CLOSING THE CYCLE: CAPTIVE BREEDING FOR THE GASTROPOD *STROMBUS*

By

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ABSTRACT

The large gastropod queen conch, *Strombus gigas,* is a valuable food source throughout the Caribbean and the Florida region. The markets for *S. gigas* are adult meat for chowders and fritters, and shells for decoration. Excessive over-fishing of *S. gigas* has led to its listing on Appendix II of CITES and mandated a statewide fishing moratorium in the Florida waters. To help preserve the species, biologists are successfully culturing queen conch from egg stage to market. However, the cycle is not closed. Egg masses are collected from reproductively active adult populations in the field. This means that the aquaculturist is heavily reliant upon spawning aggregations for continued culturing success of this threatened species.

In response to the need for specimens for aquaculture, we began a captive breeding program for *Strombus* species in June 2000 at Harbor Branch Oceanographic Institution, Ft. Pierce, Florida. A breeding arena was built in a large round tank (4.5 m dia x 0.9 m height). The tank was divided into 4 equivalent sections (4.1m^2) and filled with water to 0.42 m above the substrate. This recirculating tank system is equipped with an under-gravel filter system, comprised of coarse Bahamian aragonite sand (1-3 mm) at a depth of 10 cm. The tank was located in a greenhouse structure, allowing for natural photoperiod during our experiment. The mean water temperature was 27 °C and salinity 34 ppt.

There are seven species of *Strombus* in the Caribbean and Florida region. We chose 3 non-restricted species (*Strombus costatus*, milk conch; *Strombus raninus*, hawkwing; and *Strombus alatus*; Florida fighting conch) as well as *S. gigas* to begin our breeding program. These smaller, less threatened *Strombus* may also prove to be a premium food item and a successful aquarium animal. A total of 24 adult conch were collected: five *S. costatus* (3 female, 2 male), seven *S. raninus* (5 females, 2 males), eight *S. alatus* (4 females, 4 males), and four *S. gigas* (1 female, 3 males) from the Florida Keys and placed into their respective quadrants on June 16 and July 16 (for *S. gigas*).

The number of copulating pairs and spawning females were noted on a daily basis for the first 49 days (7 weeks). Collected egg masses were measured for size, number of eggs, and egg capsule and strand diameter. In 36 weeks we collected 401 egg masses. *S. raninus* began breeding on day 2 and continued to breed persistently through early November. *S. raninus* laid 336 egg masses. We also had success with *S. alatus*, 44 egg masses; *S. costatus* laid a total of 19 egg masses, and we were able to get *S. gigas* to lay two egg masses in mid-February. The viability of the eggs was confirmed as we successfully hatched several egg masses from all four species and raised them through juvenile stage. Based on breeding success of these species we hope to establish the commercial ability of a captive breeding program, and establish alternative aquarium and juvenile queen conch market.

ACKNOWLEDGEMENTS

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CHAPTER 1

HISTORY OF THE CONCH FISHERY

STATUS

For years conch belonging to the Family Strombidae have been harvested throughout the Caribbean region and used as a food source, for building material, and for decoration. There are seven *Strombus* species in the Caribbean and Florida region: *Strombus gigas*, *S. costatus*, *S. raninus*, *S. alatus*, *S. pugilis*, *S. gallus*, and *S. goliath* (Abbott 1974). All species can be found in the Caribbean Sea and the Western Atlantic Ocean, from Florida to South America to Central America, and some species are found in Bermuda (Figure 1).

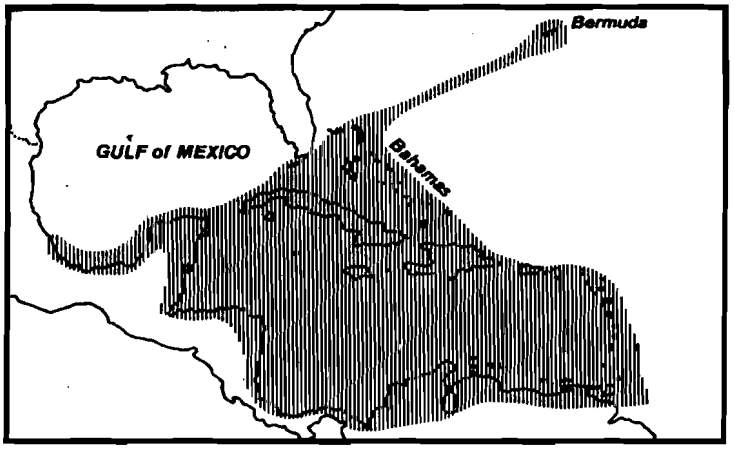

Figure 1. Geographic distribution of the queen conch, *Strombus gigas*. Also the approximate range of the other six Strombidae species. Source: Brownell and Stevely 1981.

Of these seven species, *S. gigas*, the queen conch, holds the highest commercial value as a subsistence and commercial fisheries product (Brownell 1777, Berg 1976). Nearly 4000 metric tons (mt) of meat are landed yearly from the Caribbean, making the queen conch market second only to the market for the spiny lobster, *Panuliris argus* (Appledoorn 1994).

Historically, the queen conch fishery was localized and subsistence related (Appeldoorn 1994). Beginning in the 1970's, the *S. gigas* fishery became impacted by overfishing, primarily due to the rapidly expanding tourism industry and the increase in the human population (Table 1). This overexploitation has led to the enactment of several regulations (Appeldoorn 1994). A summary of regulations from selected countries can be seen in Table 2. In Florida, a statewide moratorium, which banned all types of queen conch harvesting, began in 1985 (Stewert 1999). In 1992, *S. gigas* was added to Appendix II of the Convention for the International Trade of Endangered Species (CITES) legal mandate. Now countries that export queen conch, *S. gigas*, need permit approval from CITES management to ensure that the species is harvested at a level consistent with its fisheries population. Many Caribbean nations have implemented closed seasons (during the conch's breeding season). Likewise, the use of SCUBA has also been prohibited in several countries so that the conch have a deep water refuge to migrate to during the winter months.

Yet, management strategies alone cannot keep up with the fishing pressure on *S. gigas*. Larval rearing is now a promising means of raising juvenile queen conch for the market and for restocking efforts (Davis pers. comm. 2000). Scientists have been perfecting culture techniques for the queen conch from the larval stage to juvenile stage since the mid-1970's (Davis 1994, Creswell 1994). The juveniles can be raised for stock enhancement, rehabilitation, or more recently, for captive breeding programs. The larval cycle of the queen conch was first described over four decades ago (D'Asaro 1965), as were the breeding habits and the egg cases (Robertson 1959, Randall 1964, D'Asaro 1965).

Table 1. Approximate landings of queen conch, *Srombus gigas*, in metric tons (mt) by area (Appeldoorn, 1994).

Country	*Landings*	*Comments and Stock Status*
Venezuela	240-360 mt (1998)	From Los Roques, all illegal; Overfished
Columbia	400 mt (1988)	Down from a peak of 800 mt; Some areas probably overfished
Belize	155 mt (1990)	Overfished
Mexico	25 mt (1989)	Down from 350 mt in 1973; Overfished
Jamaica	800 mt (1990)	From Pedro Bank; Present harvest rate probably not sustainable
Cuba	1,500 mt (1990)	Almost all catch for bait; Stable, probably fully exploited but not utilized well
Turks & Caicos	431 mt (1989-90)	Data from exports only Stable; possibly somewhat overfished
Bahamas	410 mt (1991)	Overfished only in localized areas
Puerto Rico	73 mt (1989)	Down from 340 mt in 1983 Overfished
U.S. Virgin Islands	15 mt (1990-91)	From St. Croix Overfished overall; stable on St. Croix
Dominica	5 mt (1990)	Overfished
Martinique	20-30 mt (1990)	Overfished inshore; conch are plentiful in deepwater
St. Lucia	3-4 mt (1990)	Not fully exploited
Grenada	25 mt (1990)	Growth overfished

Table 2. Regulations for and management of the queen conch, *Strombus gigas*, fishery in select countries throughout the Caribbean Region (Appeldoorn, 1994).

Country	*Regulations*	*Comments*
Venezuela	1985 Fishery closed in Los Roques National Park 1989 Seasonal closure elsewhere (Mar.-Sept.) 1991 3-year closure elsewhere	Initial closure not enforced by Ministry of Fisheries Little enforcement, and fishermen do not comply; effect has been to increase direct sales to Bonaire and Martinique
Columbia	Closed season (July-Sept.) Scuba prohibited Minimum size limit (225g meat; 100g cleaned meat) Fishery closed at Quitosueno Bank	Only enforcement of closed season has been effective Poaching from other countries is a problem during closed season
Belize	Closed season (July-Sept.) Scuba prohibited Minimum size limit (125mm SL, 28g meat)	Little enforcement and fishermen do not comply; conch are exported directly to nearby countries during closed season
Mexico Yucatan Quintana Roo	Fishery closed in 1988 1985 2.5-month closed season 1990 6-month closed season (Mar.-Sept.) Annual quotas North-23 mt; Central-4 mt; South-50 mt	There is some enforcement of and compliance with regulations. Effect has been to reduce effort, but some illegal fishing continues.
Jamaica	No regulations	Industry is interested in establishing
Cuba	Fishery closed 1978-1982 Annual quota/area since 1983 (55 mt in 1991) Harvest of juveniles prohibited Closed season (April-Sept.) No recreational fishing	Good enforcement and compliance in commercial fishery, but regulations only apply to the non-bait fishery. The bait fishery is thought to take 1,500 mt/yr, and this has prevented management from being effective.
Florida	Fishery closed since 1985	Good enforcement and compliance, but stock recovery slow.

Table 2. Continued

Country	*Regulations*	*Comments*
Bermuda	Fishery closed since 1978	Little stock recovery.
Turks & Caicos	Scuba prohibited Minimum size limits (125mm SL, 227g meat)	Little enforcement or compliance with regulations, but at present fishery is stable, so effects are not obvious.
Bahamas	Harvest of juveniles prohibited 1986 No commercial export of meat Fishery closed in Exhuma Land & Sea Park	Some enforcement at sea and at points of sale. Regulations generally adhered to.
Puerto Rico	No regulations	Regulations are being considered by the government.
U.S. Virgin Islands St. Thomas-St. John St. Croix	Fishery closed in 1988 1988 Closed season (July-Sept.) Minimum size limits (230mm SL) Sale of undersized shells prohibited Recreational catch limit: 6/per/day	Some enforcement of and general compliance with regulations.
Organization of Eastern Caribbean States	Minimum size limits (180mm SL, 225g meat) Option for closed season Scuba prohibited	Enforcement and compliance variable from country to country, being better where the stocks are not stressed. Unmonitored direct export to Martinique is a problem
Martinique	Scuba prohibited	Good enforcement of and compliance with regulations.

The four species used in the research presented here include *S. gigas*, *S. costatus,* the hawkwing conch, *S. raninus*, and the Florida fighting conch, *S. alatus*. The research regarding these species behavior, development, or status is not as extensively known as that of the queen conch. The larval cycle has previously been described before and after metamorphosis for the milk conch and the hawkwing conch (Davis 1993). Bradshaw-Hawkins (1982) documented reproductive behavior and the larval cycle of *S. pugilis*, the West Indian fighting conch, a close relative of the Florida fighting conch. Scientists have been using the larval ecology data to aid in identification in the field (Davis et al. 1993), to study fisheries oceanography (Stoner 1997, Davis 1998), and to assist in determining potential larval dispersal and recruitment processes (Davis et al. 1993). To date, there is only one commercial mariculture queen conch farm and it is located in the Turks and Caicos (Davis et al. 1984).

Of the remaining six Strombidae conch species in the Caribbean, only *S. costatus* is a commercially important species, more so in Mexico than in the rest of the Caribbean (Aldana-Aranda et al. 1989). The remaining species are used primarily for subsistence fishing only (Bradshaw-Hawkins 1982).

POLICY AND MANAGEMENT

There are currently no Caribbean management plans for the any of the Strombidae conch, including the over-exploited *S. gigas*, and there is only one commercial conch farm in the world. The farm is located in the Turks and Caicos, where the queen conch fishery is considered overfished (Appledoorn 1994). There is scientific interest in establishing another conch farm in Florida, which could potentially serve as a captive breeding program as well. The remainder of this chapter will explore the current Florida aquaculture policies, and recommend a series of actions to be taken in order to establish a small scale commercial conch farm in Plantation Key, FL.

FLORIDA AQUACULTURE POLICIES (DOACS 2000)

Florida residents first embarked on the idea of tropical aquaculture in the 1950's. World War II veterans who had seen many exotic creatures during their travels oversea, had brought home an appreciation for tropical aquatic species. The semi-tropical Florida climate proved ideal for the cultivation of ornamental fish and plant species. By 1960, these entrepreneurs had mastered the art of air transport of live plants and animals, which allowed their small businesses to flourish. The Florida government became involved in 1987 when the Department of Agriculture and Consumer Services (DOACS) began permitting farmers, requiring certification, and leasing state lands to farmers. There has been a steady growth in the aquaculture business in Florida and it now includes a wide range of species such as alligators, tilapia, clams, and ornamental fish. The industry supports over 690 farms and is worth $102 million USD annually (DOACS 2000).

The Florida aquaculture industry is comprised of many constituents (Figure 2). The physical environment in this diagram consists of a hypothetical conch aquaculture facility, and therefore, some of the legal mandates and legislative groups involved are particular to either conch and/or a foreign culture species. The most significant human constituents consist of the actual commercial farmers and adjacent landowners. Adjacent landowners have property which is impacted by an aquaculture facility, with the major impacts consisting of water contamination and waste treatment. The indirect constituents are the consumers and the tourists who visit aquaculture sites. Many of the alligator farms now charge admission to tour the facilities to boost profits and educate the public.

In considering the aquaculture of the queen conch there is a legal mandate unique to this species: the CITES regulation requiring permitting of queen conch collections. The policy makers who could be involved in establishing a conch aquaculture facility would include the Florida Department of Environmental Protection (FLDEP), under which the Department of Agriculture and Consumer Services (DOACS) exists (see Appendix I). The DOACS is responsible for ensuring that aquaculture farmers remain in compliance with their Best Management Practices (BMPs) originally agreed to when they became certified. On July 1, 1998, the Florida Legislature created the program of BMPs to ensure that aquaculture farmers do not negatively impact the environment. The legislature is mainly concerned with groundwater and surface water standards, and the culture of non-native species. DOACS is required to make annual site visits to ensure compliance.

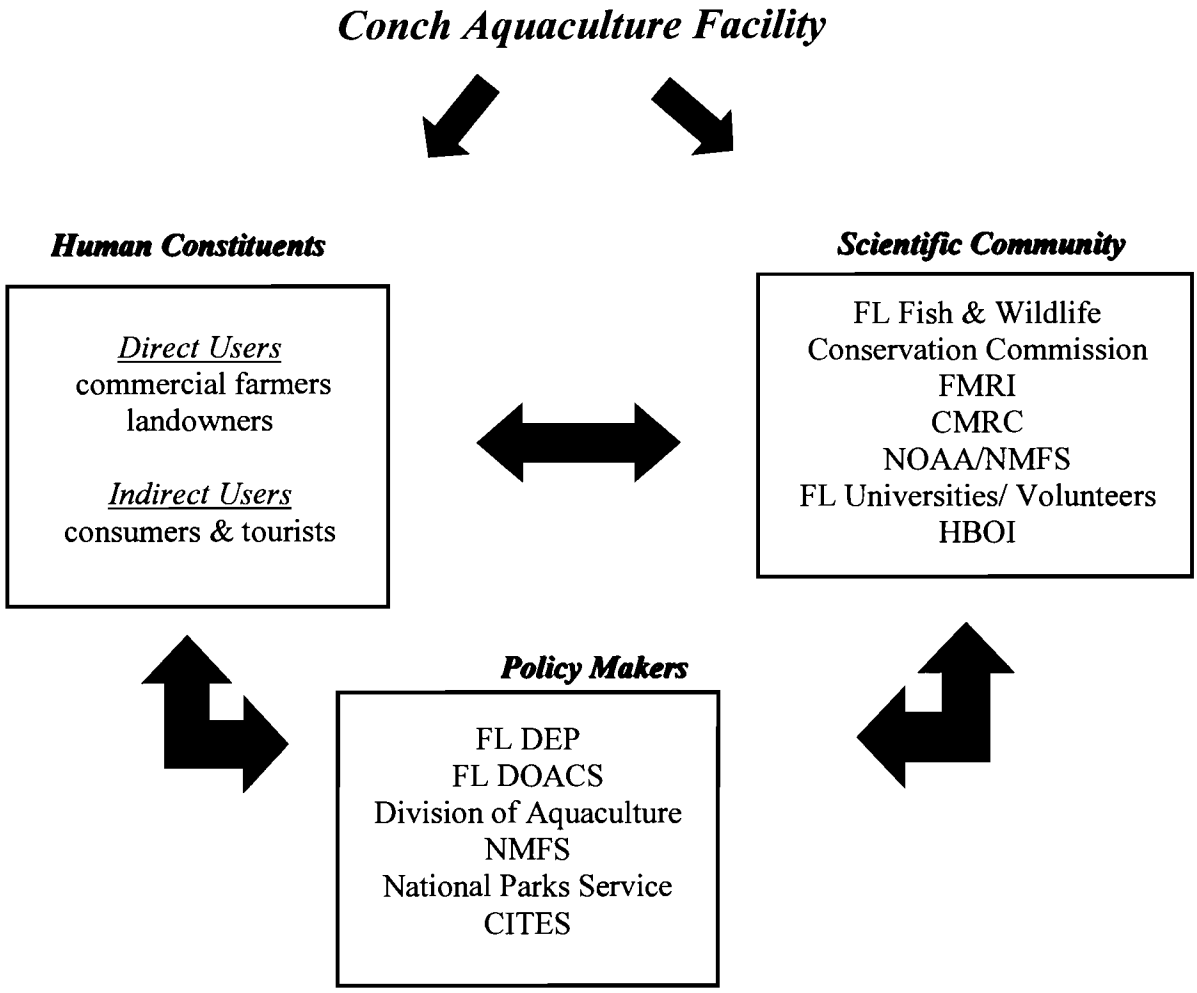

Figure 2. Human ecology diagram for an aquaculture facility in Florida.

FMRI = Florida Marine Research Institute, CMRC = Caribbean Marine Research Center, NOAA = National Oceanographic and Atmospheric Administration, NMFS = National Marine Fisheries Service, HBOI = Harbor Branch Oceanographic Institution, DEP = Department of Environmental Protection, DOACS = Department of Agriculture and Consumer Services, CITES = Convention of the International Trade of Endangered Species

In 1986, the Division of Aquaculture (DOA) was established under the DOACS. The DOA is the major agency currently responsible for regulating aquaculture farming in Florida. DOA needs to regulate (i.e. inspect and certify) aquaculture facilities and processing plants. In reference to protecting human health and the environment, DOA manages the opening and closing of shellfish harvestable waters. Florida also has a program available to aquaculture farmers where they can lease submerged state lands suitable for farming. Those farmers wishing to lease state lands need to submit a written application, which is provided by the DOA. The application is divided into four steps. First, the applicant must identify a lease site and describe the proposed activity by developing a business plan. After careful review, a site inspection made by the staff will determine whether or not the land is suitable for the development of the farm. Several surveys and site inspections may be necessary before a location is deemed suitable. Once the site survey is complete, local county and city entities are notified and the Governor and the Cabinet may approve the lease. In conjunction with the leasing program, the DOA is also responsible for ensuring that any required mitigation and restoration programs are completed when the farm is established.

Another legal mandate pertinent to all Florida aquaculture farmers falls within the DOA: the Aquaculture Policy Act (Title XXXV, Chapter 597 of the Florida Statutes). The act has two important components; first, it requires the Commissioner of Agriculture to establish an Aquaculture Review Council (ARC), and second, the act requires all Florida aquaculturists acquire a certificate of registration and abide by a set of BMPs. The ARC provides a direct link of communication between the DOACS and the aquaculture industry. The ARC consists of nine members; these include the chairs of the State Agricultural Advisory Council, and the Aquaculture Interagency Coordinating Council, along with seven other members appointed by

the Commissioner of Agriculture. The seven additional members are to include representatives from the industry. Currently members include an alligator, food fish, shellfish, tropical fish, and aquatic plant farmer, as well as a representative from the commercial fishing industry and from the aquaculture industry in general. The ARC is required to inform the Commissioner of Agriculture of recommended rules and policies, and annually to submit a list of short-term research recommendations. The ARC meets at least quarterly.

The second goal of the Aquaculture Policy Act was to require farmers to obtain an aquaculture certification. The importance of the certification program is to officially recognize the aquaculture producers and products, and allow the aquafarmer the same privileges and benefits allotted to agricultural farmers. The certification application requires four components: personal information, facility location, description of production facilities, and a list of cultured products along with an estimate of annual production. The applicant must certify the above information and agree to abide by the BMPs in place for aquaculture. The Aquaculture Certification fee is $50 a year.

Other scientific policy makers who may be involved in the process of permitting include the National Marine Fisheries Service (NMFS), and the National Parks Service (particularly for the Florida Keys). Likewise, the Florida Fish and Wildlife Conservation Commission (FLFWCC) along with the National Oceanic and Atmospheric Administration may be involved in both the policy making and within the scientific community. The FLFWCC includes the Florida Marine Research Institute (FMRI). The FMRI has a number of offices and regional field laboratories scattered throughout the state and is very active in stock assessments for the Florida waters. Stock assessment data are important when deciding whether or not a commercial farm may be feasible. The remainder of the scientific constituents

includes several Florida universities and marine research laboratories. For example, Florida Institute of Technology supports researchers and students who study at the Caribbean Marine Research Center in the Bahamas, and at Harbor Branch Oceanographic Institution located in Ft. Pierce, FL. Many students receive voluntary internships in state government and are able to assist in the volunteer stock assessment programs.

CHAPTER 2

CAPTIVE BREEDING

INTRODUCTION

My master's research was conducted at Harbor Branch Oceanographic Research Institution in Ft. Pierce, FL. Together with Dr. Megan Davis-Hodgkins, we designed a captive breeding arena, to see if we could get Strombidae conch to breed and lay fertilized eggs in captivity. Before I describe my research, I would first like to address the reproductive biology of conch as well as the history of captive breeding.

REPRODUCTIVE BIOLOGY

The reproductive biology of the Strombidae conch, particularly of *S. gigas*, is fairly well understood. Conch have separate sexes (Figure 3) with internal fertilization, and therefore have to copulate. Sex determination can be done in several ways. The easiest method is to observe a copulating pair and note their positions, or note their specific external sex organs. *S. gigas*, and perhaps other Strombidae species, adult females reach a larger size than males. Size as a distinguishing factor may be obscured in areas that are heavily fished for large adults. The most efficient way to determine the sex of the conch is to place them on their side, and note a verge (male) or an egg groove (female) when they right themselves (Davis et al. 1984). *S. gigas* reach sexual maturity at 3-4 years of age and maintain a 1:1 sex ratio in an undisturbed population (Davis 1984).

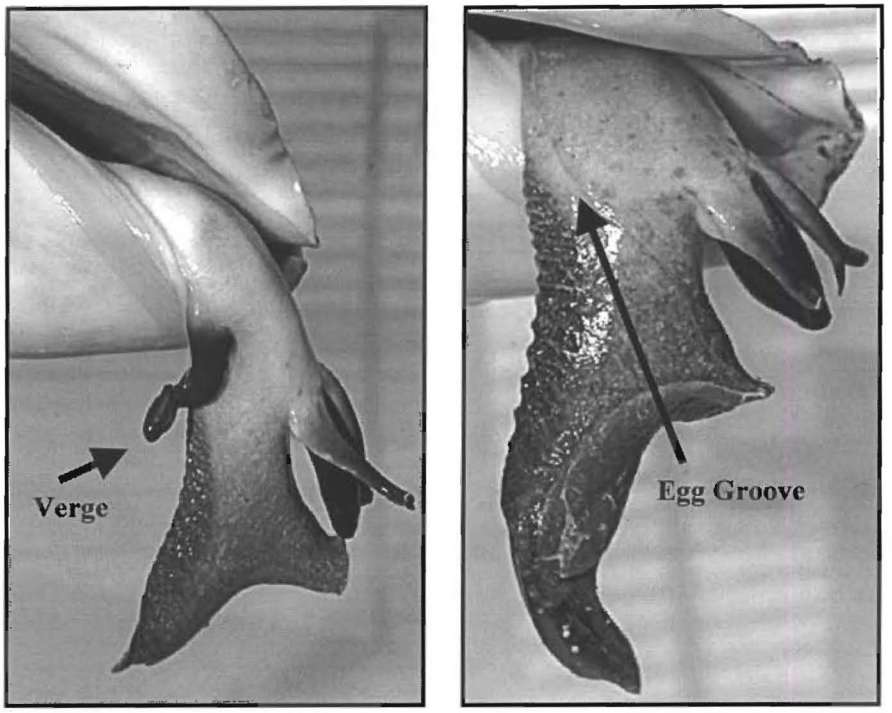

Figure 3. External sex organs for the conch family Stombidae. Shown here is a male and female *S. costatus*

The reproductive season for the Strombidae family varies throughout the Caribbean region. In the Florida Keys, *S. gigas* has a reproductive season that begins in early March and lasts until the end of September (Stoner et al. 1992). *S. costatus* have been observed spawning from November until May in Venezuela, although they breed year round in Mexico, and in the summer months in Florida (Brownell 1977). The reproductive season for *S. raninus* and *S. alatus* is also thought to be during the summer months.

When the temperature and photoperiod are suitable to induce reproduction (Stoner et al. 1992), it is known that *S. gigas* and *S. costatus* tend to migrate towards open sand patches near or surrounded by *Thalassia testudinum* beds (Brownell 1977, Robertson 1959, D'Asaro 1986, Davis et al. 1993). These aggregations are thought to occur so that the males are better able to find a female, thus increasing the number of possible copulations. The copulating pairs are

usually located at depths of 6-16 meters with temperatures in the range of 27-29°C (Stoner et al. 1992, Davis 1984, Brownell 1977). Once a male copulates with a female, egg laying will occur, although this process may follow copulation by several weeks (D'Asaro 1964). During the egg laying process, males may attempt to copulate with the females (Robertson 1959). It has been suggested by researchers that the egg laying females release a pheromone that may stimulate them to lay their eggs faster, so as to be able to spawn more often (Appeldoorn 1988). Reed (1995) dissected the reproductive tract of a female Strombidae and found that the design of her reproductive system allows her to spawn and copulate (hold sperm) at the same time.

A female S. gigas may lay an average of 9.4 egg masses during the reproductive season (Davis et al. 1984). Each Strombidae egg mass consists of a long strand of eggs tightly coiled inside a tough outer layer (Figure 4). These masses are covered with sand grains and left

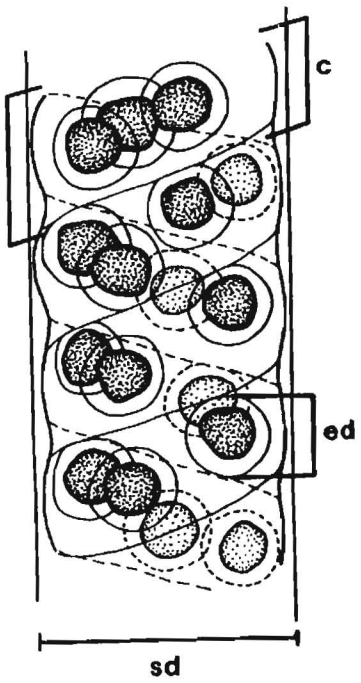

Figure 4. Egg strand section. ed, egg capsule diameter; sd, strand diameter; c, coil of the strand. Source: Davis et al. (1993)

unattended near the spawning females. Each *S. gigas* egg mass contains approximately 400,000 eggs and each *S. costatus* mass may contain 210,000 (Robertson 1959). *S. raninus* egg masses may have 240,000 eggs (Davis 1993), and *S. alatus* masses contain approximately 92,000 (D'Asaro 1986).

Behaviorally, Berg (1975) noted a "follow-touch" courtship sequence between male and female *S costatus*. The male was noted to follow a chemical trail laid down in the sand by the females, some of which may be as far away as 5m. Bradshaw-Hawkins (1982) made several behavioral observations that were unique to *S. pugilis*. These included a male "sparring" or fighting between two or three males over an egg laying female, as well as a type of male guarding behavior.

CAPTIVE BREEDING RESEARCH

INTRODUCTION

Although larviculture techniques have become fairly widespread throughout the scientific community, researchers still rely upon wild adult populations of *S. gigas* or other Strombidae species for egg mass collection. Often it can be difficult to find egg masses in the wild due limited spawning aggregations, copulation frequency, and a 6-8 month breeding season. Establishing a captive breeding program would alleviate the need to collect egg masses from the wild and possibly extend the breeding season.

Because of the permitting issues related to the harvesting and importing of S. *gigas* in Florida and in the Caribbean, we chose three non-restricted Caribbean conch species to begin the breeding study: hawking conch, *S. raninus*, Florida fighting conch, *S. alatus*, and milk conch, *S. costatus* (Figure 5). We did obtain four *S. gigas* a month into the experiment, once the CITES paperwork was completed.

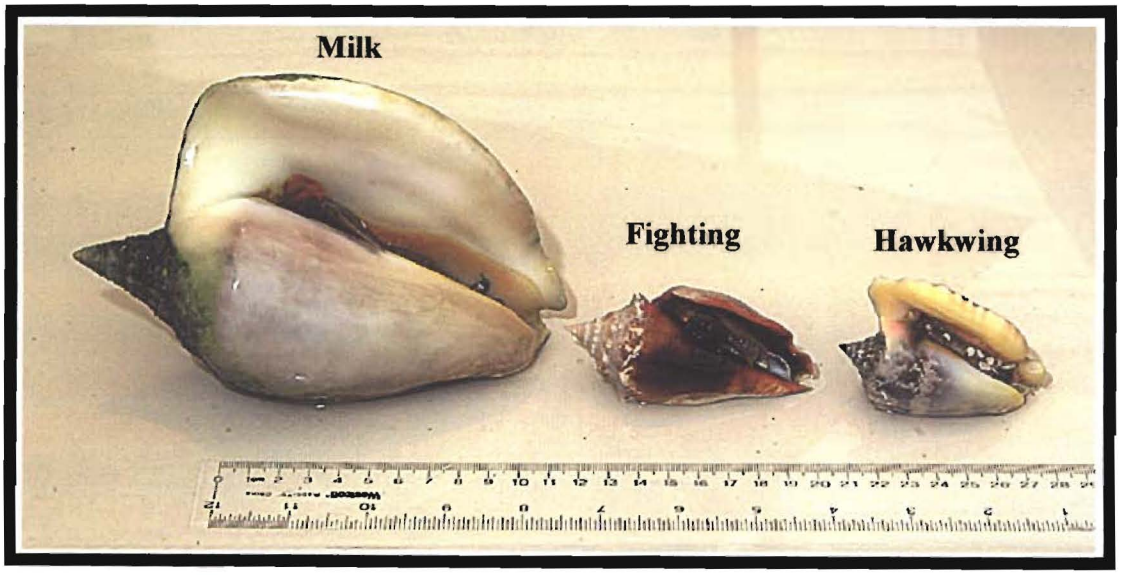

Figure 5. Three alternative, non-restricted species used in the captive breeding study.

Captive breeding and egg-laying has been observed for *S. costatus* and *S. raninus* (Bradshaw-Hawkins 1982, Reed 1995a, Reed 1995b). The majority of the conch research over the past three decades has focused on the queen conch. *S. gigas* have been observed to copulate in captivity (Davis pers. obs. 1983), however, egg mass laying had not been observed in captivity until this research was conducted.

The purpose of this captive breeding experiment was to document breeding behavior for three non-restricted Strombus species: *S. raninus, S. alatus, S. costatus*, and the restricted *S. gigas*. The number of egg masses laid per female on a weekly and daily basis, and the egg strand characteristics was recorded. The copulation frequency, and male copulation preferences were observed, and the viability of the eggs laid in captivity was assessed through larval rearing. The results from this study will assist in developing a year-round spawning program, will be tested with *S. gigas*, and may establish alternative species for the Strombus markets.

MATERIALS AND METHODS

The captive breeding study using the four Florida and Caribbean *Strombus* species: *S. raninus*, *S. alatus*, *S. costatus*, and *S. gigas* was conducted at Harbor Branch Oceanographic Institution (HBOI), Ft. Pierce, Florida from June 15 – February 19, 2001. Seven adult *S. raninus* (2 males and 5 females), and four *S. alatus* (3 males and 1 females) were collected from three sites off Plantation Key, in the Upper Florida Keys on June 15, 2000. On July 12, four more *S. alatus* (1 male and 3 females) were collected from the same location. These conch were collected in 0.3 to 1.5 m of water on a sand, algal and seagrass bottom (Table 1). Five *S. costatus* (2 males and 3 females) were collected in 2 to 2.5 m of water on a rocky hard bottom on June 1 from the Ligumiuti Channel in Indian Key, in the Upper Florida Keys. These conch

Table 4. Collection sites in the Upper Florida Keys on June 15 and July 16, 2000 (for *S. gigas*).

Site No.	Location	Depth (m)	Bottom Type	Water Temp (°C)	Salinity (ppt)	pH	Ammonia (mg/L)	Species	Sex	No.
1	Sea Oat Beach, Lower Matacumbe	1.5	Fine sand, *Batophora*, *Laurencia, Hamimeda, Thalassia, Syringoduim*	29.7	36	8.2	0.06	*S. raninus*	Male	1
2	Windley Cay, Coral Club	0.16-1.5	Soft line sand, *Syringodium*, fine diatom film, *Halimeda*	31.2	35	8.4	0.07	*S. raninus* *S. raninus* *S. alatus* *S. alatus*	Female Male Female Male	5 1 1 3
3	* Lignumuiti Channel, Indian Key	2-2.5	sandy, rocky hard bottom	N/A	N/A	N/A	N/A	*S.costatus* *S. costatus*	Female Male	3 2
4	Florida Fish and Wildlife Conservation Commission, Keys Marine Laboratory, Long Key, FL **	0.75	fiberglass holding tanks (0.75m x 3.6m)	33-42	N/A	N/A	N/A	*S. gigas* *S. gigas*	Female Male	1 3

* Collected 2 weeks prior to the collection of *S. raninus* and *S. alatus* and held in a recirculating system.

** Hatchery reared from S. gigas eggs collected in FL waters

were placed in holding tanks (18.9cm long x 14.1cm wide x 7.1cm deep) in the Florida Keys for two weeks prior to the beginning of the experiment. Four *S. gigas* (3 male and 1 female), that came from the Keys Marine Lab (FLDNR) in Marathon, FL, were added to our tank on July 12. These conch were raised from egg stage in captivity.

The sex of each conch was determined in the field on the boat. A small area on the dorsal side of the shell was scrubbed clean using sandpaper, and the sexes were numbered with fluorescent paint (teal for males and orange for females). In later stages of the experiment, the conch were remarked, and the paint was sealed with clear epoxy glue. Once the sex determination was complete, the animals were placed in a cooler filled with seawater for the remaining time in the field. For the 4 hr ride to the HBOI laboratory, the conch were kept in the cooler between towels moistened with seawater. Before being placed into the tank at the laboratory, all of the conch were weighed (g) to the nearest 0.1 g using a portable scale, and their shell length was measured (mm) using a pair of calipers (Table 4).

Table 4. Shell length (SL) and width measurements of the collected specimens.

Species	Sex	No. of conch	SL range (mm)	Avg. SL (mm)	Weight range (g)	Avg. Weight (g)
S. raninus	M	2	72.8 - 4.9	73.9	63 - 72	67.5
	F	5	77.0 - 86.4	82.8	67 - 102	85.8
S. alatus	M	4	76.0 - 92.7	86.7	51.1 - 95.4	70.2
	F	4	80.2 - 94.7	89	70.7 - 109.8	86.8
S. costatus	M	2	170 - 173	172	754 - 776	765
	F	3	165 - 195	180	965 - 1100	1030
S. gigas	M	3	180-190	183.3	1095 - 1560	1267
	F	1	190	190	1151	1151

The conch were placed in a circular tank (4.5m radius) which was divided into 4 equal units (4.1m^2) by using black plastic mesh netting 30cm high. The specimens were stocked at varying densities: *S. raninus* conch were held at 1.7 conch per m^2, *S. alatus* were stocked at 2.0 conch per m^2, S. *costatus* were at 1.2 conch per m^2, and *S. gigas* were held at 0.98 conch per m^2. Water was drawn from a salt water well, aerated, and circulated throughout the tank on the water surface and below the sand. To allow for under-gravel circulation, the sand was placed on top of ½ inch egg crating covered with window screen and supported with PVC piping and fiberglass bricks. Approximately 10cm of sand was placed on top of the crating. The sand consisted of large, course particles (1-3mm dia) of Bahamian aragonite sand as well as handfuls of bacterial sand, in order to create a live system (see Russot and Davis, in prep). Temperature, salinity, and pH were recorded daily and ammonia concentrations were determined periodically. Water temperature ranged from 21.0 to 30.5°C (averaged 27.4°C), salinity averaged 33.7 ppt, pH averaged 8.1, and ammonia remained between 0.06-0.10 mg/L (Table 5). The system was back-flushed at least every other day to aid in waste disposal.

Table 5. Water quality records from the tank and the replenishment water. The number in parentheses represents the number of samples. The replenishment water was tested at the beginning of the experiment, and flowed into the tank at 30L/hr.

	Tank Water	**Replenishment Water**
Temp (°C)	27.4 ± 2.11 (249)	28.3
Salinity (ppt)	33.7 ± 1.14 (245)	33
Ammonia (mg/L)	0.1 ± 0.02 (39)	0.08
pH	8.1 ± 0.28 (242)	7.7
Ca+ ion Concentration	429 ± 12.7 (2)	403
Dissolved Oxygen	6.2 (1)	4.09
ORP	203.1 (1)	195

The conch were fed a high quality diet rich in proteins (>30%) to maintain health and reproductive reserves. Mazuri Koi pellets were blended with *Ulva sp.* and knox gelatin to create a gel based benthic diet (Creswell pers. comm. 2000, Appendix II). The knox gelatin allows the gel food to remain stable for approximately 48 hours. The recipe consisted of 500ml of blended koi chow and 700ml of chopped *Ulva* per 1000ml of fresh water, and 700g of food grade knox gelatin. A total of 550-650g of food was fed daily, with approximately 21g per day fed to each *S. raninus*, 19g fed to each *S. alatus*, and 50-70g fed to each *S. costatus* as well as each *S. gigas* conch. More food was not added until the old food was consumed.

Egg masses production and copulatory behavior were recorded daily. On average, observations were made five times a day in intervals of 15 minutes to an hour for the first 49 days of the experiment, and approximately twice a day for the remainder of the time. When an egg mass was found, it was removed with either a net or by hand and placed onto a wet table for measurements (length, width and height). Several egg masses were stretched to determine total length of the egg strand (n=13 for *S. raninus*, n=10 for *S. alatus*, and n=10 for *S. costatus*). The two egg masses collected from *S. gigas* were not stretched since we wanted to raise the larvae, and therefore did not want to cause any harm to the eggs. The strand diameter (μm), egg capsule diameter (μm), the number of eggs per millimeter, and newly hatched veliger size (μm) were also recorded using a compound microscope (40x) equipped with a 1mm micrometer. To determine whether or not the eggs were being fertilized and were viable, several batches of each species were hatched and cultured through juvenile stage (see Davis et al. 1993 for description, see Appendix III for procedures). The number of copulations, male copulation preferences, and any "territorial" male behavior was also noted.

RESULTS

From June 16-February 21 (253 days), a total of 401 egg masses were collected. *S. raninus* had the highest productivity at 336 masses, *S. alatus* laid 44 masses, 19 egg masses from *S. costatus*, and 2 egg masses from *S. gigas* (Figure 6). During the first (136 days) weekly and daily fecundity was recorded, since egg laying frequency declined after this point. Reproductive activity stopped for *S. raninus* after week 22. During the first 22 weeks, each *S. raninus* female laid approximately 2.53 egg masses per week. *S. alatus* females laid egg masses on a regular basis for the first 15 weeks, with each individual female laying roughly 0.48 eggs masses per week. *S. alatus* underwent a period of quiescence from week 16 until week 30, and then began to lay (at a frequency of 0.3 egg masses per week per female) for weeks 31 and 32. The final egg mass collected for this experiment was taken on week 36. Each *S. costatus* female laid about 0.3 masses per week during the first 10 weeks. After this point, the *S. costatus* females did not lay eggs again until weeks 30 thru 32. At this time, the females laid at a frequency of 0.58 egg masses per week per female. The *S. gigas* female laid one egg masses each of the last two weeks (35 and 36).

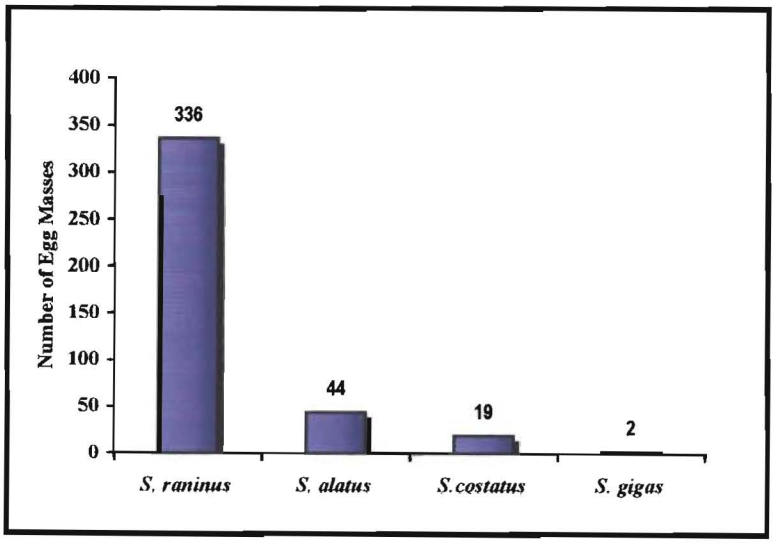

Figure 6. Total number of egg masses collected from the five S. raninus, four S. alatus, three S. costatus, and one S. gigas females from June 16 – February 21, 2001 (253 days).

The out of season reproductive activity may be related to varying temperatures throughout the experiment. Although the tank water was heated (beginning in late September), the maximum temperature attained by the heater was only 2 –3 degrees warmer than the incoming water. Temperature records show an increase in temperature which corresponds with the times when *S. alatus* and *S. costatus* began to spawn again, and when *S. gigas* began to lay eggs for the first time (Figure 7).

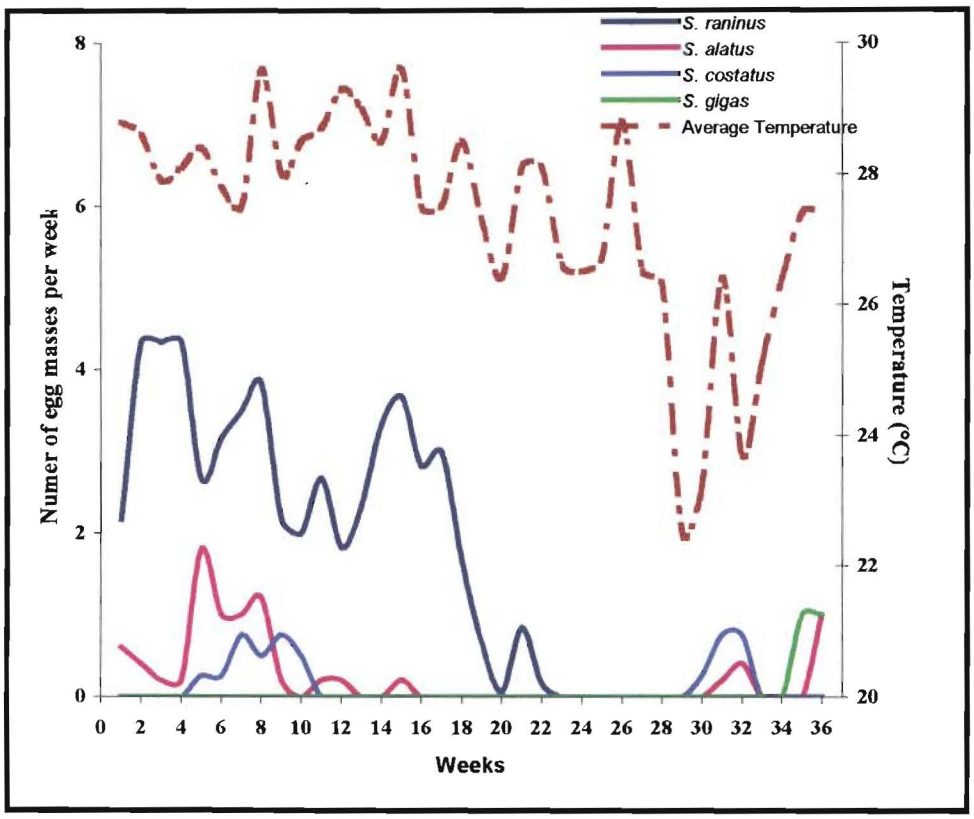

Figure 7. Average number of egg masses collected from each female on a weekly basis in comparison with weekly temperature averages for the duration of the experiment (June 16 – February 21, 2001).

Table 6. Summary of egg mass and veliger data (bolded numbers) shown in comparison to published data. The numbers in parentheses indicate the number of samples.

Variables	*S. raninus*	*S. alatus*	*S. costatus*	*S. gigas*
Length of egg mass (cm)	**3.5 - 15.5 (315)** *4-7 (4)*[c]	**4.5 - 9 (30)**	**9.5 - 30 (10)** *6-10 (2)*[c]	**9 (1)** 8-15 (9)[c]
Diameter of egg strand (μm)	**351 ± 24 (40)*** *321 ± 20 (10)*[c]	**509 ± 41 (20)*** *600*[b]	**825 ± 56 (9)*** *761 ± 18 (10)*[c]	**810 (1)** 785 ± 44 (10)[c]
Length of uncoiled strand (m)	**7.0 ± 2.0 (13)** *20*[a]	**10.8 ± 21.4 (10)** *10.7*[b]	**19 ± 9.7 (9)**	**N/A**
No. eggs/mass	**91,000-250,000 (13)** *206,000-245,000 (2)*[c] *400,000-460,000*[a]	**76,000-182,00 (10)** *92,000 (2)*[b]	**87,000-440,000 (9)** *185,000-210,000*[a]	**N/A** 313,000-485,000[d]
No. egg capsules per mm of strand length	**15 - 34 (40)*** *21-25 (15)c* *20-23*[a]	**11 - 15 (20)*** *8.6*[b]	**10 - 13 (9)*** *12-14 (10)c*	**15 (1)** 14-16 (10)[c]
Egg capsule diameter (μm)	**123 ± 10 (40)*** *140 ± 4 (30)*[a]	**181 ± 11 (20)*** *170*[b]	**250 ± 9 (9)*** *262±6 (20)*[c]	**240 (1)** 225 ± 17 (20)[c]
Newly hatched veliger length (μm)	**205 ± 10.5 (10)*** 197 ± 8 (20)[c]	**298 ± 14.2 (10)***	**370 ± 10.5 (10)*** 388 ± 14 (20)[c]	**N/A** 354 ± 15 (20)[c]

*Turkey's mulitple comparison of means test demonstrated there is a significant differences between species ($p < 0.001$), except between *S. alatus* and *S. cos* diameter of egg capsule.

[a] Robertson (1959) [b] D'Asaro (1986) [c] Davis et al. 1993 [d] Randall 1964

The egg strand calculations obtained in this experiment were compared to published literature (Table 6). A Turkey-Kramer multiple comparison test was run on the egg strand and egg capsule diameter, the number of egg capsules per mm of strand length, and the newly hatched veliger length data. Using a Turkey multiple comparison of means test, the inter-specific differences in all of the parameters were significant ($p < 0.001$) with the exception of the egg capsule diameter of *S. alatus* and *S. costatus*, in which they were grouped together. All four species proved to lay competent eggs, and each kind of veliger was raised through metamorphosis and to juvenile stage, although at the time this was printed, metamorphosis for the *S. gigas* veligers has not yet been induced. Metamorphosis was induced with the other species using the *Laurencia* procedure (Davis et al 1990), and hydrogen peroxide (Boettcher 1996).

From June 16- August 2, 2000 (first 49 days), copulatory behavior was observed. A single female was often observed copulating with more than one male while only laying one egg mass. This caused the difference in the data between the number of egg masses laid versus the number of times a female was observed copulating (Figure 8). On several occasions, males were observed copulating with egg laying females (Figure 9). *S. alatus* females were observed copulating while laying egg masses 52% of the time, whereas *S. raninus* and *S. costatus* females were observed 31% and 19% of the time, respectively.

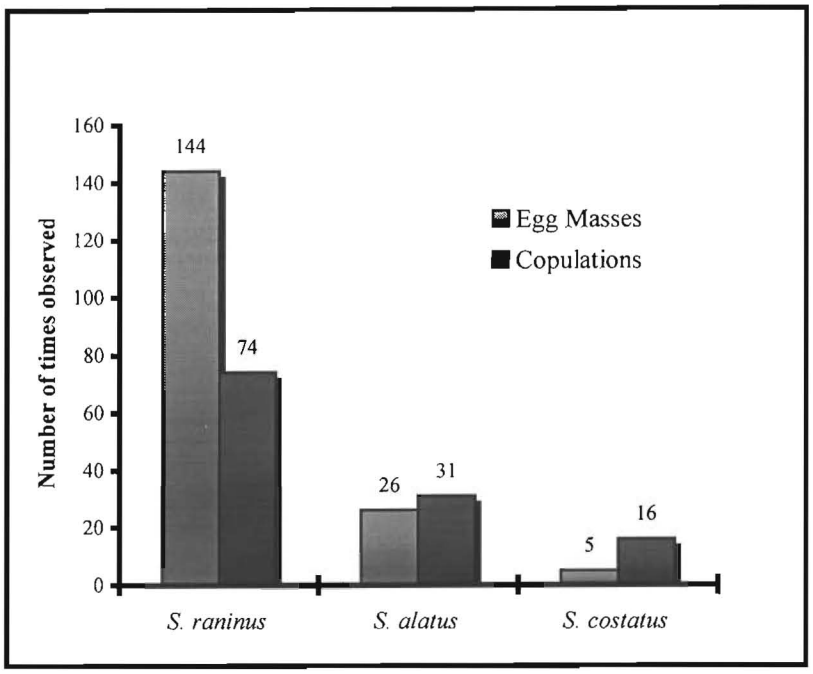

Figure 8. Number of egg masses collected versus the number of copulations observed from June 16 – August 2, 2000 (49 days)

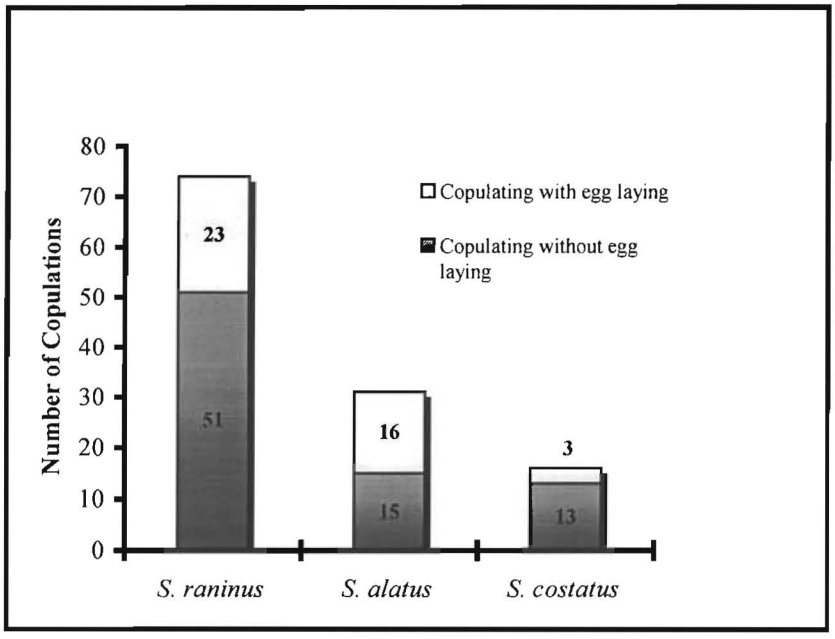

Figure 9. Number of times a female was observed to simultaneously lay eggs and copulate with one or more males. Observations were recorded for the first 49 days of the experiment (June 16 – August 2, 1000).

Male conch showed preferences for particular females (Figure 10). *S. raninus* male 1 appeared to show no preference. In contrast, *S. raninus* male 2 demonstrated a stronger mating preference especially towards females 2 and 3. The *S. alatus* males showed no particular patterns, although males 2 and 4 showed more preference towards certain females than did males 1 and 3. *S. costatus* male 1 mated with the three females with approximately the same frequency, whereas male 2 showed a preference towards female 2.

The amount of time the conch would copulate varied from 5 minutes to about 2 hours. It appeared as though the males would approach the females with their proboscis and "smell" the area underneath their lip, presumably sensing the female's pheromones. Several times the females would leap away from the male, rejecting their advances. Other times the males would follow the females around the enclosure before copulation was successful.

The *S. alatus* male conch demonstrated courting and protecting maneuvers. On several occasions, two males would prop themselves halfway on the lip of an egg laying female. Only one of the males was mating with the female while the other male appeared to be "guarding" the female. This guarding male was almost always positioned 90° to the female. One unique observation occurred when an egg laying female had three aggressive males around her. One of these males never mated with her throughout the duration of the observation. Instead he attempted to dislodge the male that was mating with her. When he was unsuccessful the first time, he used his proboscis and "sparred" with the other male. After a few minutes, the males stopped and the guarding male moved back in his position. While the two males were sparring over top of the female, another male had moved in, and was immediately chased off by the guard male. He then proceeded to attempt to dislodge the mating partner once again, but was

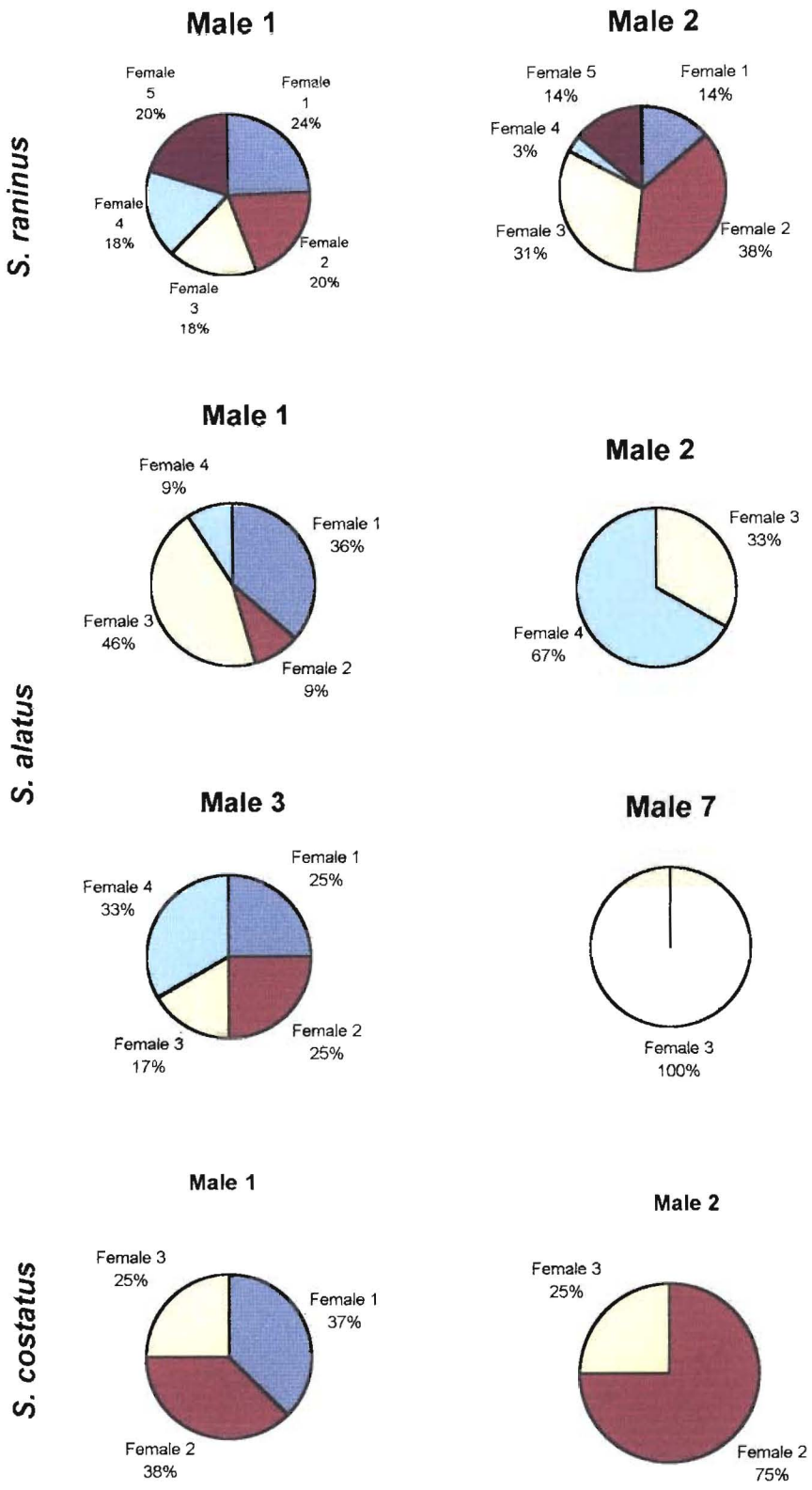

Figure 10. Male preferences towards female breeding partners. Observations were recorded for the first 49 days of the experiment (June 16 - August 2, 2000).

unsuccessful. On July 14, three more female and five more male S. alatus had been added to the tank. However, after we began to notice two males surrounding the spawning females we decided to reduce the sex ratio to 1:1 (four males and four females), to reduce the harassing of females by males.

DISCUSSION

The Caribbean queen conch, *S. gigas*, fishery is severely threatened today and alternatives for its market need to be identified. Although there have been efforts to cultivate *S. gigas* as well as replenish their populations in the wild, only one commercial queen conch farm exists, and restocking efforts have not been very successful. This experiment examined the feasibility of a captive breeding program for *S. gigas* and three alternative, non-threatened *Strombus* species. All of the four species used, *S. raninus, S. alatus*, *S. costatus*, and *S. gigas* reproduced and spawned viable eggs during their weeks in captivity. We collected a total of 401 egg masses in 36 weeks, with 336 of them coming from only five *S. raninus* females. There are two possible explanations for our success. First, we had artificially aggregated the males and females together, as they tend to do in the wild (Berg 1975, Robertson 1959), increasing the likelihood that reproduction would occur. Second, the conch were provided with a breeding arena devoid of predators, the water quality remained stable, and there was a consistent supply of food for the duration of the experiment; all conditions of which are ideal to promote conch reproduction. In early January, the water temperature spiked (from 23°C to 28°C) due to the passage of a warm front and *S. alatus* and *S. costatus* females laid egg masses again after a two months of quiescence. Similarly, the single *S. gigas* female spawned on February 12, 2001, which is not typical of a seasonal (summer) breeder. Although, it is

important to note that the female *S. gigas* appeared to be thin-lipped when she was placed into our tank. This is characteristic of a sexually immature female. We believe she may have matured during the six months in our breeding tank while no reproductive activity was taking place. In future experiments, photoperiod and temperature will be manipulated to determine if year-round spawning can be achieved.

Our research resulted in a significant amount of data on egg strands, and increased our knowledge of the reproductive biology of our three alternative species. In addition, the behavioral observations are invaluable to our understanding of reproductive behavior because little work has been published on the reproductive behavior of both *S. raninus* and *S. alatus*. In comparing our observations with those of Bradshaw-Hawkins (1982) on *S. pugilis*, I also noted some interesting territorial behaviors such as sparring and guarding with *S. alatus*. The number of times spawning females copulate will also be important once further seasonal or sex ratio data is collected.

CONCLUSIONS

Our experiments have proven that there is definitely a potential for a long-term captive breeding program. Although further research is needed, it appears as though breeding can occur in a small arena as long as there is a consistent food supply and an adequate sex ratio. We were extremely successful using a 1:2.5 male to female ratio with *S. raninus*, but we had to reduce a 1.75:1 male to female ratio in *S. alatus* to 1:1, so that the females were able to lay eggs without two or more males fighting over her. Proper nutrition is an important factor in conch reproductive health as well. *S. costatus* were originally collected from the field two weeks before they were placed in the breeding arena. It took them several weeks of intense feeding

before they began to copulate. Likewise, as was shown with our immature *S. gigas* female, the conch should have already reached sexual maturity before being placed in a breeding arena.

Further diet, temperature, and photoperiod manipulations will allow us to establish the biological parameters necessary to potentially design the first commercial conch breeding program. The use of alternative conch species in our or any future experiment may help to alleviate the fishing pressures on *S. gigas* and perhaps provide new information for fisheries management. Along with our success, we were also able to get the first captive raised female *S. gigas* to spawn viable eggs in captivity, holding promise that the reproductive cycle can be closed. With this new development, we may be able to change the strategy behind larviculture facilities, and encourage the establishment of breeding arenas as well.

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APPENDIX I

THE FLORIDA GOVERNMENT
(Department of Agriculture and Consumer Services 2000)

In order to put names and faces to all of the government constituents involved in aquaculture policy, I have compiled a short list of important personnel and pertinent information.

Bob Crawford, *Commissioner of Agriculture*

Aquaculture Review Council

Allen Register, *Alligators*
Brad McLane, *Aquatic Plants*
Alan Maxwell, *Aquaculture Industry Member At-Large*
Mike Davis, *Commercial Fishing*
Jim Harvey, *Food Fish*
Daniel Solano, *Shellfish*
Donald Drawdy, *Tropical Fish*
Tim Hennessy, *State Agricultural Advisory Council*
Sherman Wilhelm, *Aquaculture Interagency Coordinating Council*

Florida Aquaculture Industry Segments

Ornamental Species
Aquatic Plants
Shellfish
Alligators
Food Fish
Game Fish
Bait Species
Other Aquatics

Aquaculture Production and Technical Information

University of Florida, Department of Fisheries and Aquatic Sciences
Florida Cooperative Extension Service
Mitchell Aquaculture Farm
Tropical Aquaculture Laboratory
Cedar Key Field Station
Florida Aquaculture (newsletter produced by DOACS/DOA)

Financial Assistance

Small Business Administration
Farm Credit of Central Florida
Rural Business and Cooperative Services
Enterprise Florida
National Marine Fisheries Service

APPENDIX II

Gel Food

1. Using a blender, crumb the pellets of Mazuri Koi Platinum Pellets until they are in powder form, and measure out 200ml.
2. Using a blender, chop handfuls of *Ulva* mixed with salt water, then pour the blender contents into a net and squeeze the water out of the algae.
3. Measure out 300ml of chopped, packed down *Ulva*.
4. Heat 500ml of freshwater in a stainless steel saucepan on a hot plate.
5. Add *Ulva* and mix
6. Slowly add the 200ml of crumbed Koi chow and stir after each addition (may need to turn down the hot plate.)
7. Add 4-5 packets of knox gel (7 grams/packet) in small increments and continue to stir. The mixture should appear to have a milkshake like thickness.
8. Pour the contents onto a tray and spread it out so that the gel will be approximately 1/8" thick.
9. Place the tray in the refrigerator and let it cool for about 1-2 hours.
10. Cut the gel into pieces and store (in a sealed container/bag) in the refrigerator.

APPENDIX III

Laurencia Procedures

- Veligers are competent for metamorphosis approximately 21 days after hatching, when their eyes migrate outward, tentacles are of equal length, pigments of the foot change from orange to dark green, ctendium are elongated and functional, and their buccal mass has developed (Brownell 1977, Davis *et al.* 1990, Davis 1998).

- The most reliable inducer of metamorphosis in conch is an extract from the red macroalgae *Laurencia poitei.*

Extraction of *Laurencia*

1. Collect old, thick red-brown stalks from shallow, sandy-grass flats (where conch are usually found) and transport the algae to shore in a mesh bag. (The younger, yellow-orange stalks are slightly toxic to the veligers and will cause a low percentage of metamorphosis.)

2. Rinse the algae to remove any debris or predators.

3. Blend 500g of *Laurencia* and 250ml of seawater (2g:1ml) for 2 minutes until the algae is well chopped in an industrial blender.

4. Freeze the solution for a minimum of 2 days to lyse the cells and release the phycoerythrins.

5. Thaw the solution overnight.

6. Filter through a 200μm polyethylene screen.

7. Refreeze the solution in small containers until needed. (1kg of collected macroalgae will make 1.6L of blended slurry, which produces approximately 750 ml of extract.

APPENDIX III (CONT)

Dosage Test and Test Set Procedures Using Laurencia Extract

1. Three dosages are tested: 7, 10, 15ml extract/1 L of sea water, so three 100ml wash bottles will be needed.

2. Label each bottle with each of the dosages, and add 100ml of seawater along with the required dosage of *Laurencia* to the corresponding label bottle (0.7ml/100ml seawater, 1.0ml/100ml seawater, 1.5ml/100ml seawater).

3. Place 25 competent veligers for each dosage from the same tank. Place them in marked 50ml beakers labeled with the tank and dosage number (the tank number should be the same, the dosage number should be different). If doing more than one tank, there will be more than one beaker with the same dosage number, but not the same tank number.

4. To collect and place the veligers in the beakers for dosage and/or test sets, pour the water and veligers through a 100-150 micron .

5. Using the *Laurencia* wash bottle, wash veligers out of the sieve back into the beaker. Continue to add the solution until the beaker is 10ml full.

6. Let sit undisturbed for 3.5 hours.

7. After 3.5 hours, pour the *Laurencia* solution using a 149 size sieve and add sea water to fill the beaker. Observe veligers under microscope and again the next morning.

8. Observe for the following under the microscope (20 – 40X):
    - The number metamorphose- all crawling conch. Include ones with small pieces of lobe left.
    - Number of swimmers – all veligers with full lobes. Include veligers with wrinkled lobes.
    - Number dead.
    - Make comments: Healthy metamorphosed conch should be crawling and actively searching for food, not weak and pale.
    - Metamorphosis should be >60% in order to determine the dosage to use on the entire tank of veligers or to determine if the veligers in the tank are ready to go through metamorphosis.

APPENDIX III (CONT)

Hydrogen Peroxide

As cited in:

1. Boettcher, A.A. and Targett, N.M., 1996. Induction of metamorphosis in queen conch, *Strombus gigas* Linnaeus, larvae by cues associated with red algae from their nursery grounds. J. Exp. Mar. Biol. Ecol., 196: 29-52

2. Boettcher, A.A., Dyer, C., Casey, J., Targett, N.M., 1997. Hydrogen peroxide induced metamorphosis of queen conch, *Strombus gigas*: Tests at the commercial scale. Aquaculture, 148: 247-258

Small-scale assay

1. Collect competent larvae (19-24 days post hatch) with approximately 1mm SL from the same batch.

2. Place 15 larvae in 500ml polyethylene containers filled with 300ml ultraviolet sterilized, 10um filtered seawater, and 50µM of 3% pharmaceutical grade hydrogen peroxide. This is equivalent to 0.06 ml of hydrogen peroxide to 1 liter of seawater.
   - *NOTE: EM Quant peroxide test strips, which detects peroxides in the solution, should be used to monitor the seawater/peroxide solution to verify that it remained at the test level for the duration of the experiment*

3. Set up a positive and a negative control and include in each assay:
   - Positive: (The commercial inducer) an extract of Laurencia *poitei* at 0.01-0.02g wet weight algae per ml seawater, used as a measure of competency. *To avoid toxicity, only expose the larvae to the* Laurencia *extract for 5 hours, and then place them in fresh seawater for the remaining 19 hours.*
   - Negative: Seawater only, used as a test of spontaneous metamorphosis

4. Run the experiment at ambient temperature (28-29 °C) and salinity (39ppt) and under natural light conditions (12 hours light, 12 hours dark)

5. Expose the larvae to the hydrogen peroxide solution for 5-10 hours (Boettcher *et al.* 1997)
   - *NOTE: as mentioned above, the larvae in the positive control (Laurencia) need to be removed after 5 hours*

6. After the respective exposure times, the water in each container should be drained, the larvae gently rinsed with seawater, and then the container needs to be refilled with seawater.
   **For commercial scale hatcheries, it was noted by Boettcher (1997) that once the tanks were drained, rinsed, and refilled, there was not significance difference in whether or not the tanks were static, or had a flow of 5ml/sec.*

7. Percent metamorphosis is determined after 24 hours and is calculated as:

$$\frac{\text{Total number of larvae metamorphosed}}{\text{Total number recovered}}$$

**Larvae are considered to have undergone metamorphosis when they lose their velar lobes and begin to crawl using their foot*

Why use Hydrogen Peroxide?

- Studies from Boettcher et al. (1997) determine the percent metamorphosis induced by the hydrogen peroxide to be 80-95% (as compared to 45-95% with the algae extracts).
- The survival and growth rate of the newly set juveniles was comparable to those determined from the use of the algae extract.
- Provides a simple, low cost, reliable, and safe method for the commercial induction of larval conch metamorphosis.
- Hydrogen peroxide is readily available in both the reagent grade (30%) and the pharmaceutical grade (3%) stock solutions.
- The cost, using the 3% pharmaceutical grade, per batch of larvae is approximately $0.16 USD. This is low cost compared to the $15.00 USD per batch using *Laurencia*, and a cost of $5.00 USD per batch using potassium chloride.
- Easy to use peroxide indicator strips allow for the concentrations of hydrogen peroxide to be readily monitored and adjusted for each batch of larvae. Whereas *Laurencia* extracts can be highly variable, and the availability of their extracts is not always dependent.
- The use of hydrogen peroxide reduces the amount of variability readily seem in natural inducers.
- Hydrogen peroxide breaks down into oxygen and water, and therefore will not accumulate in the seawater.