Effects of Diet and Sex Ratio on the Reproductive Output of the Florida fighting conch, *Strombus alatus*

PHILLIP GILLETTE and AMBER SHAWL Harbor Branch Oceanographic Institution Aquaculture Division 5600 US 1 North Ft. Pierce, Florida 34946 USA

ABSTRACT

Florida fighting conch, Strombus alatus, are currently being raised by ORA Inc., a subsidiary of Harbor Branch Oceanographic Institution in Ft. Pierce, FL. The conch in the hatchery are fed one of two diets which partially consist of koi chow or catfish chow. This study examined the reproductive output of adult conch fed these two diets and stocked at either a 1:1 or 2:1 female to male sex ratio. The study was conducted over a period of 6 weeks (June 6- July 17, 2003). There were six replicates per diet, and the replicates for each diet treatment were further divided into three replicates of each sex ratio treatment. Egg masses were counted and measured for egg strand diameter, egg capsule diameter, and number of eggs per mm. Four egg masses from each diet treatment were hatched and the veligers were raised until metamorphosis. Veligers were measured periodically for shell length. The average veliger growth rate was 30.7 µm/d for parental conch fed koi chow, and 32.4 µm/d for parental conch fed catfish chow. There was no significant difference in the number of egg masses laid for conch fed koi chow or catfish chow, or for conch stocked at a 1:1 or 2:1 sex ratio (p > 0.05). Egg mass measurements and veliger growth rates were similar for all treatments. Since there was no difference in reproductive output, adult broodstock can be fed either diet and can be stocked at a sex ratio of 2:1, so that fewer animals are needed.

KEY WORDS: Conch, diet, Strombus alatus

Efectos del Cociente de la Dieta y del Sexo en las Capacidades Reproductivas del Caracol, *Strombus alatus*

El conch de la Florida se está cultivando por ORA, Inc., un subsidiario de Harbor Branch Oceanographic Institution en Ft. Pierce, la Florida. El conch en el criadero se alimenta una de dos dietas que consistan en parcialmente de *koi chow* o *catfish chow*. Este estudio examinó las capacidades reproductivas del caracol del adulto alimentado estas dos dietas y almacenado en cualquiera a 1:1 o 2:1 cociente hembra al masculino. El estudio fue conducido durante 6 semanas (6 de Junio hasta 17 de Julio de 2003). Había seis réplicas por dieta, y las réplicas para cada tratamiento de la dieta fueron divididas más a fondo en tres réplicas de cada tratamiento del cociente del sexo. Las masas del huevo fueron contadas y medidas para el diámetro del filamento del huevo, el

Page 948 57th Gulf and Caribbean Fisheries Institute

diámetro de la cápsula del huevo, y el número de huevos por mm. Cuatro masas del huevo de cada tratamiento de la dieta fueron tramadas y los veligers fueron levantados hasta metamorfosis. Veligers fueron medidos periódicamente para la longitud de la cáscara. El promedio veliger la tarifa de crecimiento era 30.7 μ m/d por caracol que han comido *koi chow*, y ellos que han comido *catfsh chow*, 32.4 μ m/d. No había diferencia significativa en el número de las masas del huevo puestas para el conch que han comido el *koi chow* o *catfish chow*, el caracol almacenó a conciente del sexo 1:1 o 2:1 (p>0.05). Medidas totales del huevo y índices de crecimiento para veligers eran similar para todos los tratamientos. Puesto que no había diferencia en salida reproductiva del adulto se puede alimentar cualquier dieta y se puede almacenar en conciente del sexo 2:1 de modo que menos animales son necesarios.

PALABRAS CLAVES: Caracol, sustantivo, Strombus alatus

INTRODUCTION

Six species of Strombidae can be found in the waters of Florida and the Caribbean: *Strombus. gigas, S. costatus, S. raninus, S. pugilis, S. gallus,* and *S. alatus* (Abbott 1974). The queen conch, *S. gigas,* and the milk conch, *S. costatus,* are the most economically valuable species (Davis 2000). Due to intense fishing, many conch populations throughout the Caribbean are in decline, especially populations of queen conch. As a result of declining conch populations, culture techniques have been developed for stock enhancement and for the food markets. Four of the six *Strombus* species (*S. gigas, S. costatus, S. raninus* and *S. alatus*) have been cultured in hatcheries and have laid viable egg masses in captivity (Shawl and Davis 2004). Currently, there is only one commercial *S. gigas* hatchery at the Caicos Conch Farm, TCI and one *S. alatus* hatchery located at Oceans, Reefs and Aquariums (ORA) in Ft. Pierce, FL.

The target species for this study is *S. alatus*, the Florida fighting conch. *S. alatus* is a small (7 - 10 cm) herbivorous conch found along the coasts of North Carolina to Florida and throughout the Gulf of Mexico (Shawl and Spring 2003). *S. alatus* have a similar life cycle and culture characteristics of queen conch, however, the adults tend to have a more complex breeding behavior. Multiple males have been observed copulating with one female at the same time (Reed 1995, Shawl and Davis 2004). Likewise, a male *S. alatus* often guards the female with which he mated, and may also encourage "sparring contests" with other males that attempt to mate with her (Shawl and Davis 2004). Males also tend to following females, and have even attempted to copulate with an unguarded female while she was laying an egg mass (Shawl and Davis 2004). These types of breeding competitions may be a cause of stress to egg laying females, and therefore may interfere with the egg laying process and thus the success of a commercial hatchery.

The purpose of this study was to look at the effects of diet and sex ratio on the reproductive output of *S. alatus*. The conch food used at ORA is produced exclusively at Harbor Branch Oceanographic Institution (Harbor Branch). One of the main ingredients in the adult conch food is koi chow, while catfish chow

Page 949

is used in the juvenile food. In order to help streamline the culture of conch, this study looked at the effects of using catfish chow to feed broodstock. In addition to the two diets, the conch were also stocked at different female: male sex ratios; 1:1 and 2:1. If the conch held at a 2:1 ratio laid as many or more eggs than those at a 1:1 ratio, then less animals (less males) need to be kept. Having fewer males could alleviate problems associated with the stresses of male guarding and sparring, and may also encourage more prolific egg laying for the commercial hatchery.

MATERIALS AND METHODS

This study was conducted at Harbor Branch from June 5 to July 17, 2003, a period of six weeks. A total of 144 *S. alatus* individuals were used for the study. Seventy-one conch were collected April 14, 2003 from the Gulf of Mexico, and placed in troughs for the remainder of the study. These animals were randomly assigned to diet treatments, and feeding began on April 15, 2003. Prior to June 6, 2003, all conch in a diet treatment were kept in a single large holding trough, and no eggs were collected. The remaining 73 animals were taken from the broodstock tanks at Harbor Branch for the study and assigned to diet treatments.

Conch were assigned to diet and sex treatments by means of a random number generator. In each diet treatment, there were six replicates with 12 conch per treatment. In addition to the diet treatments, the animals in each replicate were stocked at one of two different sex ratios. Three of the replicates for each diet treatment were stocked at a female: male sex ratio of 6:6 (1:1), while the other three replicates were stocked at an 8:4 (2:1) ratio. The females for each replicate were then numbered with enamel paint in order to record egg laying activity for each female.

Conch were kept in 0.53 m x 2.36 m troughs with a raised aragonite sand substrate, at a density of 7.3 conch/m². The troughs were all part of a large recirculating system. The water in the system was drawn from a salt water well and then charcoal filtered, micron filtered, and UV sterilized. Each trough was divided in half with a polypropylene mesh (1 cm openings), allowing two treatments per trough. A water-jet provided air to one half of the trough and water flow for the whole trough, and an air-stone was used to provide air for the other half of the trough. The troughs were located inside a slightly shaded greenhouse. In order to reduce the growth of algae, screen covers were made and placed over the troughs. The conch were exposed to a natural 12 hour light cycle.

Temperature (°C) and pH were recorded daily for the system, and the salinity (ppt), dissolved oxygen (mg/L), ammonia (mg/L), nitrite (mg/L), and nitrate (mg/L) were measured once a week. All replicates in both diet treatments were fed the same amount of food daily - 20 g. Any variation from this amount was noted. Before feeding, any old food or fungus in the trough was broken up. The troughs were checked twice a day (morning and afternoon) for any egg masses. If an egg mass was found, it was collected, the replicate number was recorded, and, if possible, it was noted which female laid the egg mass. The egg masses were gently shaken to expel any loose sand, and

then measured for overall length, width, and height to calculate the volume (cm³). A small egg strand was pulled from the mass and used to measure the strand diameter (μ m), egg capsule diameter (μ m), and the number of eggs per mm of strand. These measurements were made using a 40X dissecting microscope with an eyepiece micrometer. The egg strand parameters were measured three times per egg mass.

After being measured, the collected eggs were placed in an incubator and kept at a temperature of 27-28°C for two to three days, after which they were placed in conical larval tanks for culture. Veligers were cultured according to the guidelines set forth in Davis (2000). Ten veligers from each tank were measured periodically throughout the larval cycle using a 40X dissecting microscope equipped with a micrometer. Veligers were raised through metamorphosis to juvenile stage.

A 2-way analysis of variance was used to compare the diet treatments and sex ratio treatments. The ANOVA tested differences between the two diet treatments, between the two sex ratios, and between the sex ratio and diet treatments.

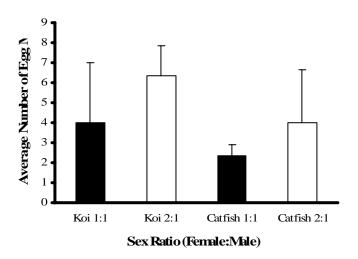
RESULTS

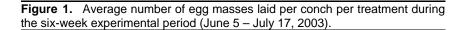
During the six-week experimental period (June 5 to July 17, 2003), there were 50 egg masses collected from all treatments. A total of 19 egg masses were collected from conch fed catfish chow, while 31 egg masses were collected from conch fed koi chow. The average number of egg masses laid per replicate was 5.16 ± 2.48 in the koi chow treatments and 3.16 ± 1.98 in the catfish chow treatments (Figure 1). The average number of egg masses laid per female was 0.12 ± 0.06 for conch fed koi chow, and was 0.08 ± 0.05 for conch fed catfish chow. There was not a significant difference between the two diet treatments (p = 1.475).

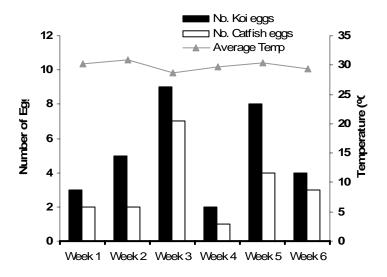
The average number of eggs laid per replicate for conch stocked at a sex ratio of 1:1 was 3.16 ± 2.13 , with an individual average of 0.52 ± 0.36 egg masses per female. For conch stocked at a 2:1 ratio, the average per replicate was 5.16 ± 2.31 and the average per female was 0.64 ± 0.29 (Figure 1). There was no significant difference in the number of egg masses laid by conch stocked at the two sex ratios (p = 1.475).

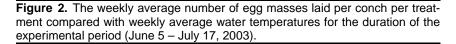
Water quality parameters remained relatively constant throughout the experimental period. Over the six week experimental period, temperature averaged $29.9^{\circ}C \pm 1.6^{\circ}$, salinity averaged 33.2 ± 0.97 ‰, and pH averaged 7.7 ± 0.16 . There appeared to be no correlation between temperature and the number of egg masses laid (Figure 2).

There was no indication that any one female laid the majority of eggs in a replicate. The egg strand diameter, capsule diameter, and the number of eggs per mm were similar for conch fed koi chow and catfish chow (Table 1). The data on egg strand diameter, capsule diameter, and the number of eggs per mm are similar to those reported by Shawl and Davis (2004) for captive raised *S. alatus*.









Variable	Koi Chow Diet	Catfish Diet
Volume of egg mass (cm)	16.9 ± 9.7 (29)	17.8 ± 8.7 (18)
Diameter of egg strand (µm)	493.2 ± 56.3 (21)	500.5 ± 49.7 (14)
Egg capsule diameter (µm)	203.5 ± 24.7 (21)	195.2 ± 13.4 (14)
No. egg capsules per mm	12.1 ± 1.6 (21)	12.5 ± 1.6 (14)

Table 1. Summary of egg mass data from June 5 - July 17, 2003. Results are expressed as average \pm standard deviation (n = samples).

Veliger size was recorded periodically throughout the culture period and the averages of all cultures from each diet treatment were combined (Figure 3). The average growth rate of veligers (μ m/d) in the koi chow treatment was 40.2 \pm 21.8, which was similar to the catfish chow treatment at 37.4 \pm 18.5 (Table 2). All cultures reached developmental stages at approximately the same age, and metamorphic competency was reached around day 25. The number of days until metamorphosis and the shell length of veligers at metamorphosis were similar to the data presented for *S. alatus* in Shawl and Davis (2004).

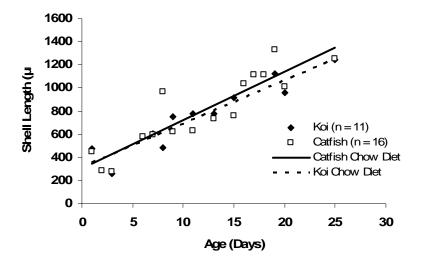


Figure 3. Average *S. alatus* veliger shell length in relation to age for each treatment.

Table 2. *S. alatus* veliger characteristics for the two diet treatments June 5 - July 17, 2003. Results are expressed as average \pm standard deviation (n = samples).

Variable	Koi Chow Diet	Catfish Diet
Shell length at hatch (µm)	480 ± 21.2 (2)	447.5 ± 81.3 (2)
Shell length at metamorphosis (µm)	1247.5 (1)	1258.3 (1)
Growth rate (µm/d)	40.2 ± 21.8 (4)	37.4 ± 18.5 (4)

DISCUSSION

Although visually there appears to be a difference in the number of eggs laid between the two diet treatments, an analysis of variance showed that there is no significant difference. The variance between replicates was very high, which helps to explain the insignificance of the results. Since there was not a significant difference between the diet treatments, broodstock can be fed either the koi chow or the catfish chow, and it will not have an effect on the number of egg masses produced nor on the health of the veligers.

There was no significant difference found between the number of eggs laid by females stocked at the two sex ratios either. Conch held at a 1:1 density statistically laid as many eggs as conch held at a 2:1 sex ratio. It can therefore be assumed that it would be advantageous to hold conch at a sex ratio of 2:1. Having two females to every one male would mean that hatcheries could stock a higher number of females, thus increasing the egg mass output for the hatchery.

There was no difference in any of the measurements taken of the egg masses or egg strands. There was also no difference found in the growth or health of veligers. It can be assumed that the eggs produced from both diet treatments were of the same quality. Veligers had similar growth rates and health between the treatments as in previous studies (Shawl and Davis 2004). There is no reason to believe there will be any difference in the health or survival of juveniles raised from broodstock and fed either diet.

ACKNOWLEDGEMENTS

The authors would like to thank Megan Davis for advice, help and guidance. Harbor Branch Oceanographic Institution for use of facilities and equipment, and the Link Foundation for funding the project. This is a Harbor Branch contribution 1573.

LITERATURE CITED

- Abbott, R.T. 1974. *American Seashells*, 2nd *Edition*. Van Nostrand Reinhold Company, New York, New York USA. 663 pp.
- Davis, M. 2000. Queen Conch (*Strombus gigas*) culture techniques for research, stock enhancement and growout markets. Pages 127-159 in: M. Fingerman and R. Nagabhushanam (eds.). *Marine Biotechnology*. Science Publishers, Inc., St. Louis, Missouri USA.
- Shawl, A. and A. Spring. 2003. Culturing the Florida fighting conch *Strombus* alatus. Tropical Fish Hobbyist L1 5:94-97.
- Shawl, A.L. and M. Davis. 2004. Captive breeding behavior of four Strombidae conch. *Journal of Shellfish Research* 23(1):157-164.
- Reed, S.E. 1995. Reproductive Anatomy and Biology of the Genus Strombus in the Caribbean: II. Females. Journal of Shellfish Research 14(2):331-336.