Aquaculture of Marine Invertebrates for the Marine Ornamental Trade, Year 3

General Information

Reporting Period

October 1, 2004–September 30, 2005; no-cost extension through March 31, 2006 (final report)

Funding Level

Year	Amount
1	$55,000
2	$35,000
3	**$35,000**
TOTAL	$125,000

Participants

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Objectives

Overall Project Goal

Develop and transfer culture techniques for Hawaiian marine invertebrates to promote economic opportunities without dependence on wild-caught specimens.

Project Objectives

1. Validation of captive maturation and spawning at pilot scale.
2. Determination of settlement substrate for mass culture of feather-duster worms.
3. Field testing growout techniques of hatchery-produced feather-duster seed.
4. Production of a "How to" manual on the artificial propagation of feather-duster worms.
5. Technology transfer in the form of journal articles, newsletter articles, and workshops.

Principal Accomplishments

Objective 1: Validation of captive maturation and spawning at pilot scale.

Oceanic Institute (OI): Captive maturation trials have been ongoing at the Oceanic Institute for two consecutive years, combining both indoor (Year 2) and outdoor (Year 3) trials. Food sources investigated have been both the use of cultured phytoplankton and shrimp pond effluent. The shrimp pond effluent was found to be excessive when used directly and even when diluted. Current outdoor trials are testing the efficacy of two commercial phytoplankton diets, *Nannochloropsis oculata* and a Shellfish Diet, both from Reed Mariculture. The shellfish diet is a combination of four algaes. The results to date reveal that maturation of captive broodstock worms can occur when fed *Nannochloropsis oculata* for both the male and female sex. However, the stage of maturity in which spawning was induced in wild-caught worms has yet to be achieved under the culture conditions tested to date. Likewise, no natural spawning of captive broodstock has been achieved to date with both indoor and outdoor captive broodstock trials. Captive maturation and natural spawning remain challenges that require further investigation.

Hawaii Institute of Marine Biology (HIMB): For unknown reasons, spawning trials held during the initial period (October 2004–December 2004) of the Year 3 project did not result in successful spawnings, as reported in previous years. A closer examination of the appropriate stage of maturity for inducing spawning was decided as the corrective action.

Wild-caught worms in waters surrounding HIMB have been undergoing routine histological examination for the possession of sexually mature gametes for at least two consecutive years. A description of the various stages of sexual maturation for both sexes was conducted during the no-cost extension period. Examples of the various histological stages of maturation are summarized in Figure 1. The maturation stages are outlined below:

Female

Stage 1: No germ cells or coelomocytes are visible in the coelomic cavity (Sex cannot be differentiated).
Stage 2: Only coelomocytes can be seen free floating in the coelom (Sex cannot be differentiated).
Stage 3: Immature oocytes (io) and coelomocytes are visible in the coelomic cavity.
Stage 4: Mature oocytes pack the coelomic cavity.

Male

Stage 3: Characterized by the presence of coelomocytes and spermatids.
Stage 4: Differentiated by a coelom densely packed with mature spermatozoa.

During the previous reporting period the slides were examined and the data summarized in order to determine the extent of the natural spawning season of this worm in Hawaiian waters. During the no-cost extension period the changes in stages of maturity were also examined in relation to environmental changes (e.g., water temperature and day length). This information until now has yet to be determined. The data indicate that a small percent of the wild population can be found at the stage of maturation (Stage 4) where spawning can be induced between March through December. This observation means spawning can be induced over a much broader time period than previously thought (e.g., October through December). The average stage of maturation for both males and females was plotted against temporal changes in day length and average monthly water temperatures in Kaneohe Bay. Various regression analyses of the data was conducted in order to find a statistical model that would account for the wide variation in maturation that was observed. No correlation could be detected in the temporal changes in maturational stages versus day length. In contrast, one model was found that could explain a significant amount of the temporal variation observed in maturity of both sexes and water temperature. The model: $y=[(12.59(X) - 0.249\,(X^2)] - 156.33$, where y = mean maturation stage and X = mean water temperature significantly accounts for 63% of the variation observed in changes in average stages of maturation $R^2 = 0.63$; $P < 0.001$. As can be seen in Figure 2, the shape of the statistical model also indicates a broad peak in reproductive activity during the months that have average water temperatures between 25 °C–26 °C.

The histological data also revealed conclusively that the feather-duster worm is a hermaphrodite with sperm and eggs present in the same individual. Early indications are that the feather-duster worm is a sequential protandrous hermaphrodite starting out with a preponderance of males and changing into females as they grow in size (Figure 3). Confirmation, however, will require a much more intensive sampling scheme that covers worms of all sizes and is currently beyond the scope of the current project. Still, it should be pointed out that this finding is a new one for this species of worm.. Likewise, it is not known whether the hermaphrodite responds to the technique of inducing it to spawn and may have been a contributing factor to the poor responses experienced earlier in the project.

Spawning trials were attempted in June, July, and August of 2005, and successful induction of spawning was achieved during the months of June and July, although at a very low percent of success (1 of 4 trials). Spawning was achieved only when female worms where characterized as Stage 4 (e.g., possessed abundant oocytes that were freely floating in the coelomic cavity). Although spawning could be achieved, finding individuals of the appropriate stage of maturity was a challenge during this time of year. For example, in the June trials, only three females out of 60 worms were found to be at a suitable stage of maturation; in July, only three females out of 58 and, in August, out of 60 worms none were found to be at Stage 4. Using spawning data from all three years of the CTSA-supported project, the percent success in achieving spawning was significantly highest in the months of October, November, and December (Figure 4). It would appear that while there are individuals that are capable of spawning in the wild over a much broader time period than previous thought, the peak period for spawning activity clearly is October through December, a period that coincides with a drop in water temperature.

Objective 2: Determination of settlement substrate for mass culture of feather-duster worms.

Because of the poor spawning results during the earlier portion of the project, this objective had to be put on hold in 2004. The off season spawning trials, however, provided an opportunity to conduct preliminary settlement trials, one of which was initiated in July 2005. The larvae that resulted from the one successful spawning were stocked into a 200-L fiberglass tank that was equipped with a single airstone for aeration and plastic mesh netting as well as filled with filtered seawater in a closed system. Feeding of larvae occurred on the seventh day after hatching or during the time period when settling is known to be taking place. Larvae were fed live *Chaetoceros* sp. once a week. A very encouraging result of this trial has only recently been obtained and is summarized in Figure 5. Settled worms are clearly visible on the bottom of the tank, along the sides of the tank, and among the plastic mesh netting. However, a clear preference for settlement was observed for the three inch airstone, as indicated in the photograph (Figure 5). The density of settled worms on the various substrata is also summarized in Table 1.

While there is a larger number of settled worms on the plastic mesh netting when expressed in terms of area (e.g., ft^2) than the number on the sides or bottom of the rearing tank, the observed density pales in comparison to what is observed on the airstone. The number of settled worms extrapolates to more than 1,800 individuals, when calculated on a per square foot basis. It should be pointed out that air was continuously being pumped through the airstone and the larval feather-duster worms would have to actively swim and settle on the stone to remain at the density observed. This result has been confirmed during the no-cost extension period with an additional replicated trial with similar results. Clearly, the airstones, with or without aeration, provide a desired substrate for settlement by these worms.

Objective 3: Field testing growout techniques of hatchery produced feather-duster seed.

While additional settlement experiments still need to be carried out, other items that are important in the growout of cultured worms can be inferred from investigations on wild worms. For example, the distribution and abundance of feather-duster worms in a protected marine environment like HIMB (Figure 6) should provide some indication of what the growout requirements might be for this species.

From the information obtained from this activity, it is clear that the south edge of Coconut Island is a preferred habitat of the feather-duster worm. Water quality parameters and total suspended solids (TSS) were found to be similar with other sites. Water motion and occurrence of coral substrate, however, was found to be significantly higher at this particular location. The increased water motion is consistent with a higher density of worms as food in the water column has a higher probability of reaching the worms. The 100 ppm TSS value detected around the waters of HIMB may also serve as an indicator of the level of food required to support feather-duster worms in the wild. It also provides a baseline for determining the optimal amount for feeding worms under cultured conditions.

The kinds of substrate that feather-duster worms inhabited were also investigated to provide insight into the settling substrate and possible locations of placing a growout farm; this work is summarized in Figure 7. Clearly, the association of the worms with live coral is one factor that needs to be taken into consideration to optimize successful growout of the worms. It is thought that the coral provides protection from predation, resulting in a higher abundance. It is interesting to note that the feather-duster worm also settles on floating dock structures, indicating that larvae freely swim and can utilize a floating platform for growout. Thus, a growout facility could utilize more than a single dimension in its design. The data presented has been summarized in a manuscript and was submitted to the peer-reviewed journal *Pacific Science* for publication during the no-cost extension period.

Feeding trials using *Chaetoceros* sp. have been repeated at HIMB with this trial, including sufficient replication to insure acceptance in a peer-reviewed journal. A summary of the results obtained is provided in Figure 8. The data are consistent with the previous trial conducted during the Year 1 project: when worms are provided with a live food at levels above that found in the wild, they will grow at a significantly ($P < 0.01$) elevated rate and achieve market size within one year. A manuscript summarizing the results obtained under this objective is in preparation for submission to a peer-reviewed journal.

Objective 4: Production of a "How to" manual on the artificial propagation of feather-duster worms.

This objective has been put on hold, as not all of the information required to produce a quality manual has yet to be obtained. However, during the no-cost extension period, an AquTips article summarizing the highlights of the information to date was prepared and printed in the March 2006 issue of CTSA's *Regional Notes*.

Objective 5: Technology transfer in the form of journal articles, newsletter articles, and workshops:

Although no workshops have been held to date, the other forms of disseminating the technology developed during the project period included two presentations and four published articles (see the Publications section of this report). Four more manuscripts are either submitted to a journal or in preparation (see below):

Bybee, D. R., J. H. Bailey-Brock, and C. S. Tamaru**.** Submitted. Distribution of the fan worm *Sabellastarte spectabilis* around Moku O Lo'e (Coconut Island) in Kane'ohe Bay, (O'ahu, Hawai'i*). Pacific Science*.

———. In Prep. Gametogenesis and spawning periodicity in the fan worm *Sabellastarte spectabilis* (Grube 1878).

———. In Prep. Settlement and substrate preferences of the fan worm *Sabellastarte spectabilis* in controlled conditions.

———**.** In Prep. Molecular and morphological evidence of relationships among the *Sabellastarte* (Polychaeta: Annelida).

Impacts

Advancements in the artificial propagation of marine species have led to the belief that culturing marine ornamental organisms can alleviate some of the fishing pressure on wild stocks as well as create small- or large-scale industries. Commercially cultured feather-duster worms would provide an alternative source of animals for the aquarium trade and would also ease the burden on coral reefs caused by current collecting practices. High demand suggests that this type of aquaculture also has the potential to provide substantial economic benefits to commercial farmers

(DLNR catch data 1986–1994). Realization of this potential, however, will hinge upon the successful development of culture technologies that are cost effective enough to overcome the economic constraints of doing business in Hawaii. Progress made over the course of the projects has also resulted in an increased knowledge base in the reproduction and ecology of the species. While there are no economic impacts to date, because of the paucity of information on the biology of the species and for polychaete worms in general, the spawning technique, larval development, distribution and abundance have broadened the base of scientific information.

Recommended Follow-up Activities

- Additional funding is being solicited to complete the work started. Currently, an RFP has been submitted to CTSA for review.
- Dave Bybee is currently summarizing the remaining data and preparing for the completion of his dissertation in partial fulfillment of his Ph.D. from the Department of Zoology, University of Hawaii at Manoa.

Publications in Print, Manuscripts, and Papers Presented

Bybee, D. R. 2005. Spawning periodicity and gametogenesis in the fan worm *Sabellastarte spectabilis*. Abstract in *Pacific Science* 55(1):100.

Bybee, D. R. 2005. Vermiculture of feather-duster worms in Hawaii. Presentation made at the Hawaii Aquaculture Association Annual Conference, May 26, Windward Community College in Kanehoe, Hawaii.

Bybee, D. R., J. H. Bailey-Brock, and C. S. Tamaru. 2004. Reproduction and larval development of *Sabellastarte spectabilis* (Grube 1878) (*Polychaeta:Sabellidae*) in Hawaiian waters. In *Proceedings of the 8th International Polychaete Conference*, July 5–9, in Madrid, Spain.

———. Forthcoming. Larval Development of Sabellastarte spectabilis in Hawaiian waters. *Scientia Marina*.

———. Forthcoming. Evidence for sequential hermaphroditism in the fan worm *Sabellastarte spectabilis*. *Pacific Science*.

Tamaru, C. S., D. Bybee, J. Bailey-Brock, D. Ziemann, and T. Ogawa. 2006. AquaTips: Developing techniques for the artificial propagation of the feather-duster worm (*Sabellastarte spectabilis*) in Hawaii. Center for Tropical and Subtropical Aquaculture *Regional Notes* 17(1):4–6.

Appendices

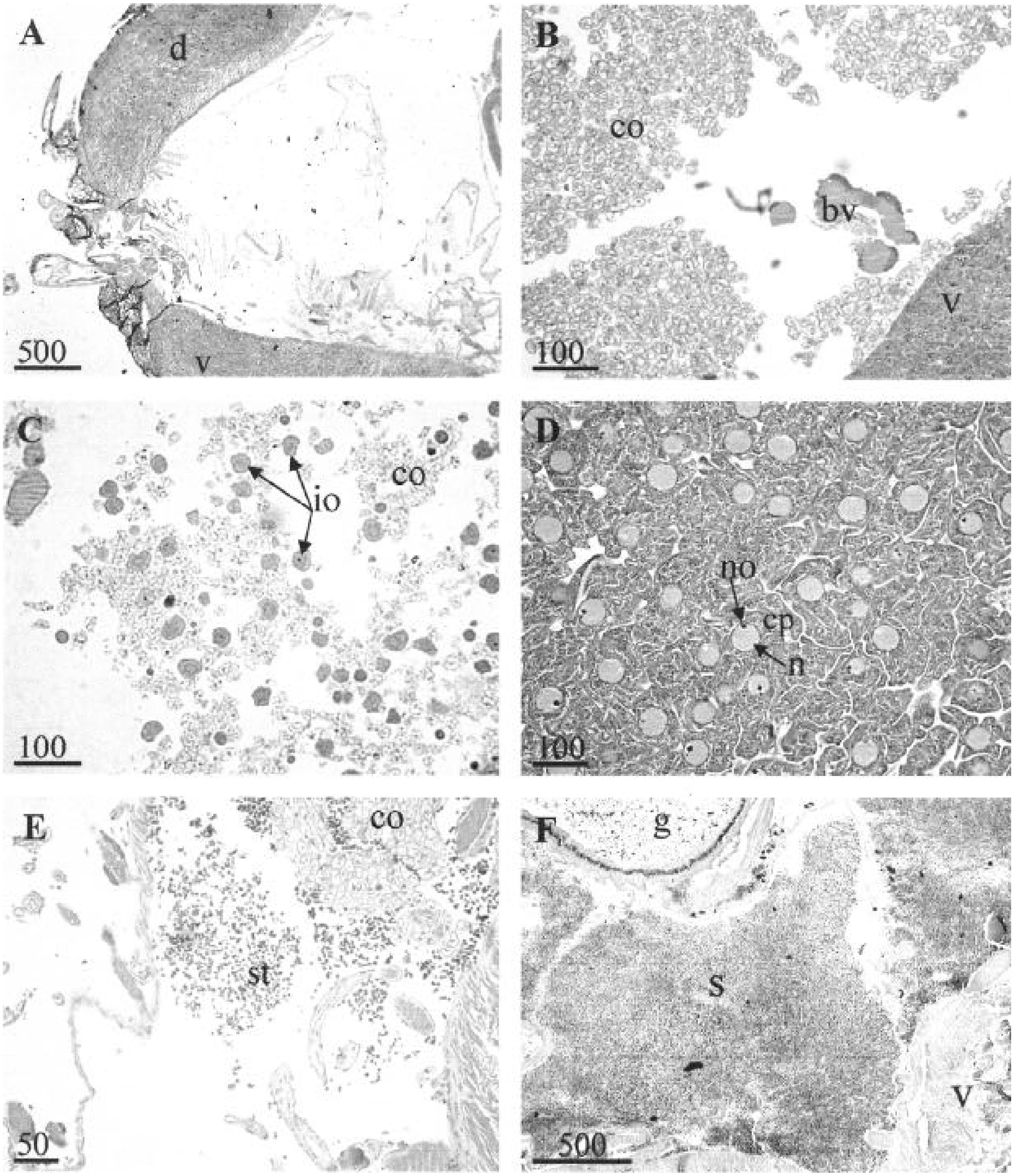


FIGURE 1. (A) Stage 1 in which no germ cells or coelomocytes are visible in the coelom, d = dorsal epidermis and v = ventral epidermis. (B) Stage 2 in which only coelomocytes (co) can be seen freely floating in the coelom, bv = blood vessel. (C) Female Stage 3 in which immature oocytes (io) and coelomocytes are visible. (D) Female Stage 4 in which the coelom is full of mature oocytes, n = nucleus, no = nucleolus, cp = cytoplasm. (E) Male Stage 3 is characterized by the presence of coelomocytes and spermatids (st). (F) Male Stage 4 is differentiated by a coelom densely packed with mature spermatozoa (s), g = gut.

Substrate	Density (individuals/ft ^{2})
Side of Tank	0.9
Bottom of Tank	1.1
Plastic Mesh Netting	8.0
Airstone	1,881.0

TABLE 1. Summary of density of settled feather-duster worms on various substrates.

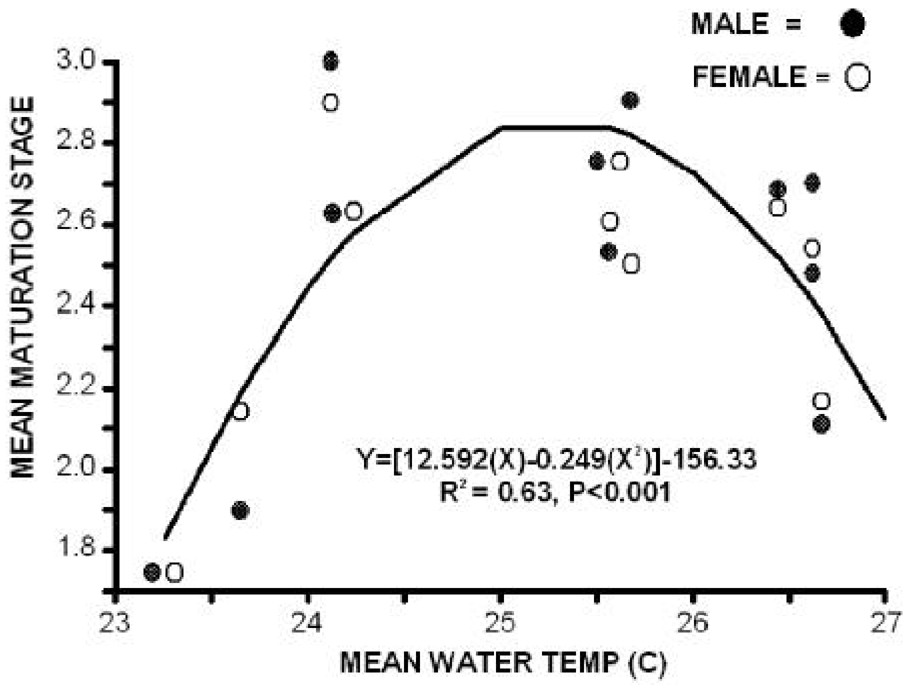


FIGURE 2. Correlation of mean maturation stages for male and female feather-duster worms against average monthly water temperatures.

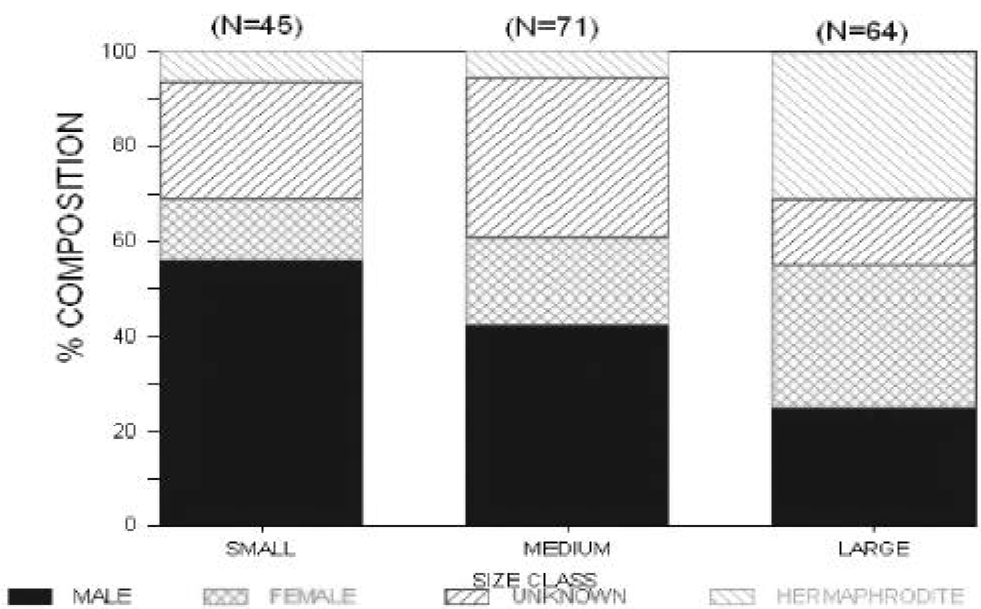
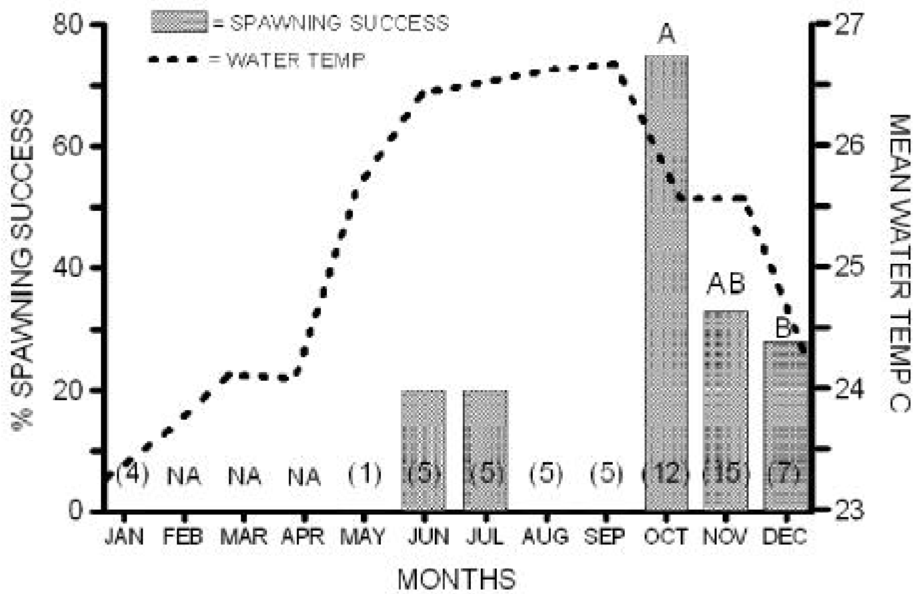


FIGURE 3. Change in percent composition of sexes in relation to size class of the feather-duster worms. Numbers in parenthesis indicate number of individuals examined. (small = 6–8 mm, medium = 9–10 mm and large 11–13 mm).

FIGURE 4. Summary of percent spawning success obtained during various months of the calendar year. Values in parenthesis represent spawning attempts, and bars that do not share the same alphabet are significantly (P < 0.05) different from each other. (NA = No Attempts due to lack of mature individuals)



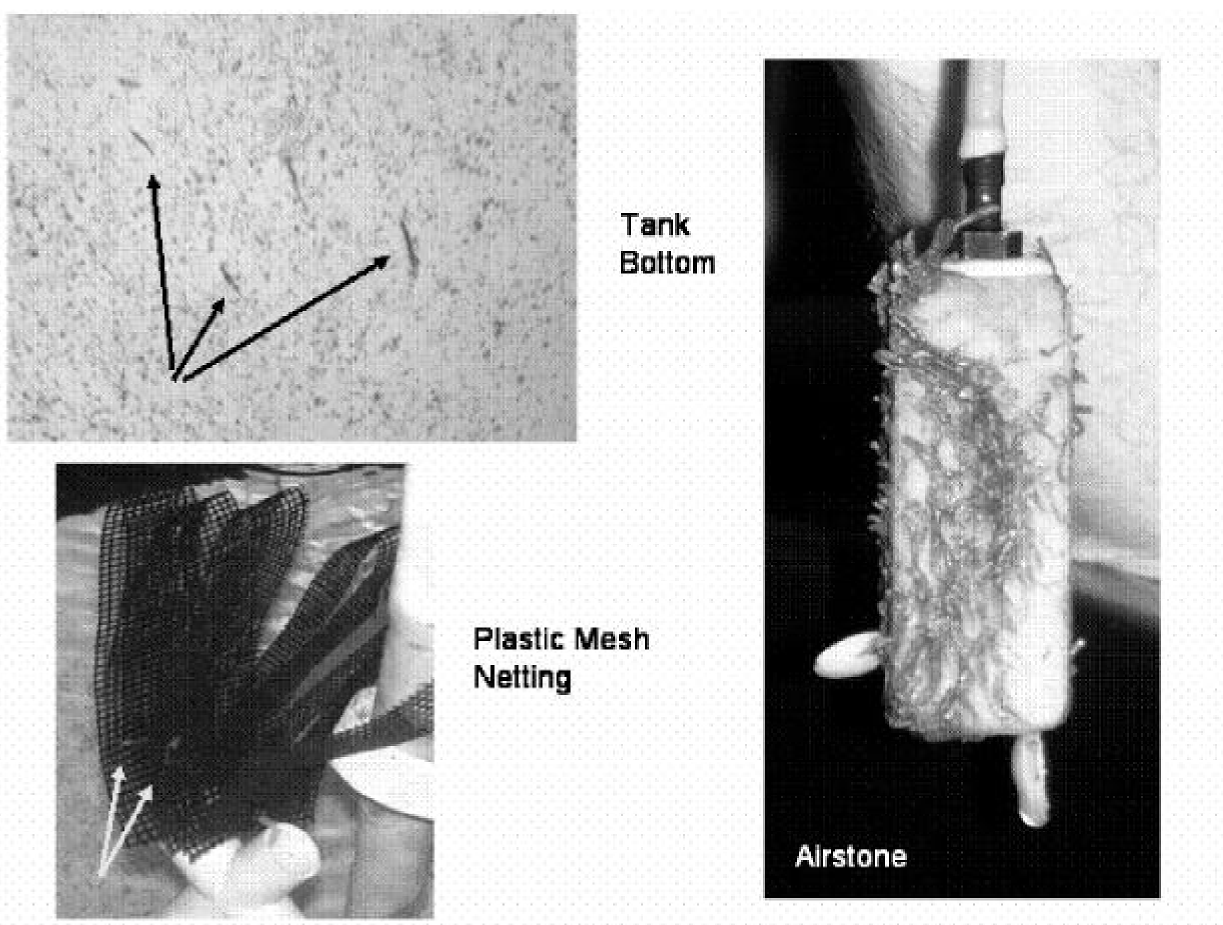


FIGURE 5. Photographs of settled worms (arrows) on various objects in a fiberglass larval rearing tank.

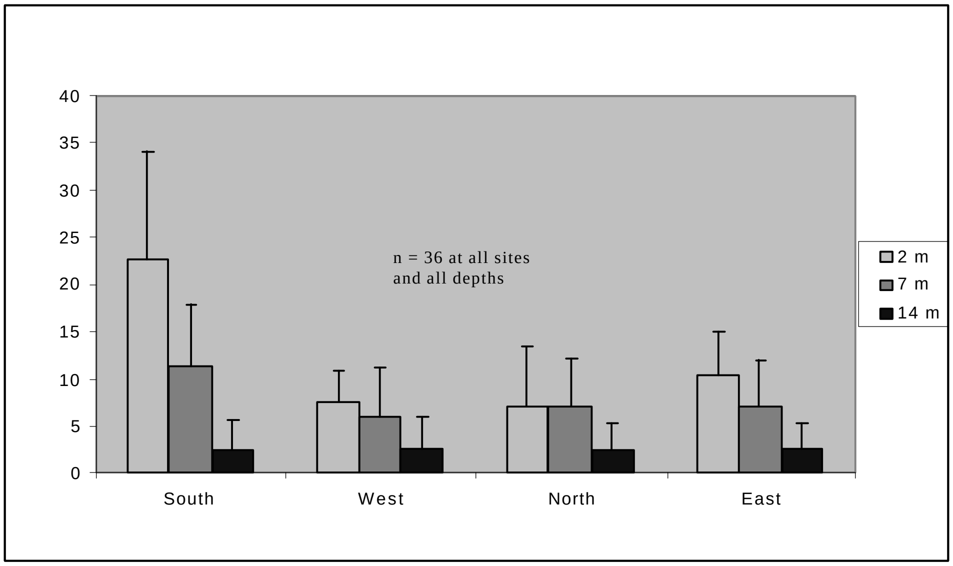


FIGURE 6. At three different depths in the surrounding waters of Moku o lo'e, feather-duster worms or fan worms have different vertical distribution and abundance. The y-axis is a measure of mean individuals per m² (n = number of quadrats) and the x-axis represents site location. Lines above bars are standard deviations.

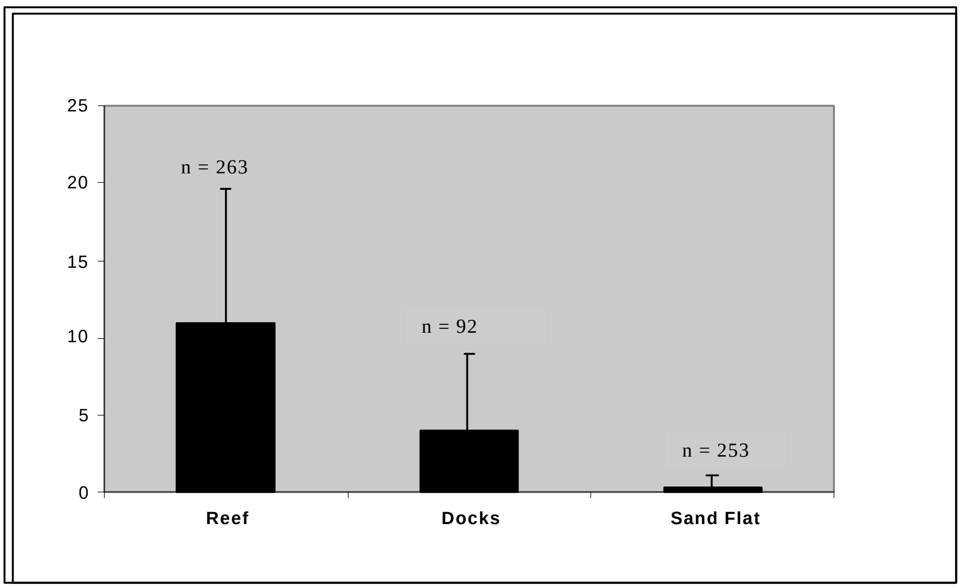


FIGURE 7. Summary of abundance on various substrates located at the Moku O Lo'e Island. The y-axis is a measure of mean individuals per m² (n = number of quadrates) and the x-axis represents substrate type. Lines above bars are standard deviations. All values were significantly different (P < 0.001) from each other.

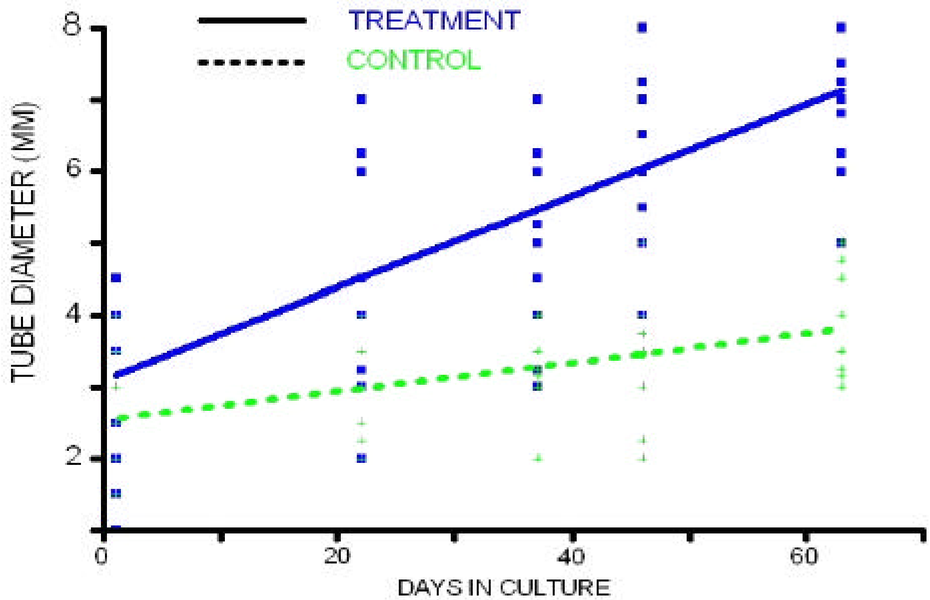


FIGURE 8. Comparison of the growth between feather-duster worms provided a live phytoplankton (e.g., Chaetoceros *sp.) and those receiving seawater from Kaneohe Bay.*