. However, the culture attempts undertaken do provide valuable insights into conducting future culture trials with deep 'soft' corals. Accordingly, this component is reported here in Appendix form to help guide future undertakings in such experimental culturing.

## INTRODUCTION

The skeletal growth rates of Isidae and *Primnoa* gorgonians have only been calculated after specimen collection using ring counts and radiometric dating (Andrews et al., 2002; Roark et al., 2005). There have been no growth studies, either *in situ* or in aquaria. This a posteriori approach has two significant limitations: 1) none of the environmental variables are controlled (or known very well in some cases), and 2) growth rates are not directly measured but inferred from the interpretation of proxy information such as growth rings and radiometric measurements. Grange and Goldberg (1994) attempted to address growth rates more directly in a study on tropical antipatharian utilizing uniformly labelled  $^{14}\text{C}$ -D-glucosamine and  $^{14}\text{C}$ -L-histidine tracers (both precursors for skeletogenesis). However, the authors were unable to determine if growth rings were annual. In addition, skeletal stable isotopic variation of all deep-sea organisms have only loosely been correlated to changes in environmental and/or coral biology as direct calibrations under known geochemical and environmental conditions do not exist. Calibration of growth ring formation and skeletal geochemistry between the different deep-water organisms would expand the geographic area from which we can obtain records, and strengthen the interpretation of existing and future deep-water coral proxy records.

## METHODS

Specimens were collected on September 17<sup>th</sup> through 19<sup>th</sup> from the Gulf of Mexico using the DSRV Johnson Sea Link (JSL) from three different locations: VK-906/862 from depths 1010 to 1123 ft, VK-826 from depths 1442 to 1490 ft, and VK-826 from depths 1509 to 1571 ft. Eight specimens of *Lophelia pertusa*, five Isidae, one unidentified gorgonian, and six antipatharians were collected (Table 1).

Specimens were collected using the JSL and put into the biobox at depth, then brought to the surface. At the surface, specimens were transferred to the onboard cold room and into insulated tanks (Fig. 1) filled with seawater collected at depth in the biobox. Each tank was aerated using aquarium aeration stones. Specimens were attached to 2 by 2 inch square tiles using a two part marine epoxy, and stained with Calcein and Alizarin Red for three hours each (Fig. 2). After staining, the corals were rinsed and the seawater within each tank was replaced with fresh seawater. Specimens were fed with natural phytoplankton and organic matter found in the seawater. Seawater was changed every two or three days throughout the cruise.

On September 21<sup>st</sup>, all of the tanks containing all of the stained specimens were transferred to our vehicle and transported to the Ohio State University (OSU). During transport, tanks were placed in coolers lined with ice and the water was aerated with airstones. At OSU specimens were maintained in a series of glass aquarium tanks under recirculating flow conditions (Fig. 3). Each tank contained three specimens (Fig. 4). The transported seawater was mixed with Instant Ocean seawater mix. Specimens were rotated daily within a tank and among tanks every three days to minimize tanks effects on their growth rates. Every three days, the corals were fed two-day-old brine shrimp nauplii (approximately 200  $\mu\text{m}$  in size, hatched from one tablespoon of dry cysts in aerated seawater). Corals were kept in these tanks and under these conditions for the duration of the experiment.

### Objectives

1. Live collection of 8 gorgonian specimens, 8 antipatharian specimens, and 8 *Lophelia pertusa* specimens.
2. Stain all specimens using Alizarin Red and Calcein and rear them in tanks in the Aquatic Ecology Laboratory at the OSU for six months.
3. Measure the experimental growth rates in all three organisms.
4. Measure the periodicity and morphology of growth rings and the possible relationship of ring formation with temperature.
5. Calibrate stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ) of the skeleton to temperature conditions and/or coral biology.

### RESULTS/DISCUSSION

A sufficient number of specimens were collected during the cruise. All specimens appeared to have survived the staining procedure and most survived the first week of growth within the tanks onboard the R/V Seward Johnson II while supplied with natural seawater. However, all of the specimens died within the first two weeks of the experiment. The specimens appear to have starved to death. Though fed regularly, the soft corals did not seem able to eat the brine shrimp (a technique that has worked very well with scleractinian corals). We suspect that deep-water soft corals eat particles smaller than 200  $\mu\text{m}$  and quite possibly feed on particulate and dissolved organic matter (POM and DOM, respectively), which is abundant in marine snow.

If we were to repeat this experiment, we would need to develop a material similar in consistency, size, and nutritional composition of the DOM found in deep-water sites. In addition, death occurred slowly in the specimens. There was visible wasting of the tissues and the organisms metabolized their own tissues to survive in the absence of adequate heterotrophic food supply. Such wasting would have altered the isotopic composition of the tissues due to respiratory fractionation effects and would no longer be representative of the natural isotopic composition of specimens found *in situ*. Such fractionation directly interfered with any possibility of calibrating the stable isotopes of the specimens. Specimens are currently stored in a -80°C freezer for potential future use yet to be determined. Though a disappointing result, we have gathered enough information on how to maintain such organisms under tank conditions and we feel that given a second opportunity, we would be successful.

#### ACKNOWLEDGMENTS

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#### DISCLAIMER

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Appendix F Table.1. List of specimens collected during Gulf of Mexico 2005 research cruise.

<b>Date</b>	<b>Dive #</b>	<b>Specimen</b>	<b>Site</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Collection depth (ft)</b>
9/17/05	4875	<i>Lophelia</i>	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Antipatharian (red tissue)	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Bamboo ( <i>Keratoisis</i> )	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Bamboo ( <i>Keratoisis</i> )	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Bamboo ( <i>Keratoisis</i> )	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Antipatharian (white tissue)	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Bamboo ( <i>Keratoisis</i> )	VK906/862	29.105	-88.384	1010-1123
9/17/05	4875	Bamboo ( <i>Keratoisis</i> )	VK906/862	29.105	-88.384	1010-1123
9/17/05	4876	Antipatharian (red tissue)	VK906/862	29.107	-88.384	1011-1071
9/17/05	4876	Antipatharian (orange tissue)	VK906/862	29.107	-88.384	1011-1071
9/18/05	4877	Antipatharian (white tissue)	VK826	29.170	-88.010	1509-1571
9/18/05	4877	Antipatharian (white tissue)	VK826	29.170	-88.010	1509-1571
9/18/05	4877	<i>Lophelia</i>	VK826	29.170	-88.010	1509-1571
9/18/05	4877	<i>Lophelia</i>	VK826	29.170	-88.010	1509-1571
9/18/05	4877	<i>Lophelia</i>	VK826	29.170	-88.010	1509-1571
9/18/05	4877	<i>Lophelia</i>	VK826	29.170	-88.010	1509-1571
9/18/05	4877	<i>Lophelia</i>	VK826	29.170	-88.010	1509-1571
9/18/05	4877	Gorgonian (unknown sp.)	VK826	29.170	-88.010	1509-1571
9/19/05	4879	<i>Lophelia</i>	VK826	29.160	-88.019	1442-1490
9/19/05	4879	<i>Lophelia</i>	VK826	29.160	-88.019	1442-1490

**FIGURE LEGENDS**

- App. F, Figure F.1. Specimens in chilled tanks onboard the Seward Johnson.
- App. F, Figure F.2. Green and red dye of the Calcein and Alizarin Red in each chilled tank. Specimens were left in these tanks to stain for three hours.
- App. F, Figure F.3. Recirculating seawater tanks at OSU maintained in 10°C chamber.
- App. F, Figure F.4. Three specimens (left to right: antipatharian, *Lophelia*, and bamboo coral) in one of the tanks at OSU.



App. F, Figure F.1. Specimens in chilled tanks onboard the Seward Johnson.



App. F, Figure F.2. Green and red dye of the Calcein and Alizarin Red in each chilled tank. Specimens were left in these tanks to stain for three hours.





App. F, Figure F.3. Recirculating seawater tanks at OSU maintained in 10°C chamber.



App. F, Figure F.1. Three specimens (left to right: antipatharian, *Lophelia*, and bamboo coral) in one of the tanks at OSU.

