

# An Investigation of the Current Status of the Hawaiian Black Coral Fishery Using Historical and New Perspectives

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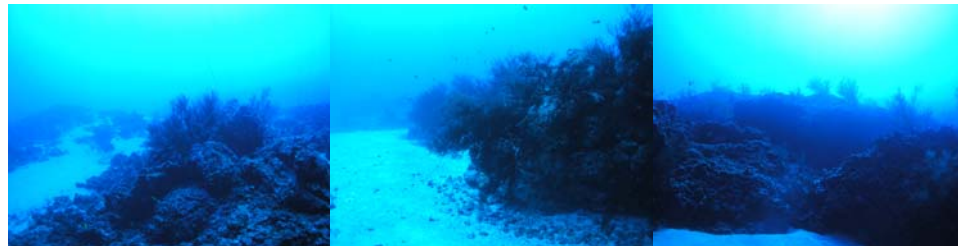
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*Antipathes grandis*, Auau Channel, Maui (65 m)



*A. cf. curvata*, south Kona,  
Hawaii (61 m)

*A. cf. curvata*, Makahuena  
Point, Kauai (61 m)

*A. cf. curvata*, Auau  
Channel, Maui (55 m)



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**Abstract:**

This project summarizes the current status of the Hawaiian black coral fishery and compares results with historical measurements. To achieve this, historical methodology where possible was used to make comparisons as similar as possible. The goals and objectives of this project included: 1) measure black coral colonies to determine the current age structure, 2) collect samples to measure genetic relatedness of black corals across the Main Hawaiian Islands to address possible distinct populations, 3) measure the density of black coral colonies in the areas sampled for population structure, 4) determine the growth rate and compare with the historically determined growth rate for the targeted species, and 5) develop a draft management plan based on all available information (new and historical).

Age frequency data collected shows a significant change in population structure over time. Post-harvest age class mortality rates have increased since 1998 suggesting an increase from fishing pressure while pre-harvest age class mortality rates appear to be zero. It is concluded from the analysis of change in pre-harvest age classes and an understanding of the assumptions with the use of age structure data that recruitment has decreased since 1998. The explanation of a drop in recruitment is unclear, but may be a result of multiple stressors on the population.

Early measurements of growth rates seem to generally support the established growth rate of 6.42 cm/ year, while density measurements can not be compared over time. Methodological differences between time periods seem to confound interpretation of differences in density over time.

As has been observed in other anthozoans, mitochondrial gene sequences show relatively low levels of variation among taxa, and no variation was observed among more than 75 colonies of *Antipathes cf. curvata* collected from four Hawaiian Islands. This lack of variation is likely a reflection of low mutation rates and an indication that mitochondrial genes are not useful for distinguishing population-level differences of black corals. It should not be used to imply that gene flow and dispersal is extensive among the islands. Comparative analyses showed that mitochondrial genes may be useful as species markers. Future genetic-based approaches for delineating population structure must concentrate on nuclear gene markers. Primer design for

several nuclear genes was initiated during the course of this project, and comparative analyses are ongoing. A suitable tissue sample size was amassed from this project such that an investment into design and testing of frequency-based markers, e.g., microsatellites or AFLPs, is feasible. Neither of these marker types has previously been applied to antipatharians and therefore no preliminary data are available; however microsatellites are commonly applied to other taxa for population-level questions. The application of AFLPs to marine invertebrates is still in its infancy.

The results of significant changes in age structure over time have been an instrumental dataset in managing this fishery. It is clear that the sustainability of this fishery is in question without more restrictive measures put in place. To this end, these results have been presented to the Western Pacific Regional Fishery Management Council. The Council is currently in the process of eliminating an exemption effectively increasing the minimum size for all fishery participants. The State of Hawaii will also increase its regulation for its minimum size, but will also include other management options in revised rules. The options include creating closed areas in State waters where black coral is readily abundant and potentially a location for harvesting. However, the State will also consider creating a special permit and potentially a limited entry system for this fishery. These options have not been put into draft rule, but will be considered in the rule revision.

## **Introduction:**

Black corals (Order Antipatharia) are colonial anthozoans found in all oceans, usually at depths greater than 30 m (Grigg, 1965). They are poorly studied due to their deeper habitat and scarcity. Their common name is derived from the color of the horny (proteinaceous and chitin) skeleton, which is commercially harvested for use in the jewelry trade. The outer tissue color varies depending on species, and may be yellow, green, red, orange, white, or brown. The growth forms found among black corals range from whips to fans to bottle-brushes to trees, and their height can exceed 3 meters. The growth rates of black corals range from less than 3 cm/yr (Grange, 1997) to just over 6 cm/yr (Grigg, 1976). Little information has been published on reproduction of antipatharians, apart from work on the fjord-endemic *Antipathella fiordensis* of New Zealand (Miller and Grange, 1997; Miller, 1997; Parker et al., 1997; and Miller, 1996). The critical habitat for antipatharians is not fully understood; however, there have been some suggested essential environmental parameters for *Antipathes* cf. *curvata* (formerly known as *Antipathes dichotoma*). These parameters include low light levels, low surge motion, substantial current, and low levels of sedimentation (Grigg, 1965).

Not only the ecology of antipatharians are poorly known, but also few genetic analyses of antipatharians have been conducted to date. The only population genetic study of a black coral examined the fjord-endemic *A. fiordensis* in New Zealand using allozymes (Miller, 1998). The data suggested that patch size was relatively small and that larval dispersal between patches is restricted. The only DNA-based data come from systematic studies of higher level groups that include the Order Antipatharia (e.g., France et al., 1996; Berntson et al., 1999; Collins, 1998; Medina et al., 2001), and only 10 DNA sequences can be found in the GenBank database (as of 9/16/2003). Any new sequence data will be a valuable contribution to the database and would likely prove useful beyond the goals of the current study. Recent research has completed sequencing the entire mitochondrial genome of a deep-sea black coral (Brugler and France, 2006) as well as an initial study to explore patterns of mitochondrial DNA sequence variation within and among antipatharian species. Although recent studies have revealed that octocorals and some other anthozoans have low rates of mutation in the mitochondrial genome (Shearer et al., 2002), antipatharians have not yet been examined (prior to this current project).

Black corals have historically been used to make jewelry items, and this practice continues today. Native Hawaiians used black corals as a medicinal powder (Ticomb, 1978). Black corals have been commercially harvested in many localities around the world, including Hawaii (Grigg, 1976), Tonga (Department of Lands, Survey and Natural Resources, Nuku'alofa, Tonga, 1985) Ecuador (Romero, 1997), and throughout the Caribbean (Wells et al., 1983). Some of these localities have been depleted of black corals due to over-harvesting. The life history characteristics (slow growing, long life span, older age of maturity) of antipatharians increases the potential for over-harvesting, unless regulated at some level. This has caused black corals to be listed as commercially threatened by the International Union for Conservation of Nature and Natural Resources (IUCN) in 1983. They have also been listed on Appendix 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). CITES appendix 2 animals are not endangered, but they may become endangered if action is not taken. The actual extinction of black corals seems unlikely due to their depth distributions. Most species that are commercially sought can be found at depths beyond typical sport divers' limits. However, divers collecting for curios or jewelry have significantly reduced shallow-water populations in some parts of the world. Advanced technology allowing humans to go deeper with reduced risk also may threaten more populations without regulations (Montgomery, 2002).

The harvesting of black corals throughout the world has been intense over the last few decades. Many areas have been overexploited. Areas such as Barbados, Jamaica, U.S. Virgin Islands, the Netherlands Antilles, Cozumel, and other parts of Mexico have all been subjected to heavy harvesting. Most Caribbean nations have passed legislation to prohibit or limit the taking of black corals (Wells et al., 1983). Over the past 20 years, the black coral *Myriopathes panamensis* has been harvested so heavily in Ecuador that it has become commercially extinct there (Romero, 1997).

Hawaii has had an active fishery for black coral since 1958 when this resource was discovered in abundance off Lahaina, Maui (Grigg, 1965). The majority (90%) of the harvesting targets *A. cf. curvata*; however, two other species are also commercially sought: *Antipathes grandis* (9%) and *Myriopathes ulex* (1%) (Oishi, 1990). However, recent discussions with the fisherman suggest that *A. grandis* and *M. ulex* compose far less than 10% of the current fishery (Robin Lee,

personal communication). This fishery is currently valued at \$30 million at the retail level (Grigg, 2004). However, sales have slowed since September 11, 2001 due to changes in the global economy (Carl Marsh, personal communication).

Currently, the Division of Aquatic Resources (DAR) of the Department of Land and Natural Resources (DLNR), State of Hawaii, and the National Oceanic and Atmospheric Administration (NOAA) through the Western Pacific Regional Fishery Management Council (WPRFMC), manage the harvest of black corals around the Hawaiian Islands. The State of Hawaii limits the harvest of black corals to a minimum basal diameter of  $\frac{3}{4}$ " (Hawaii Administrative Rules: Title 13, Chapter 91) while the federal regulations limit the harvest with a minimum basal diameter of 1" or 48" height (Federal Register 50 CFR Part 660 subpart F). One exemption to the federal regulations is for divers that have been active in the fishery prior to the federal regulations being implemented which allows them to harvest coral with a  $\frac{3}{4}$ " basal diameter or 36" height.

For the past 30 years, the collection of data for the Hawaiian fishery has primarily come from one researcher, Dr Richard Grigg of the University of Hawaii at Manoa. Dr. Grigg has conducted three separate studies on the status of this fishery since 1975 (Grigg, 1976; Grigg, 2001; and Grigg, 2004), and these studies have laid a foundation for the current management of the fishery. The most recent work (Grigg, 2004) suggested some changes are occurring in the fishery that should be addressed. In particular, Grigg (2004) stated that recruitment appears to be decreasing. However, it is not certain if this decrease is an artifact of sampling due to a different measurement methodology, or a true trend in the fishery. Grigg (2004) also noted three changes in the fishery: 1) an increase in demand for black corals, 2) a gradual reduction in black coral biomass from years of harvesting, and 3) an invasion of a non-native soft coral (*Carijoa riisei*) in certain areas of the black coral habitat. The invasion of *C. riisei* has been suggested to be extensive in the deep portion of the Auau Channel off Maui (Grigg, 2003).

These changes, in combination with a possibility that recruitment has decreased, have brought more attention to the management of this fishery. Dr. Grigg, through the WPRFMC process, has requested an increase in the regulations of black coral harvesting (make a 48" height or 1" basal diameter minimum size). Prior to this study, the 118<sup>th</sup> Council's final decision was to defer on

increased regulations until the State of Hawaii and the federal representatives could meet with researchers and the fishermen to develop a consistent and more appropriate management strategy. The outcome of this meeting was a recommendation to defer further action temporarily. It was agreed that more research needed to be conducted in order for a proper management strategy to be developed.

### **Goals and Objectives:**

This project had five main objectives:

- 1) Measure black coral colonies for height in order to determine age structure,
- 2) Collect and analysis black coral samples from at least four islands (Kauai, Oahu, Maui, and Hawaii) for genetic relatedness to determine potential connectivity,
- 3) Measure black coral densities at key study sites to compare to historical values,
- 4) Measure growth rates to compare to historical values, and
- 5) Develop criteria for a draft management plan to be used as a guide for the development of new harvesting regulations.

**Objective 1** was to measure black coral colonies (*A. cf. curvata* and *A. grandis*) to determine the current age structure in areas heavily harvested by fisherman as well as areas less heavily harvested by fisherman in the Auau Channel. Areas previously measured by Grigg (1976; 2001) will also be sampled to allow for comparison with historical age structures. Our null hypothesis is that there no significant difference in age structure among sites with different amounts of fishing pressure as well as between sites over time (1975, 1998 and present). Our alternative hypothesis is that a significant difference in age structure does exist between heavily harvested sites and less heavily harvested sites, and there is a significant difference in age structure between the present and 1975 and 1998.

In order to accomplish this objective 1421 colonies were measured for height in multiple locations in the Auau Channel (some sites were as close as possible to Grigg's historical sites). This data was then able to be graphed in a age frequency distribution to show the age structure (see Project Accomplishments section for full description of methods).



**Objective 2** was to collect samples (*A. cf. curvata*) to measure genetic relatedness of black corals across the Main Hawaiian Islands to answer the following questions: 1) What constitutes a black coral population, and 2) What is the genetic exchange among possible various black coral populations. Our null hypothesis is that there is no genetic difference between populations on Kauai, Oahu, Maui, and Hawaii; therefore, these populations are not reproductively isolated. Our alternate hypothesis is that there is significant genetic difference between these populations; therefore, some of these populations are reproductively isolated.

In order to complete this task, 182 samples were collected from Kauai, Oahu, Maui, and Hawaii. The samples were analyzed using mitochondrial DNA sequence variation and intraspecific variation to the nuclear genome.

**Objective 3** was to measure the density of black coral colonies (*A. cf. curvata* and *A. grandis*) in the areas sampled for population structure and compare to historical measurements. Our null hypothesis is that the density of black coral colonies is not significantly different between sites with different amounts of fishing pressure as well as between sites over time (1975, 2001, and 2004). Our alternate hypothesis is that there is a significant difference in colony densities between sites with different amount of fishing pressure and among sites over time (1975, 2001, and 2004). This objective was completed by conducting 30-100 m<sup>2</sup> transects at various depths.

**Objective 4** was to determine the growth rate of *A. cf. curvata* and compare with the historically determined growth rate of 6.42 cm/year (Grigg, 1976). Our null hypothesis is that the measured growth rate will not differ from the historical value. Our alternate hypothesis is that there will be a difference in the measured growth rate and the historical growth rate. This objective was accomplished by tagging 39 individual colonies and measured their growth over time.

**Objective 5** was to develop a draft management plan based on all available information (new and historical). This objective was to analyze existing data and use it to create a set of criteria to be used for the management of this fishery. This was accomplished by looking at the future of this fishery and develop a strategy (or criteria for a strategy) to manage this fishery for future generations.

## **Completed Work:**

The work conducted to accomplish the stated goals and objectives of this project require 53 deep open circuit scuba dives using trimix and 6 dives using nitrox. Work on individual dives included collecting samples for both genetic analysis and taxonomic consideration, height measurement of colonies, photo documenting habitat, tagging colonies for growth, and measuring density of colonies.

In addition to using divers as a data collection tool, drop camera surveys were completed to scout new areas of interest. This technique utilized by Dr. Parrish proved to be an essential tool before scuba dives were completed. This technique allowed 100% of scuba dives to be successful, meaning zero dives were aborted due to missing dive site. A total of 33 individual drop camera surveys were completed.

This project involved approximately 7 weeks in the field and many more for laboratory work and data analysis.

## **Project Collaborations:**

During the course of this project, several components included the participation of several partners. The first is of course co-PI Dr. Scott France and his assistant Dr. Mercer Burglar at the University of Louisiana at Lafayette. Dr. France's lab ran all of the genetic analysis on collected samples and provided data summaries for all reports. The other major partnership was with Dr. Frank Parrish and Mr. Raymond Boland at NOAA's Pacific Islands Fisheries Science Center. Through the cooperation of Dr. Parrish this project was able to utilize five sea days on board the R/V Oscar Elton Sette (September 8-13, 2004). In addition, both Dr. Parrish and Mr. Boland were able to dive and assist in data collection making these 5 days very productive and valuable. We thank Dr Parrish for this extraordinary opportunity and partnership and look forward to future collaborative efforts.

Other collaborators included Mr. Robin Lee, black coral fisherman. Mr. Lee provide valuable input into his impression to site selection. In addition, Mr. Lee provided the PI with 5 years of

logbook data for exact coordinates of fished areas. Although this dataset is not part of this report, it is recognized that this data is invaluable in many ways.

In addition, Dr. Richard Grigg of the University of Hawaii also provided valuable input during the course of this project. He was able to provide additional detail (over and beyond published information) into methodology and areas of interest. His historical data for this fishery has and will continue to be tremendously important as this fishery is analyzed critically for future sustainability.

## **Project Accomplishments:**

### **Objective 1 – Introduction**

This report analyzes data collected in 2004 and draws conclusions about the current status of the black coral population in the Auau Channel located between Maui and Lanai. DAR conducted a series of surveys in the fall of 2004. The main focus of this objective is to compare current data with historical data on the Auau Channel black coral population. This approach is aimed at giving insight into any potential changes that have occurred in the population.

### **Historical Data – 1975, 1998, 2001, and 2004**

Historical data from the black coral population in the Auau Channel has been collected and published by Grigg (Grigg, 1976; Grigg, 2001, and Grigg, 2004). This represents the majority of data from the Auau Channel population. Grigg's studies concentrated on using age frequency distributions created by measuring the height of individual colonies (by divers in 1975 and 1998 and by submersible in 2001) and converting the height into estimated age. Grigg used age frequency distributions to calculate the slopes of linear regressions of log-transformed abundance data versus age to determine an estimate of mortality rate.

Grigg's 1998 surveys provided data that appear to show the fishery was sustainable. Grigg concluded, when comparing the 1975 data to the 1998 data, that the larger, older colonies had been reduced in the population but that recruitment and overall mortality had been relatively stable since the 1975 survey. It was suggested that the fishery was sustainable due to voluntary compliance by fisherman to a suggested harvest size of 48 inches (1.2 meters) height (estimated

19 years old), and because there was an unfished deeper population of black coral (Grigg, 2001) which provided a breeding stock reservoir. Figure 1 shows the age distributions for Grigg's datasets.

Grigg's 2001 surveys showed further changes in the population structure since 1998, including a continued decline in the larger, older age classes and a recent decrease in the proportion of corals in age classes under 5. The population structure was dominated by intermediate age classes. The continued decline in larger, older ages was attributed to fishing. However, the cause of the decline in younger age classes was attributed to some combination of: (1) harvesting; and (2) overgrowth of black coral by the invasive soft coral, *Carijoa riisei*, in the deeper (unfished) population. Grigg suggested the future sustainability of the fishery might be in jeopardy unless more restrictive regulations are put in place (Grigg, 2004).

Although the results of Grigg's 2001 survey (Grigg, 2004) show dramatic differences with earlier populations, there is reason to suspect differences in survey methodology between 2001 and previous surveys could account, at least in part, for the apparent low level of recruitment evident in Grigg's 2001 surveys. Since there is a call to action to increase regulations, the Division of Aquatic Resources needed to gather more data to verify the current status of the population.

Most recently, Kahng and Grigg (2005) reported on the impact *Carijoa riisei* has had on black coral in the Auau Channel. They reported the greatest impact to colonies deeper than 80 meters and greater than 75 cm in height, which represents the estimated size of maturity. They also report an increase in black coral colony infestation between 2001 and 2004 (~65% and 90% respectively). This may indicate that the entire (or almost entire) mature population below 80 meters may be lost with time. This could effectively remove the breeding stock reservoir proposed by Grigg.

## **Methodology**

In order to directly compare newly collected age frequency distribution data with historical age frequency distribution data, the same methods employed by Grigg in 1975 and 1998 were used

by DAR in the 2004 surveys. The height of all black coral colonies was taken by measuring colonies with a 1.2-meter rod and recorded on an underwater data sheet. **The height was defined as the longest continuous branch of the colony regardless of the direction of growth.** The height was then converted into age by dividing the height by the reported species-specific growth rate (*Antipathes* cf. *curvata* = 6.42 cm/year and *A. grandis* = 6.12 cm/year). The current dataset (2004) contains 1421 samples (both species combined) from depths of 30 to 65 meters; however, samples from depths of 45 to 55 meters were pooled to generate the data in Figure 2 in order to make a better comparison with historical data (which were collected in the 45 to 55 meter isobath).

Following the approach of Grigg<sup>1</sup> (1976, 2001), changes in regression slopes through time are used to indicate changes in mortality rate<sup>2</sup> and therefore to assess whether there is support or not for the idea that population structure has changed through time.

This analysis will address the following questions:

- 1) Have there been changes in the age structure across all ages?
- 2) Are there differences in post-harvest age structure (>14 years)?
- 3) Are there differences in pre-harvest age structure (<14 years)?
- 4) Has recruitment in younger ages classes diminished?

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<sup>1</sup> There are a couple of noteworthy points about the method used to calculate the regression here, which may differ somewhat from Grigg's method: (1) age classes 0 and 1 were dropped as they appear to be under-sampled due to difficulty in finding juveniles under 13 cm in height (~2 years of age); (2) if an age class did not have any observed individuals, that age class was averaged with the previous or next year which did have individuals (due to the impossibility of calculating a log of a zero value); and (3) regressions were carried out only across age classes for which there was reasonably continuous data (i.e. no more than 2 years without any individuals)

<sup>2</sup> The method of estimating survival and mortality rates from age composition has been used in human demography for generations. However, this method was more recently used in this century when fish populations were able to be aged (Ricker, 1975). When using this methodology three assumptions need to be made (Krebs, 1999). They are: 1) Constant survival rate over a period of time; 2) Constant recruitment for all age groups; and 3) Sampled population is representative of the entire population.

Further, scientist have also used catch curves to estimate mortality and survival of fish (Ricker, 1975). This method uses log frequency against size to estimate mortality. This method has the following assumptions (Krebs, 1999): 1) Mortality rate is uniform with age; 2) Mortality rate is constant over time; 3) Sample is random; and Constant recruitment for all age groups.

Both methods can estimate mortality; however, using the catch curve method allows the data to be made linear and it is believed that these methodologies can be applied to black coral demographics.

### **Age structure across all ages:**

Visual (qualitative) analysis of the age frequency distributions (Figure 2) suggests two changes in the population: (1) a continuing decline in the proportion of larger, older colonies - those above about age 19 and (2) fewer corals in age classes less than 9 years in the 2004 dataset. These analyses are only qualitative, so there is a need for more quantitative analysis to verify these qualitative observations.

Figure 3 shows the regression of the 1975, 1998 (note: 1975 and 1998 size distribution data were extracted from graphs in Grigg 1976 and 2001), and 2004 data and suggests that there have been changes in the population. However, it is difficult to determine what changes have taken place, as a presumption of a common mortality rate across all age classes is simplistic particularly for a harvested population. It seems logical to break the data into pre-harvest and post-harvest age groups and separately estimate mortality within those ranges. The current minimum size for harvesting is 3/4" base diameter (~ 14 years); therefore, the following analysis is for 'pre-harvest' age classes (<14 years old) and 'post-harvest' age classes (>14 years old).

### **Post-harvest age structure:**

If the post-harvest age classes have declined in comparison to historical populations, a higher mortality rate across the post-harvest age range would be expected. Figure 4 (note the relatively high  $r^2$  value indicating a good fit for the regression line) shows a trend of increasing mortality rate as the post-harvest mortality rate increased from 1975 to 1998 and then again in 2004. Since there were no observed colonies infested with *Carijoa riisei*, an increasing mortality rate suggests that the harvestable age classes are increasingly being affected by fishing. The mortality rate between 1975 and 1998 only increased slightly: from 17.3% to 19.7%, but by 2004 the mortality rate of harvestable age classes had increased to 30.9%.

### **Pre-harvest age structure:**

Age frequency distribution in pre-harvest age classes is worth examining as it may show changes in relative recruitment. An assumption of the regression technique used to calculate IRM is that recruitment is constant among years and declines in age class frequencies as age increases are a reflection of real mortality rates. If that assumption is violated more than mildly, the calculated

mortality rate will not be realistic. As a corollary of that, an unrealistic mortality rate may indicate the steady state recruitment assumption has been violated.

The regression analysis of pre-harvest age classes in the 1975, 1998 and 2004 datasets suggests there was minimal if any change in the recruitment between 1975 and 1998 (same conclusion as in Grigg, 2001). However, the estimated mortality for 2004 was almost 0 (a flat slope). An assumption of zero mortality is not realistic based on historical datasets, especially in young age classes; a more likely explanation would be that recruitment into the early age classes has decreased during the period between about 1998 and 2004. The regression (note the  $r^2$  values for the 1975 and 2004 regression lines are low) is shown in Figure 5. This suggests variability in age class abundance for the 2004 regression. The short period of low recruitment in 1975 (ages 3 to 5) may contribute to its lower  $r^2$  value. It is difficult to know how significant this apparent period of low recruitment – 1998 to 2004 – is, since low numbers of corals in age classes 3 to 5 in the 1975 dataset indicates that a few years of low recruitment is not unprecedented.

**Nevertheless an apparently sustained period of low recruitment is a matter of some concern.**

Grigg (2004) suggested the age frequency distribution measured in 2001 showed signs of diminished recruitment in age classes under 5. Age distribution data collected in 2004 suggests diminished recruitment in age classes under 9 (somewhere between 5 to 9 years). If each of these datasets were back calculated to the time period of possible recruitment slow down, they both tend to suggest a similar timing of the onset of low recruitment (mid-late 1990s). **It therefore seems reasonable to assume that a wide-scale slow down in recruitment occurred in the mid-late 1990s.**

#### **Colonies-counted per unit of time:**

Another way to analyze changes in the population is to calculate the number of measured individuals<sup>3</sup> per unit of time or area. Area-surveyed was not measured on these or previous

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<sup>3</sup> Grigg's 1975 dataset was collected in 4 dives total utilizing 2 divers measuring colonies as a team. Grigg's 1998 dataset was collected with 5 dives utilizing the same buddy system (Grigg, pers. comm.; Grigg, 1976; 2001). The 2004 data was collected in 6 dives with two divers collecting data independently. Grigg's dives ranged from 10 to 13 minutes per dive team while the 2004 dives were either 20 or 25 minutes per diver.

surveys, but survey time was closely watched and close to fully utilized (due to safety reasons for calculating decompression). Number of colonies recorded per unit of survey time may therefore be a useful indicator of coral density in various age class categories (<9, 9-14, 14-19, and >19).

Analyzing this data (Figure 6) for age classes under 9 suggests that there are fewer individuals under age 9 in 2004 as in previous years (1975 and 1998). Also, note that the number of individuals in age classes 9 to 14 is approximately the same in all survey periods. Age classes 9 to 14 would be expected to remain similar over time as they are not subjected to harvesting pressure and are older than the age classes seemingly affected by diminished recruitment (in other words, they provide a sort of test of this analytical approach). Age classes 14 to 19 seem to be approximately equally abundant in all survey periods too. However, the age classes above age 19 have clearly decreased since 1975 and 1998.

Figure 6 shows that in 2004 there were fewer colonies under age 9, almost the same number of individuals between ages 9 to 14 and 14 to 19, and fewer colonies over age 19 when compared to previous surveys. This is not an exact method to estimate the population structure changes, but does coincide with the regression analysis. This analysis is valuable as the different analytical approaches suggest similar changes in population structures.

### **Conclusions – Objective 1**

- 1) Age structure for all ages has changed between 1975 and 2004 as shown by an increased mortality rate (from 10.2% to 15.9%).
- 2) Post-harvest age structure has significantly changed with an increase in mortality rate (particularly between 1998 and 2004) and fewer large colonies. The cause is believed to be from fishing pressure (1975=17.3%, 1998=19.7%, and 2004=30.9%).
- 3) Pre-harvest age structure has also changed by having fewer recruit and juveniles in the populations presumably indicating a drop in recruitment. The cause of this is unknown but may be a combination of fishing, *Carijoa* infestation and natural slow down in recruitment.



4) Analysis using colonies counted per minute of dive time support the conclusions made for changes in the population structure for pre and post-harvest sages.

5) Analysis of the 2004 surveys seems to support Grigg's conclusion of diminished recruitment. The reduction of older age classes is most likely due to harvesting, as surveys detected very little *Carijoa* (and zero deaths were attributed to *Carijoa*). However, the cause of diminished recruitment is much more uncertain. We do not have a sound understanding of the reproductive input supplied by the deeper population of black coral (the portion being mostly effected by *Carijoa*), and we do know that harvesting has impacted the larger colonies in depths that *Carijoa* has not impacted directly. The most likely cause of diminished recruitment is a combination of *Carijoa* impacts and harvesting, but also recognizing the possibility that natural fluctuation in recruitment may be a factor. We may never know the cause, but it seems like the phenomenon is real.

## **Objective 2**

Tissue fragments were received from 182 *Antipathes* cf. *curvata* individuals that were collected from various localities near Kauai, Oahu, Maui, and Hawaii (Table 1). Genomic DNA has been extracted from 154 of the 182 *A. cf. curvata* individuals. A majority of the Oahu samples were only recently obtained, and extractions and PCR amplifications of the targeted set of genes (see below) are ongoing.

It is not believed that any previous or concurrent study has examined DNA sequence variation of black corals at the intraspecific (population) level, and very few interspecific studies have been done. Therefore, initial efforts have been to assess variation within black coral taxa. These analyses incorporated additional species from all seven families of the order Antipatharia to provide a systematic context for the variation, and to assess utility of the chosen gene regions for species identification. Sequencing the mitochondrial genome of a black coral was recently completed (Brugler & France, 2006) and therefore conducted an initial search for a suitable DNA marker within this widely-used genome. The following mitochondrial gene regions were amplified and sequenced: cytochrome oxidase subunit I (*cox1*, 658 base pairs in length in

*Chrysopathes formosa* genome), large subunit ribosomal RNA gene (*rnl*, 523 bp), and five intergenic spacers (*trnW-nad2*, 448 bp; *nad5-nad1*, 407 bp; *rns-cytB*, 900 bp with 122 bp non-coding; *cytB-rnl*,  $\approx$ 210 bp [two short ( $\approx$ 80 bp) non-coding regions flanking tRNA Met]). At the intraspecific level, it was predicted that the larger intergenic spacers would be the most informative.

As anticipated, maximum genetic distances were observed among taxa when comparing sequences of the *nad5-nad1* (max 27.99%) and *trnW-nad2* (max 21.26%) intergenic-spacers. The *cox1* gene, which is being promoted as the ideal gene to barcode the ‘Tree of Life,’ showed a maximum interspecific divergence of 12.25%. Among Hawaiian species within the family Antipathidae, encompassing three genera (*Antipathes*, *Cirripathes*, *Stichopathes*), the *nad5-nad1* and *trnW-nad2* intergenic-spacers showed maximum distances of 1.4% and 0.8%, respectively (Table 2). The genetic distances between the two Hawaiian *Antipathes* species were greater than that between *A. cf. curvata* and a nominal *Stichopathes* (Table 2), which suggests difficulty in identifying these specimens or potential problems with the taxonomy of the Hawaiian Antipathidae. Indeed, at the beginning of this study the species name *Antipathes dichotoma* was being applied to what is now referred to as *Antipathes cf. curvata*, based on a reassessment of morphology by a leading black coral specialist, Dennis Opresko. We were able to amplify and sequence a known specimen of *A. dichotoma* collected from the Mediterranean Sea and found it to be  $\approx$ 14% different from the Hawaiian *Antipathes* at the *trnW-nad2* intergenic-spacer region, supporting the renaming of the Hawaiian specimens. We also sequenced a specimen of *A. curvata* from Hong Kong and found it to be <1.5% divergent from the Hawaiian *A. cf. curvata*, which further supports the morphology-based identification as a species similar to *A. curvata*, although there is an even smaller genetic distance between the Hawaiian *A. grandis* and *A. cf. curvata*.

In the course of this study, DNA sequence variants of *A. cf. curvata* were revealed as specimens of *A. grandis* that had been misidentified. This information provided new insight into the distribution of *A. grandis*. No sequence variation was observed at the intraspecific level for any mitochondrial gene region, despite sequencing more than 75 *A. cf. curvata* individuals for both intergenic regions. We do not believe this is necessarily reflective of patterns of gene flow and

population structure. Rather, there is insufficient variation in the mitochondrial genome to address the issue. Low mitochondrial DNA sequence variation has been documented in other anthozoans (e.g., Shearer et al., 2002), although this is the first such examination of antipatharians. This does suggest that mitochondrial sequence variants may be reliable markers of different species.

Efforts were expanded to search for intraspecific variation in the nuclear genome, initially sequencing the internal transcribed spacers (ITS1 and ITS2) of the ribosomal DNA. Results showed intragenomic variation for ITS1 in 17 *A. cf. curvata* individuals. The ITS region is known to exhibit within-individual variation because of the large number of copies within the nuclear genome (e.g., Forsman et al., 2005). Thus, the ITS marker was excluded from further analysis.

Degenerate primers were developed from available anthozoan sequence data to the following nuclear genes: actin, tubulin, heat shock protein 90, elongation factor, growth hormone receptor, and calmodulin. Sequence data was successfully obtained from PCR fragments amplified using beta-actin, beta-tubulin, and calmodulin primers, although an insufficient number of individuals have been analyzed to date to determine suitability as an intraspecific marker (Table 3). No introns have been amplified within either the beta-actin or beta-tubulin gene sequences; introns are typically more variable than the protein-coding portion of the gene. Testing of nuclear gene regions using existing tissue samples will continue. Additional primers to nuclear gene regions will be made available by the NSF-sponsored Cnidarian Tree of Life project, and these will be tested for the presence of variable introns. Microsatellite markers are used extensively in population genetic analyses, but they are too expensive and time-consuming to develop in the absence of a large population sample. Given the relatively large sample size of tissues for genetics made available by this study, the development of microsatellite markers for *A. cf. curvata* is of interest.

## **Conclusion – Objective 2**

These results provide the first survey of DNA sequence variation in black corals. As has been observed in other anthozoans, mitochondrial gene sequences show relatively low levels of

variation among taxa, and no variation was observed among more than 75 colonies of *Antipathes cf. curvata* collected from four Hawaiian islands. This lack of variation is likely a reflection of low mutation rates and an indication that mitochondrial genes are not useful for distinguishing population-level differences of black corals. It should not be used to imply that gene flow and dispersal is extensive among the islands. Comparative analyses showed that mitochondrial genes may be useful as species markers. Future genetic-based approaches for delineating population structure must concentrate on nuclear gene markers. Primer design was initiated for several nuclear genes, and comparative analyses are ongoing. A suitable tissue sample size from this project was collected such that an investment into design and testing of frequency-based markers, e.g., microsatellites or AFLPs, is feasible. Neither of these marker types has previously been applied to antipatharians and therefore no preliminary data are available; however microsatellites are commonly applied to other taxa for population-level questions. The application of AFLPs to marine invertebrates is still in its infancy.

### **Objective 3**

Density of black coral colonies can be difficult to measure due to the patchiness of their distributions. However, a good estimate of density needs to be done in order to calculate a maximum sustainable yield for the fishery (notwithstanding an ecosystem approach). Previous surveys by Grigg have reported densities from 0.05 colonies/m<sup>2</sup> at 40 –70 meter depth (Grigg, 1976) to 0.10 colonies/m<sup>2</sup> at 40 –110 depth (Grigg, 2004). The methodology of the density reported in Grigg, 1976 is unknown (not reported). However, the density reported in Grigg, 2004 used a submersible and ROV over large stretches of black coral habitat.

Density measurements collected in this study used a 25 m x 4 m belt transect with divers counting all colonies in the area. The sites were similar sites (or in some cases identical) to sites chosen for size frequency data collection. This means that typically the sites chosen were sites with relatively high black coral abundance. Drop camera surveys (although not quantitative) suggest many areas of perceived suitable habitat do not support many black coral colonies (sometimes suitable habitat was observed to have zero colonies). This also supports the idea of a patchy distribution.

This project collected black coral density on 30 transects (100 m<sup>2</sup>) ranging from depths of 32 – 60 meter depth. The average density was 0.25 colonies/ m<sup>2</sup>. The difference in density over time (0.05 in 1975 to 0.10 in 2001 to 0.25 in 2004) cannot be compared in any meaningful way. The variations in methodology and specific sites make any comparisons meaningless. However, there may be some interesting comparisons within the 2004 dataset. Each transect has a depth recorded with the density (figure 7). There appears to be a difference (although no statistical analysis has been used) between the shallower black coral (less than 40 meters) than the deeper black corals (greater than 40 meters). The density on top of the ledge system in the Auau Channel has a density of 0.09 colonies /m<sup>2</sup> above 38 meters while below 38 meters has a density of .37 colonies / m<sup>2</sup> (figure 8). This requires more analysis to make any firm conclusions.

### **Conclusion – Objective 3**

The recent data clearly show a need for a standardized method in collecting density information on black coral. However, it must be determined how this data will be used in the management of this resource before this method can be developed. It was the intention of this project to compare recent density information with historical values. The data suggest either an increase in densities or a bias in the data. It is believed that differences in methodology account for any biases.

### **Objective 4**

In 2003 (previous to this project) and 2004, 39 black coral colonies were tagged on Oahu (9 total) and Maui (30 total). These colonies were measured using height as an indicator for growth. During this time period, some colonies did not show any linear growth in height, while others showed significant growth (Table 4).

### **Conclusions – Objective 4:**

- 1) Colonies tagged similar in size to Grigg, 1976.
- 2) Growth rates ranged from 5.12 cm/year (including all colonies tagged) to 8.35 cm/year (dropping all colonies who showed zero or negative growth) averaging approximately 6.74 cm/year. It is assumed that the ‘real’ growth rate may be in this range and may represent an average growth rate.

- 3) Given the timeframe of this study, these growth rates do not vary significantly from Grigg 1976 -- 6.42 cm/year (Grigg) compared to 6.74 cm/year (Montgomery).
- 4) Growth seems highly variable; however, this isn't a surprise based on Grigg's measurements in 1976.
- 5) Although environmental factors may affect growth, these average growth rates may have a resemblance of the population as a whole. However, additional time and work are required to monitor growth rates.

### **Objective 5**

The management plan for the harvest of black coral is to put regulations in place that will keep the fishery sustainable especially for future generations. The current fishermen in the industry average 59 years in age. Given the physical demands of this profession, it is safe to assume this generation of fishermen will not continue to be active for more than a few years. If the current fishermen were to retire, this would create a niche for possible new fishermen. Maui divers, currently the only buyer of raw material would also have an unfilled demand for raw material. Currently, there is a large stockpile held by Maui Divers, but this will not last indefinitely. Eventually, there will be a new generation of divers and may use superior technology and techniques. Given the historical slow response of management agencies to respond to increased pressure on a resource, new regulations need to consider a potential new generation of fishers that can remove more coral in a shorter time frame. This fishery would most likely be unable to be sustainable for any length of time if harvesting pressure was to increase dramatically. To this end, the following criteria will be the basis of new regulations. However, it is important to note that these regulations have not been written and the State of Hawaii has an extensive process of Attorney General and public review before regulations are put in place. Criteria includes:

- 1) Change minimum size requirements from  $\frac{3}{4}$ " base diameter to 1" base diameter or 48" in height. This requirement will be consistent with federal changes create a uniform minimum size in both federal and state waters.
- 2) Use an area based management framework to protect a set amount of black coral habitat. During the black coral workshop held in April 2006, fishers and industry agreed that this approach would be appropriate and felt that a designated area in the Auau Channel should set aside for no black coral harvest. On Maui, the area known as Stone Wall was

suggested by fishers to designate. This would potentially allow an area to recover to preharvest conditions (pre 1958) and may allow more insight into historical black coral age structure.

- 3) Create a permit based fishery where all fishers would require a special precious coral fishing permit and be required to report landings individually (rather than under the captains per as allowed currently). This would also allow managers to a better handle on who is fishing and watch for any new fishermen entering the fishery.
- 4) Set a control notify the public that this fishery may create a limited entry or a limited access program in order to utilized this fishery. The options to limit the entry or take of black coral could be somewhat complicated as it is with any limited entry fishery. However, the black coral fishery would probably be the more straight-forward limited entry fishery one could image. There is limited demand, limited interest in participation, a well defined and monitored fishing area, and a strong dialogue between state and federal managers as well as the industry stakeholders.

A limited entry program may be the only way to actually keep this fishery alive if the current trends continue. The drop in larger age classes with an apparent reduction in younger age classes could be extremely significant if any combination of *Carijoa* impact or increased fishing were to occur. Given time, the juvenile ages (in reduced numbers) will reach maturity and harvestable size. If the recruitment reduction does not increase over the next several years, we could be faced with expedited drop in the population, i.e. fewer colonies reach maturity, therefore decreasing population spawning potential compounded by a long continued drop in recruitment could have a devastating consequences in approximately 10 years. Of course, this is a worse case scenario, but it is important for resource managers to be aware of this potential situation. It is hoped with the proposed set of management criteria that more colonies will be allowed to spawn longer and reach a higher fecundity rate, thereby increasing recruitment. Continued monitoring will give future insight into the short-term sustainability of this fishery.

### **Samples Collected:**

During the course of this project, 182 samples of black coral have been collected and deposited at the France lab at the University of Louisiana at Lafayette. In addition, approximately 12 live

specimens were sent to the France lab for fresh tissue analysis. Two entire colonies (approximately 70 cm in height) were collected and donated to the Smithsonian Museum for taxonomic studies. All collections were overseen and authorized by a Department of Land and Natural Resources Biologist.

### **Identification of significant problems:**

The only component of this project that was unable to be completed was the development of amplified fragment-length polymorphism (AFLP) markers. The genetic analysis became more focused on designing and testing PCR primers to nuclear gene regions that would amplify across a range of black coral species (all the primer design has been trickier than expected). After the start of the project, it was determined that it is more important to understand more about sequence-based marker variation across different taxonomic levels than working with markers that are less well known in a group of taxa whose relationships were unclear. However, it is important to note that the information gathered in this project will greatly help with working on AFLP markers in the future.

### **Future Needs and Plans:**

- 1) The Auau Channel population must be monitored for continued changes in age frequency distribution. It is recommended to monitor the population every 3 to 5 years.
- 2) Standardized methodology must be developed and implemented to monitor specifically for recruitment. Ideally, this method would be completed yearly initially and eventually less frequent; however, based on logistical constraints, it should be completed at a minimum of every 3 years.
- 3) Continued work must continue for the development of microsatellite markers in order to determine the connectivity of black coral populations between islands.
- 4) Standardized protocols must be developed to monitor potential changes in black coral densities. These protocols must address the ultimate use of such data and potential location and harvesting variables.
- 5) Tagged colonies must be monitored continually to verify growth rates over a long period of time. Ideally, colonies would be tracked for 10 years or greater.



- 6) Other research needs to be completed on *A. cf. curvata* in order to manage this fishery properly: 1) description of *A. cf. curvata* reproductive biology, 2) recruitment limitations looking at Kauai populations along with Maui populations, and 3) *A. cf. curvata*'s ecological role with other species.
- 7) Future fishery and ecological models should be addressed through GIS based approaches and tools.

### **Result Dissemination:**

Results from objective 1 were presented to multiple sessions of the council including the Precious Coral Plan Team, Scientific and Statistical Committee (multiple meetings), and the full Western Pacific Fishery Management Council meeting (WPFMC) (multiple times). The results created much discussion at the council about the appropriate action and ultimately led to the final decision of the council to remove the exemption to fishers (which effectively increases minimum harvest size). These results, which supported Grigg's early results also were a reason the Scientific and Statistical Committee recommended a 5-year moratorium on the fishery (see <http://starbulletin.com/2005/05/29/news/story4.html> ).

In addition, the WPFMC and the Hawaii State Department of Land and Natural Resources held a workshop to analyze the current status of black coral research and management in Hawaii. This dataset was presented to the researchers and fishermen. The fishermen listened to this data as well as other datasets such as fishery landings data and impacts from *Carijoa* and expressed a willingness to agree to an area based management strategy. For example, they agreed that an area off Maui, known as Stone Walls (an area visited during this project), would be a reasonable area to establish a no take black coral area. This area, within state waters, will be considered in revised rules to create a no harvest black coral area.

During the 3<sup>rd</sup> International Symposium on Deep-Sea Corals held in Miami, Florida on November 28 – December 2, 2005, two presentations were made as a result of this project.

- 1) Brugler, Mercer and S. C. France. 2005. Low Sequence Variability within Anthozoan Mitochondrial Genomes: Are Antipatharian noncoding Regions the

Exception? Book of Abstracts 3<sup>rd</sup> International Symposium on Deep-Sea Corals held in Miami, Florida on November 28 – December 2, 2005. page 4.

- 2) Montgomery, A.D. 2005. Spatial and Temporal Differences in Size Structure of Hawaiian Black Corals. Book of Abstracts 3<sup>rd</sup> International Symposium on Deep-Sea Corals held in Miami, Florida on November 28 – December 2, 2005. page 170.

The genetic study was also presented at the Evolution 2006 symposium. Brugler, Mercer and S. C. France. 2006. Have We Discovered A “Fountain Of Variation?” An Analysis Of Non-Coding Regions Within The Black Coral (Cnidaria: Anthozoa) Mitochondrial Genome. Book of Abstracts Evolution 2006, Stony Brook, NY, June 23-27 2006. page 61.

The DNA sequences will be submitted to an online database (NCBI’s GenBank).

Finally, both the genetics studies and age frequency data will be submitted for publication.

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# Figures and Tables

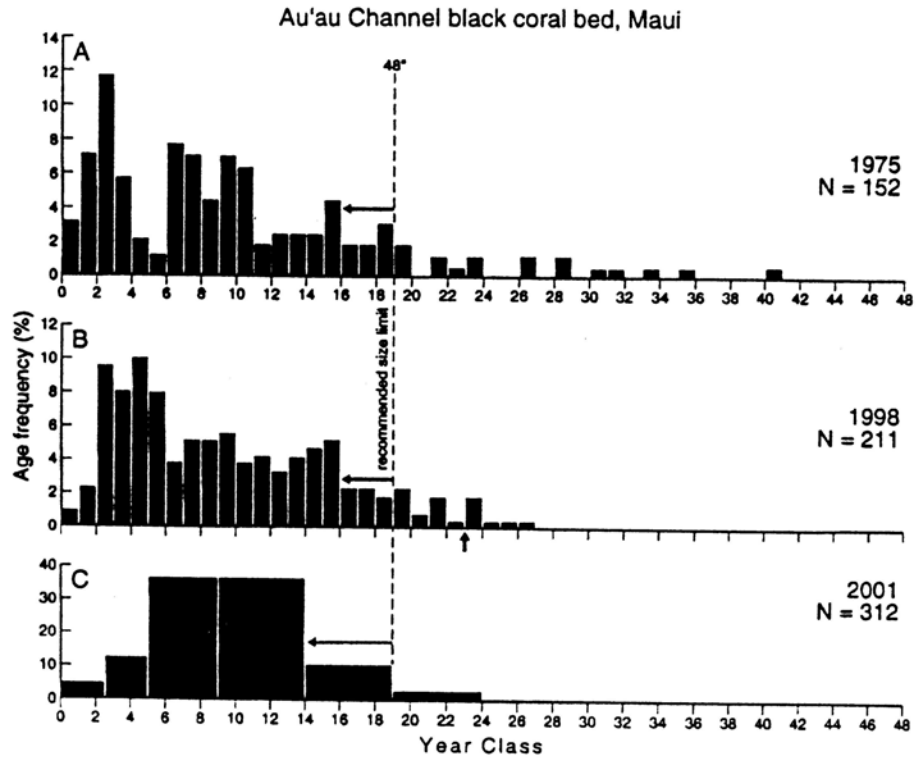


Figure 1. Grigg's dataset from 1976, 2001, and 2004.

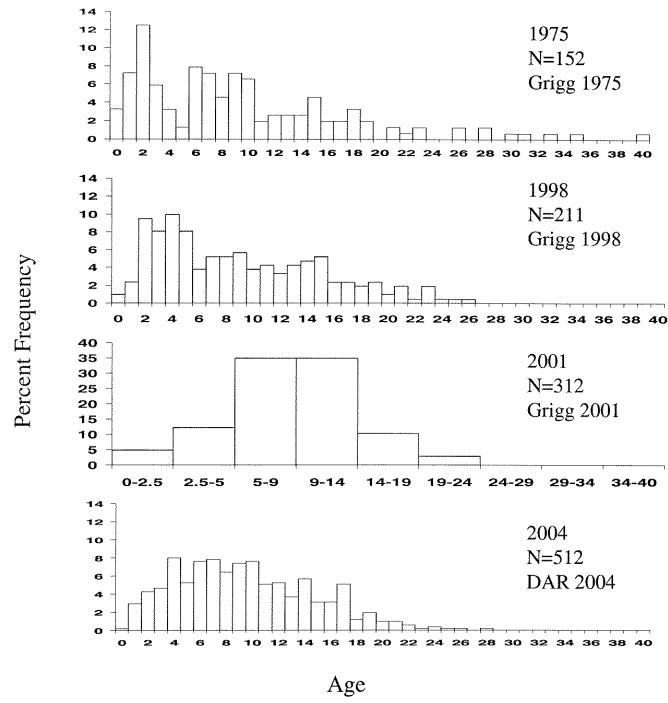


Figure 2. Age frequency distribution for 1975 (Grigg, 1976), 1998 (Grigg, 2001), 2001 (Grigg, 2004), and 2004 (current study)

### Regression of Age Frequency Distribution for All Ages

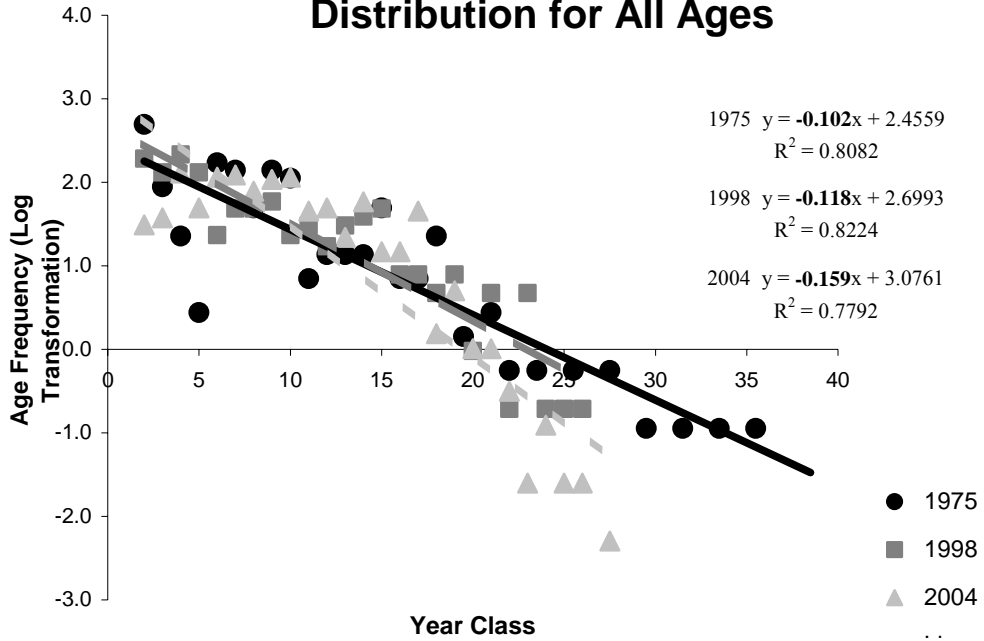


Figure 3. Regression analysis for all age classes.

### Regression of Post-Harvest Age Frequency Distribution

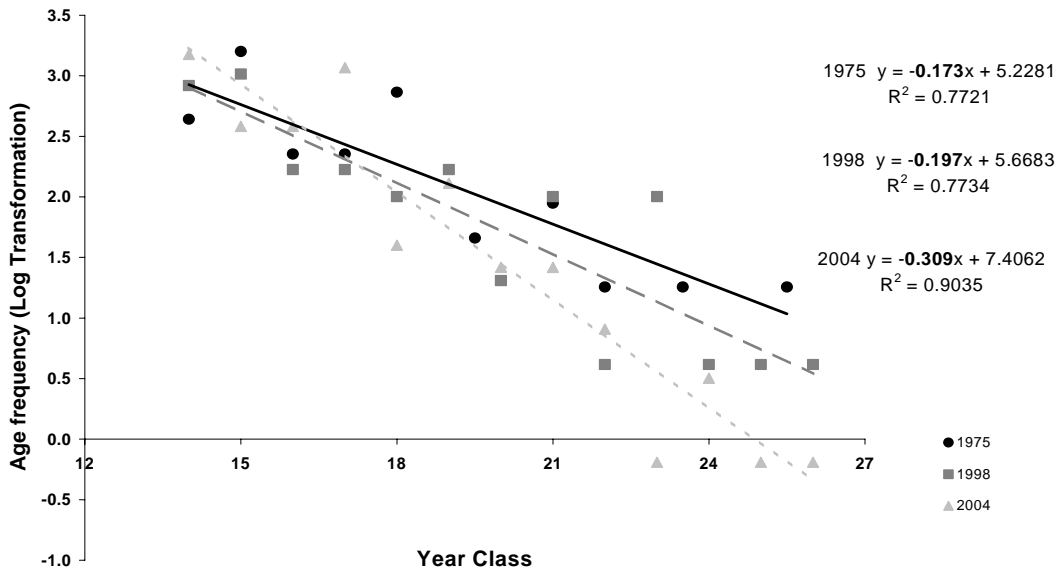


Figure 4. Regression of post harvest age classes.

## Regression of Pre-Harvest Age Frequency Distribution

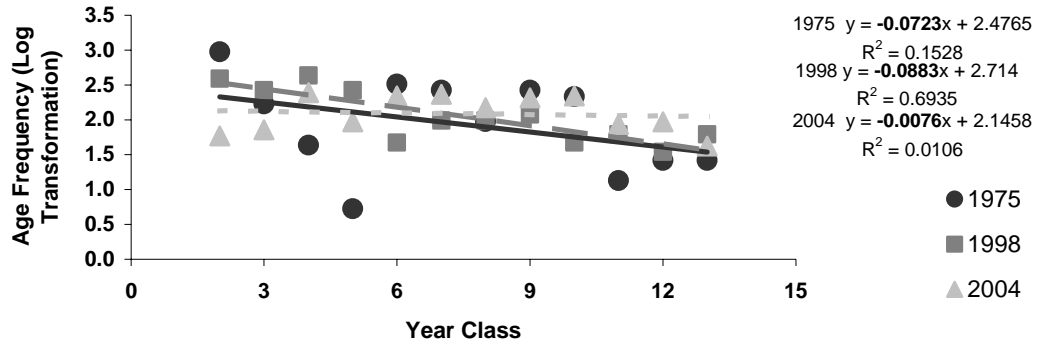


Figure 5. Regression analysis for pre-harvest age classes.

## Colonies Measured per Minute Dive Time

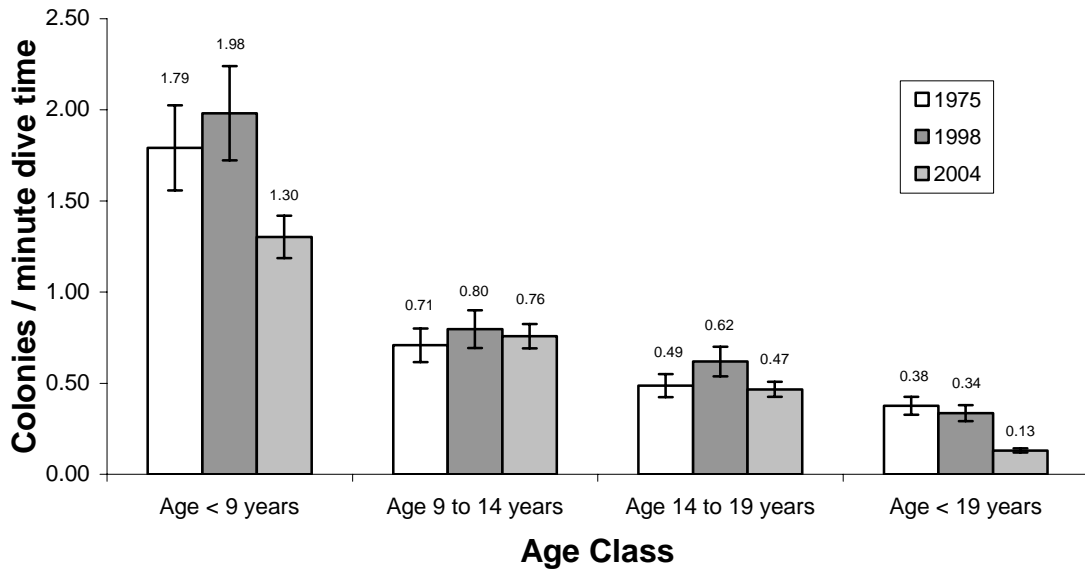


Figure 6. Colonies counted per minute of dive time for each age class.



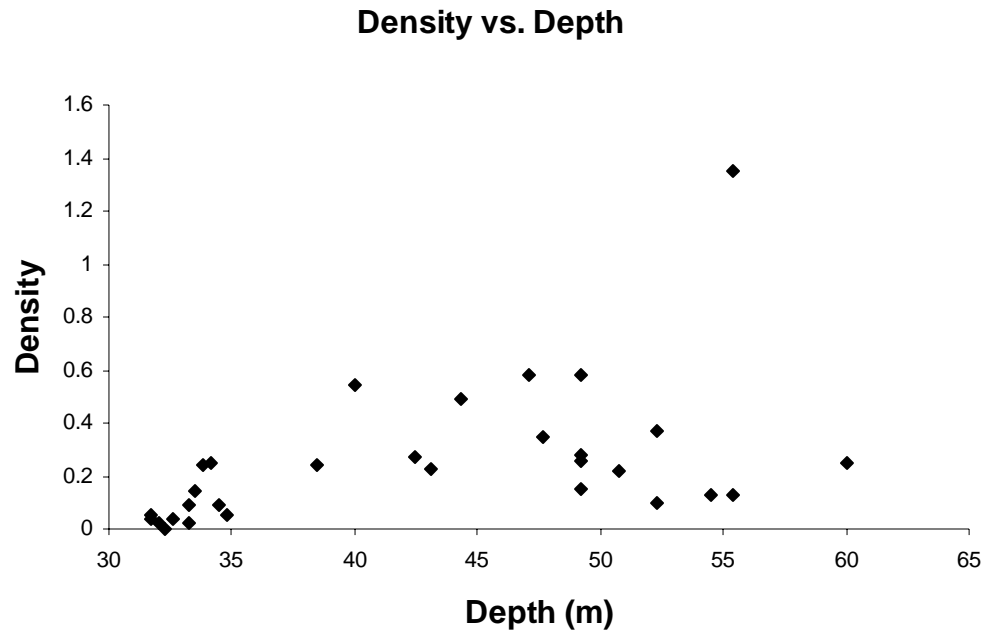


Figure 7. Density measurements conducted at each respective depth.

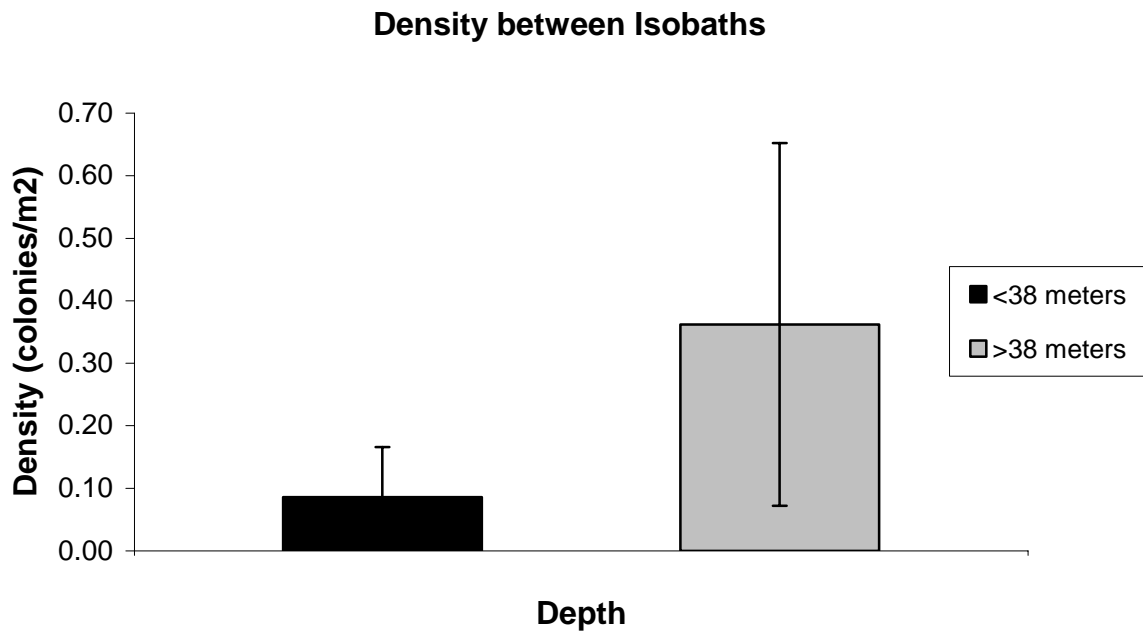


Figure 8. Density comparing 2 different isobaths. Less than 38 meters is on top of the Stone Wall ledge system and greater than 38 meters is the slope of the ledge.



**Table 1.** Summary table of genetic data for *Antipathes* cf. *curvata* (formerly named *Antipathes dichotoma*). Values in columns are the number of individual colonies for which 1) tissue samples were available; 2) DNA was extracted; and 3) sequenced for a given gene.

Location	Sample size	Extracted DNA	<i>trnW-nad2</i>	<i>nad5-nad1</i>	<i>cox1</i>	<i>rns</i>	<i>rnl</i>	ITS 1	ITS 2	$\beta$ -Actin	$\beta$ -Tubulin	Calmodulin
<b>Maui</b>												
Most Radicals	15	15	12	12	6	6	6	1	0	1	0	1
Stone Walls	43	43	19	19	6	11	12	3	0	0	0	0
Parrish Pinnacle	13	5	1	1	0	2	2	0	0	0	0	0
<b>Oahu</b>												
Artificial Reef	3	3	0	0	0	0	0	0	0	0	0	0
Kahala Barge	23	3	0	0	0	0	0	0	0	0	0	0
Maunalua Bay	6	6	0	0	0	0	0	0	0	0	0	0
Other	4	4	4	4	0	0	0	3	1	0	0	0
<b>Kona, Hawaii</b>												
South Point	30	30	13	13	0	3	3	2	1	0	0	0
Town	17	17	13	13	0	0	0	1	0	1	0	0
Other	3	3	2	3	0	0	0	1	0	0	0	0
<b>Kauai</b>												
Other	25	25	12	12	5	0	0	6	0	1	0	0
<b>TOTAL</b>	182	154	76	77	17	19	20	17	2	3	0	1

**Table 2.** Uncorrected genetic distances ( $p$ -distance) between black coral species. Values above and below the diagonal are for the *nad5-nad1* and *trnW-nad* intergenic regions, respectively. For *Antipathes* cf. *curvata* and *Antipathes grandis*, intraspecific comparisons are shown on the diagonal (no differences). Specimens from Hawaii were collected specifically for this study; remaining specimens collected elsewhere were included for comparative purposes. *Antipathes*, *Stichopathes* and *Cirripathes* belong to the family Antipathidae; *Elatopathes* – Aphanipathidae; *Tanacetipathes* – Myriopathidae; *Chrysopathes* – Cladopathidae; *Stauropathes* – Schizopathidae; *Leiopathes* – Leiopathidae.

	<i>A. cf. c</i>	<i>A.g.</i>	<i>A.c.</i>	<i>A.d.</i>	<i>S.sp.</i>	<i>C.a.</i>	<i>E.a.</i>	T.t	<i>C.f.</i>	<i>Sta.sp.</i>	<i>L.sp.</i>
<i>Antipathes</i> cf. <i>curvata</i> (Hawaii)	0/0	0.005	0.014	N/A	0.002	0.014	0.190	0.198	0.185	0.181	0.192
<i>Antipathes grandis</i> (Hawaii)	0.004	0/–	0.012	N/A	0.002	0.012	0.190	0.198	0.182	0.178	0.190
<i>Antipathes curvata</i> (Hong Kong)	0.008	0.008	–	N/A	0.012	0.000	0.191	0.199	0.185	0.181	0.192
<i>Antipathes dichotoma</i> (Mediterranean)	0.139	0.142	0.141	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>Stichopathes</i> sp. (Hawaii)	0.000	0.004	0.008	0.140	–	0.012	0.186	0.194	0.182	0.178	0.190
<i>Cirripathes anguina</i> (Hawaii)	0.008	0.008	0.004	0.141	0.008	–	0.191	0.199	0.185	0.181	0.192
<i>Elatopathes abientina</i> (Gulf of Mexico)	0.119	0.117	0.119	0.141	0.119	0.121	–	0.159	0.149	0.153	0.140
<i>Tanacetipathes tenacetum</i> (Gulf of Mexico)	0.136	0.134	0.140	0.171	0.134	0.147	0.140	–	0.159	0.169	0.147
<i>Chrysopathes formosa</i> (Fieberling Guyot)	0.105	0.109	0.111	0.132	0.107	0.115	0.112	0.110	–	0.057	0.090
<i>Stauropathes</i> sp. (North Atlantic)	0.120	0.123	0.119	0.156	0.119	0.123	0.130	0.136	0.065	–	0.077
<i>Leiopathes</i> sp. (North Atlantic)	0.139	0.146	0.147	0.201	0.145	0.150	0.099	0.116	0.118	0.098	–

**Table 3.** *Antipathes cf. curvata* - select nuclear genes successfully amplified but not yet sequenced.

Location	Sample size	Extracted DNA	$\beta$ -Actin	$\beta$ -Tubulin	Calmodulin
<b>Maui</b>					
Most Radicals	15	15	1	1	1
Stone Walls	43	43	2	0	0
Parrish Pinnacle	13	5	2	0	0
<b>Oahu</b>					
Artificial Reef	3	3	1	0	0
Kahala Barge	23	3	0	0	0
Maunalua Bay	6	6	1	0	0
Other	4	4	1	1	1
<b>Kona, Hawaii</b>					
South Point	30	30	1	0	0
Town	17	17	0	1	1
Other	3	3	0	0	0
<b>Kauai</b>					
Other	25	25	0	1	1
<b>TOTAL</b>	182	154	9	4	4

Table 4. Summary for growth study and comparison with Grigg, 1976.

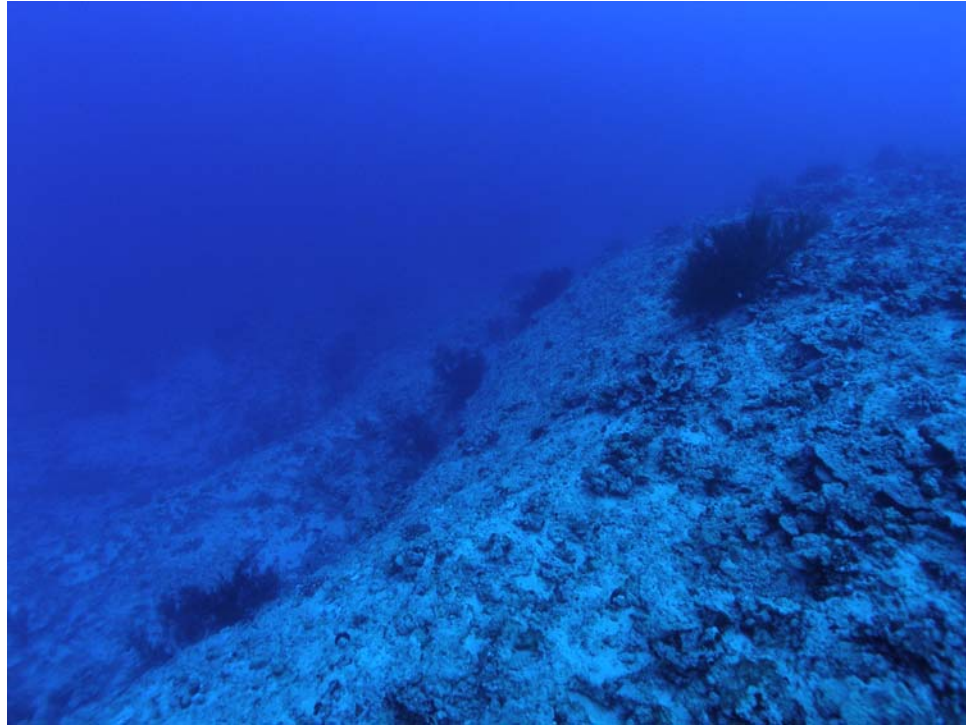
<b>Site</b>	<b>Oahu</b>	<b>Maui – Stone Walls</b>	<b>Maui - Pinnacle</b>	<b>Maui – Grigg 1976</b>
<b>N</b>	9	20	10	16
<b>Time monitored</b>	31 months	12 months	12 months	42 months
<b>Growth rate (cm/year)</b>	4.78 – 5.52	4.0 – 11.6	7.56	6.42
<b>Size range (cm)</b>	12-59	53-138	26-100	30-125
<b>Percent not showing growth</b>	11%	45%	0%	0% (not verified)



Black Coral habitat in the AuAu Channel, Maui



Black Coral habitat in the AuAu Channel, Maui



Black Coral habitat in the AuAu Channel, Maui



Black Coral habitat in the AuAu Channel, Maui

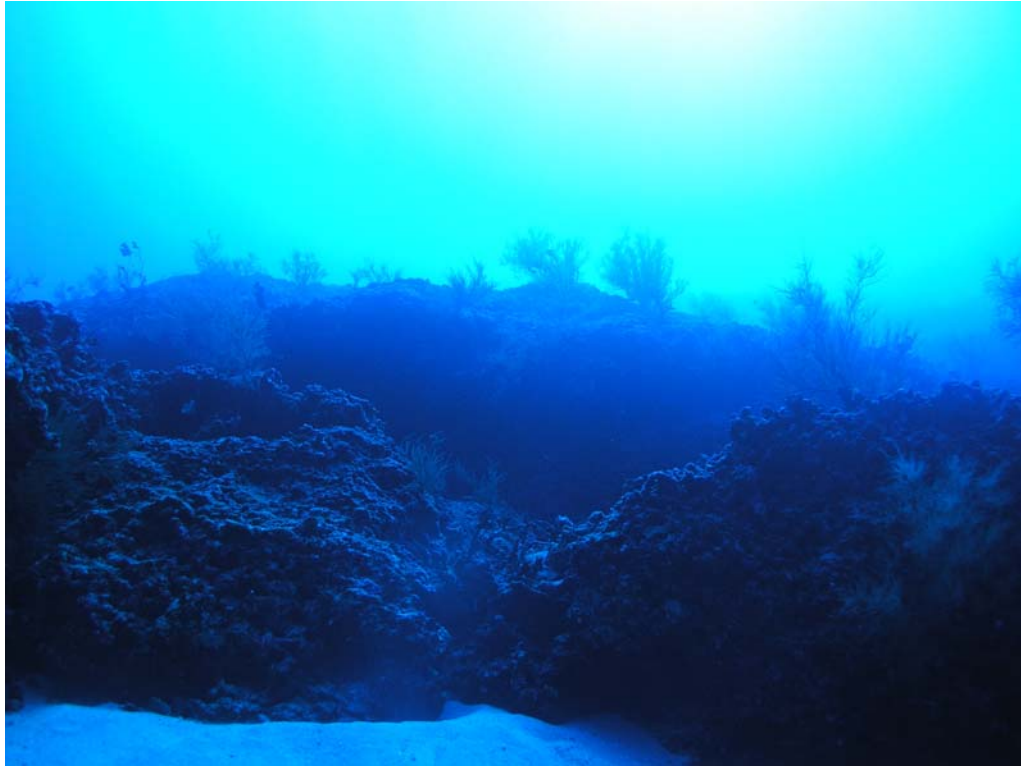




*Antipathes cf. curvata* in the AuAu Channel Maui



*Antipathes grandis* in the AuAu Channel Maui



Black coral distribution between shallow and deep ledges (top = 140', bottom = 180')



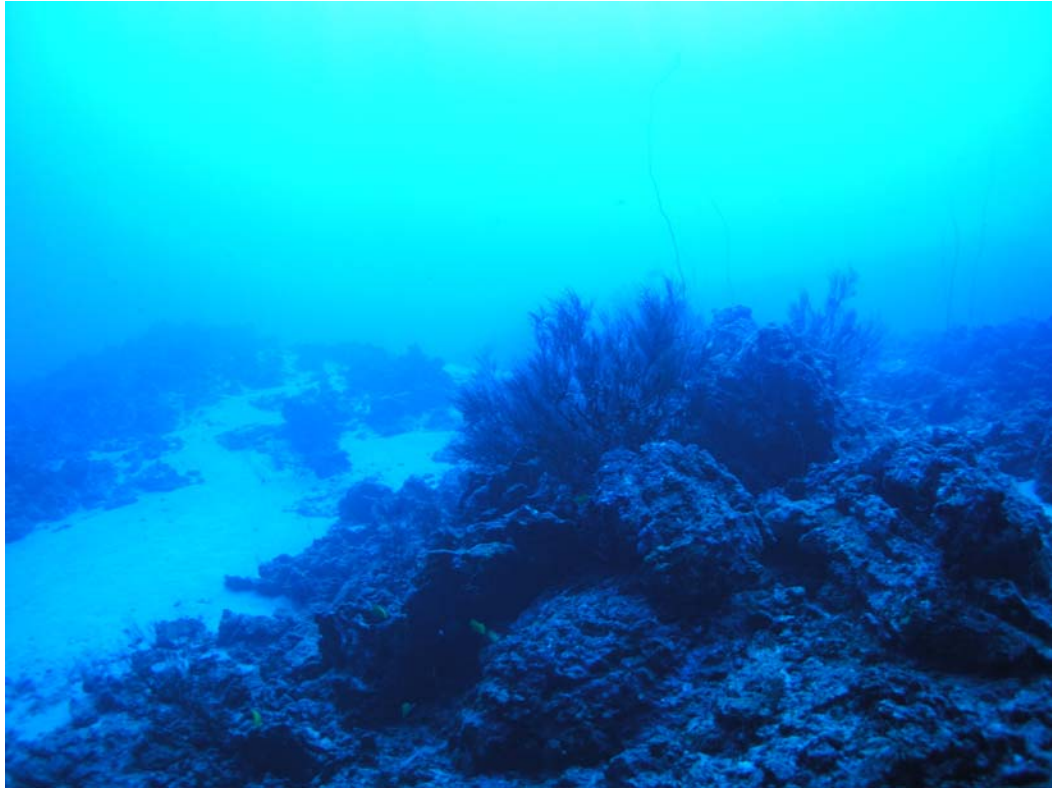
Tagged colony of *Antipathes* cf. *curvata*



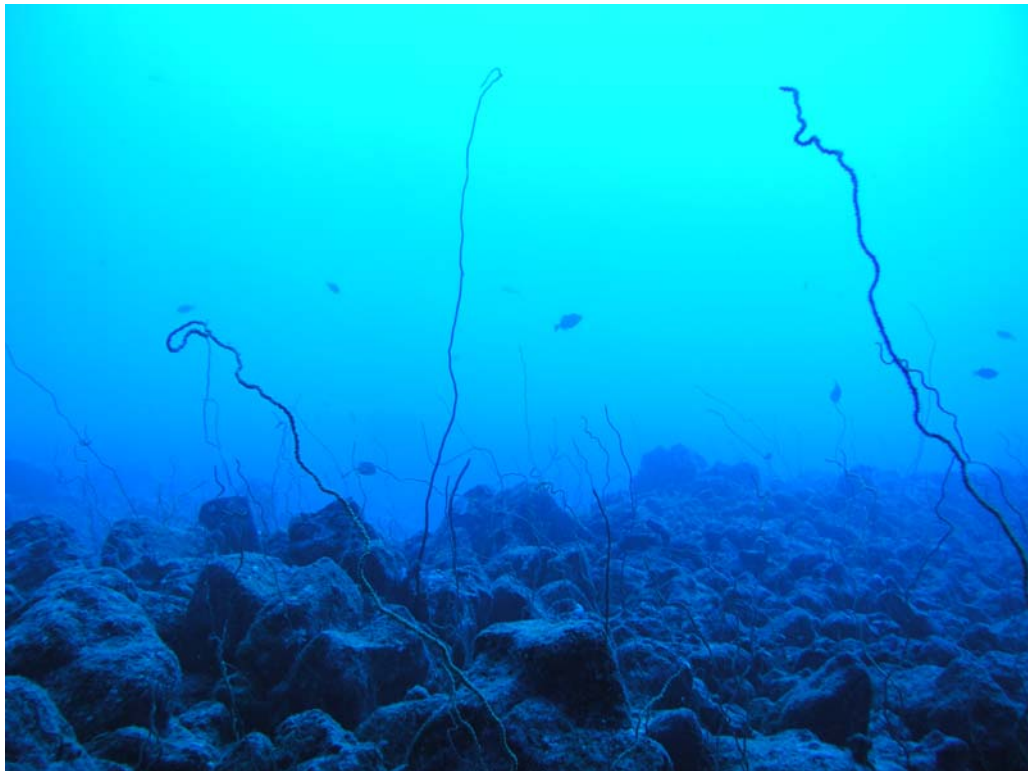
Tony Montgomery measuring *Antipathes* cf. *curvata*



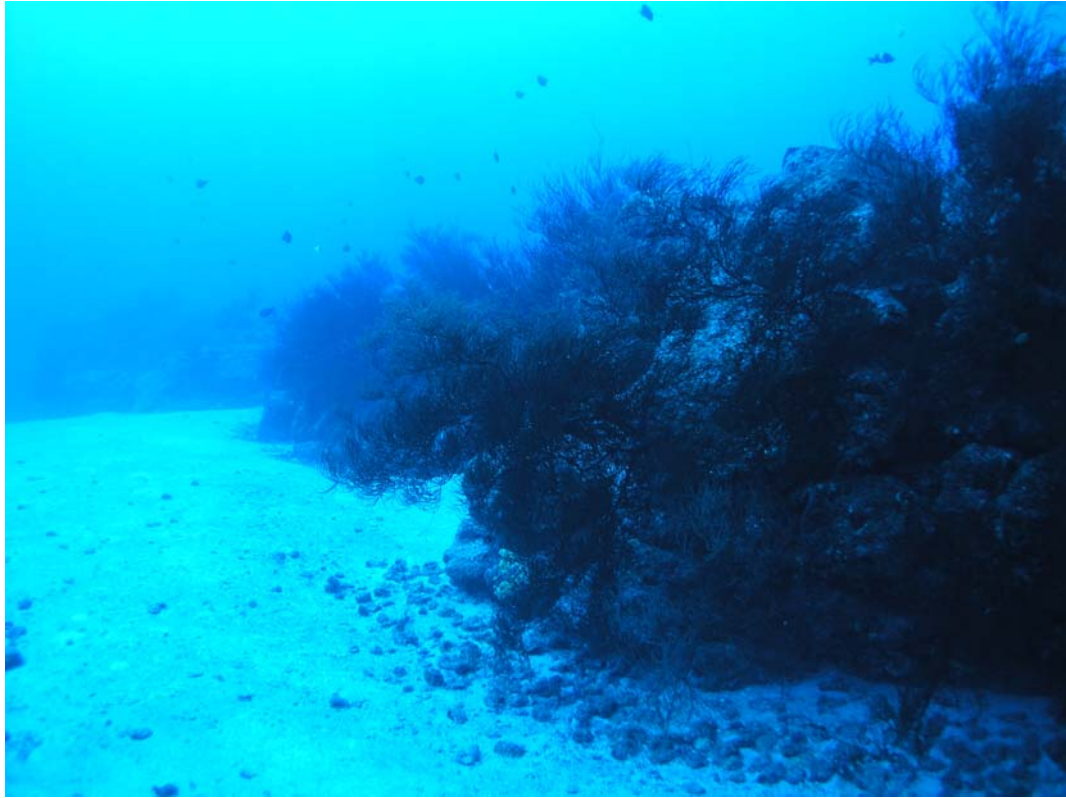
Andy Burnell counting black coral colonies along a transect



*Antipathes cf. curvata* at 210' in south Hawaii



Wire coral forest in Kona, Hawaii



*Antipathes cf. curvata* at 210' on south Kauai



Swarms of fish on black coral bed on southern Kauai



Technical scuba gear ready to be deployed off the NOAA ship, Oscar Elton Sette



Andy Burnell, DAR, and Ray Boland, NOAA, ready to conduct deep mix gas scuba dive



Jason Leonard collecting black coral genetic sample



Jason Leonard decompressing with safety diver Paul Murakawa in background



Tony Montgomery (left) decompressing with safety diver Paul Murakawa (right)



Tony Montgomery measuring largest wire coral seen (approximately 12' in length)





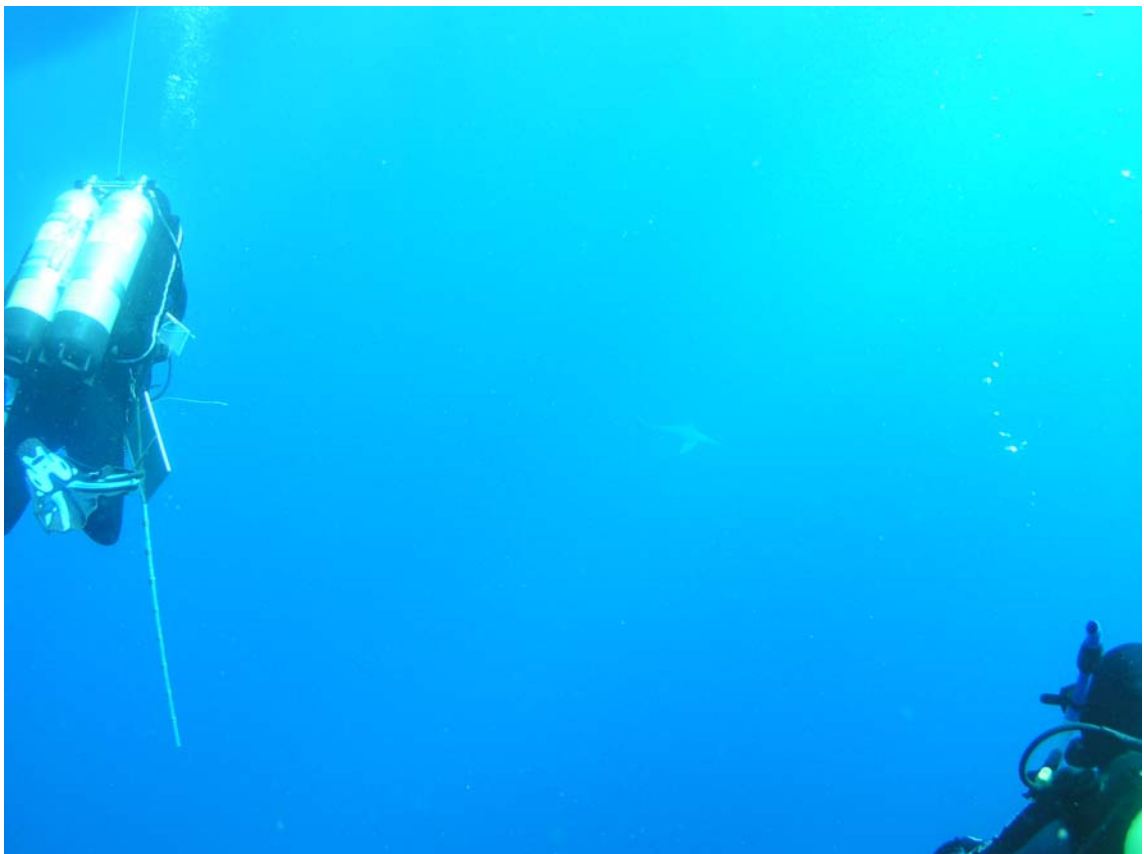
Deep air diver (150'), Paul Murakawa, collecting black coral genetic sample



Divers decompressing in Kona



Andy Burnell (left) decompressing with safety diver Shawn Fujimoto (right)



Divers decompressing with Gray Reef Sharks