Culture Potential for the Four-sided Sea Cucumber, *Isostichopus badionotus*, in Bermuda: An Approach for Conserving Natural Populations

Primeras Experiencias para el Cultivo de Pepino de Mar, *Isostichopus badionotus*, en Bermuda: Un Enfoque para la Conservación de su Poblaciones Naturales

Premières Investigations sur L'élevage Larvaire du Concombre de Mer, *Isostichopus badionotus*, aux Bermudes: Le Besoin D'assurer la Conservation des Populations Naturelles

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EXTENDED ABSTRACT

Introduction

Demand in Asian countries for sea cucumbers and their products has been growing since 1980, leading to the depletion of this resource in the traditional fishing grounds close to Asia; with an ever expanding market over the last ten years, this activity has moved to new and more distant fishing grounds, leading to the overexploitation of the species worldwide (Purcell et al. 2011). Natural populations of *Isostichopus fuscus* on the Pacific coasts of Mexico and Ecuador have recently been reported to be at critical levels due to overfishing (Toral-Granda 2008). Currently, Atlantic populations of sea cucumber are being targeted, and more specifically those in the Caribbean Region. Serious concern for long-term sustainability of natural populations of the Atlantic species *Isostichopus badionotus* (four-sided sea cucumber; three-rowed sea cucumber) has been expressed by governments of the Wider Caribbean Region, as they face increasing pressure to supply the Asian market (INFOPESCA 2014). Trends in the industry suggest that high prices and overfishing of the natural sea cucumber populations will continue; for this reason, aquaculture appears to be the most sustainable source of this product in the long term. *I. badionotus* was identified by FAO as a top culture candidate in the Wider Caribbean following an aquaculture focused workshop held in Jamaica in 2010 (Lovatelli and Sarkis 2011). The four-sided sea cucumber species is widespread throughout the Caribbean (Guzman et al. 2003), occurring from North Carolina on the SE Coast of the U.S., to northern Brazil, and east to the mid-Atlantic including Bermuda, Ascension Island, and the Gulf of Guinea in western Africa (Hendler et al. 1995). *I. badionotus* is a relatively large species, attaining 45 cm in length; its larval life has been described by Zacarias-Soto et al. (2013), who confirmed that the species is easily adaptable to culture conditions resulting in competent larvae and successful metamorphosis, critical stages of the culture cycle. Their work has demonstrated the feasibility of juvenile production under controlled conditions. More research is required to further develop and optimize culture techniques for *I. badionotus*, improving survival rates and cost-effectiveness of methods at all stages.

The current study aims to scale-up the production of Atlantic sea cucumber juveniles, by adapting well tested flow-through larval systems developed for bivalve culture to this holothurians species.

Standard larval rearing conducted in static systems generally yield satisfactory results in terms of growth and survival rate; however, this method has drawbacks including a partial reliance on antibiotics and high labour demand. A flow-through system leads to an antibiotic-free system, and where labour involved in regular water changes is greatly minimized; in addition, flow-through systems provide the potential for rearing a larger number of larvae in a given space. Both labour costs and space availability are two limiting factors in developing aquaculture in the Caribbean Region, as identified by FAO (Lovatelli and Sarkis 2011).

A flow-through system designed for tropical scallop species was tested for the rearing of *I. badionotus*; its performance was compared to that obtained in a standard static rearing system, assessed by growth and survival rates to metamorphosis.

Methodology

Mature adults of *Isostichopus badionotus* were collected from the natural environment during peak spawning period; field observations indicated that natural spawning of this species in Bermuda occurred during the summer months between June and September; first release of gametes was observed 4 - 5 days prior to the full moon, with peak spawning 3 days before (Oshea Meyer, *pers.comm.*); no spawning activity was observed after the full moon. Adult sea cucumbers were washed with filtered seawater and transferred directly into 600 L spawning tanks. Both male and female individuals were placed in the same tank. Gametes were released following thermal shock, and fertilized eggs were collected by siphoning onto a 150 µm sieve; eggs were washed with 1 µm filtered seawater, and passed through a 500 µm sieve into the larval tanks to remove detritus.

Protocols for static and flow-through systems differed mainly with respect to frequency of complete water exchange. Rearing of *I. badionotus* in static systems followed standard procedures used for bivalve species where water of rearing tanks is changed 3x a week, and food is distributed by batch once a day (Sarkis and Lovatelli 2007).

A continuous flow-through system was used as described in Sarkis et al. (2006) (Figure 1); flow rate was maintained at 600 L/min. For a 480 L tank, this results in an overall capacity of 900 L over 24 h. For a standard larval density of 6 larvae/ml, a flow-through system with this flow rate enables an increase in rearing density to 10 larvae/ml. One complete water change was conducted at Day 8 after fertilization for a thorough assessment of the culture.

Temperature was maintained at ambient or 24°C. Larval length was assessed every other day to coincide with that of static system, with percent of larvae reaching settlement determined at the end of the larval life.

Food ration ranged from 15 cells/µl to a maximum of 36 cells/µl for Late Auricularia stage and decreasing to 21 cells/µl by the end of larval life. Food ration consisted of a mixture of microalgal species, *Isochrysis galbana* (clone TIso), *Chaetoceros muelleri*, and *Tetraselmis chuii*. Food ration for static system was distributed in one batch at the beginning of the day; for the flow-through system, half of the ration was given as batch and the second half given as drip-feed over a 24 hour period.

Results

Mean wet weight of I. *badionotus* broodstock was 2.28 ± 0.55 kg. Mean size of newly fertilized eggs was measured at 254.8 ± 15.5 µm. *I. badionotus* larvae followed five distinct stages of development, three stages of Auricularia, Doliolaria and Pentactula, as described by Zacarias-Soto (2013). Early Auricularia larvae developed one day after fertilization, and continued to develop through mid and Late Auricularia for the next 15 days. The first Doliolaria larvae appeared on Day 6 after fertilization, and first Pentactula were recorded on Day 13 after fertilization.

Growth rates obtained when rearing in both flow-through and static systems were comparable throughout the stages; Late Auricularia larvae reached a maximum length of 704 ± 69 µm; thereafter length decreased as Doliolaria larvae typically resumed a barrel shape (410 ± 42 µm) and metamorphosed into Pentactula. Zacarias-Soto (2013) report slightly higher lengths for all stages.

In the static system, percent survival to Day 2 Early Auricularia averaged 35%, associated with observed bacterial contamination, whereas survival rate was

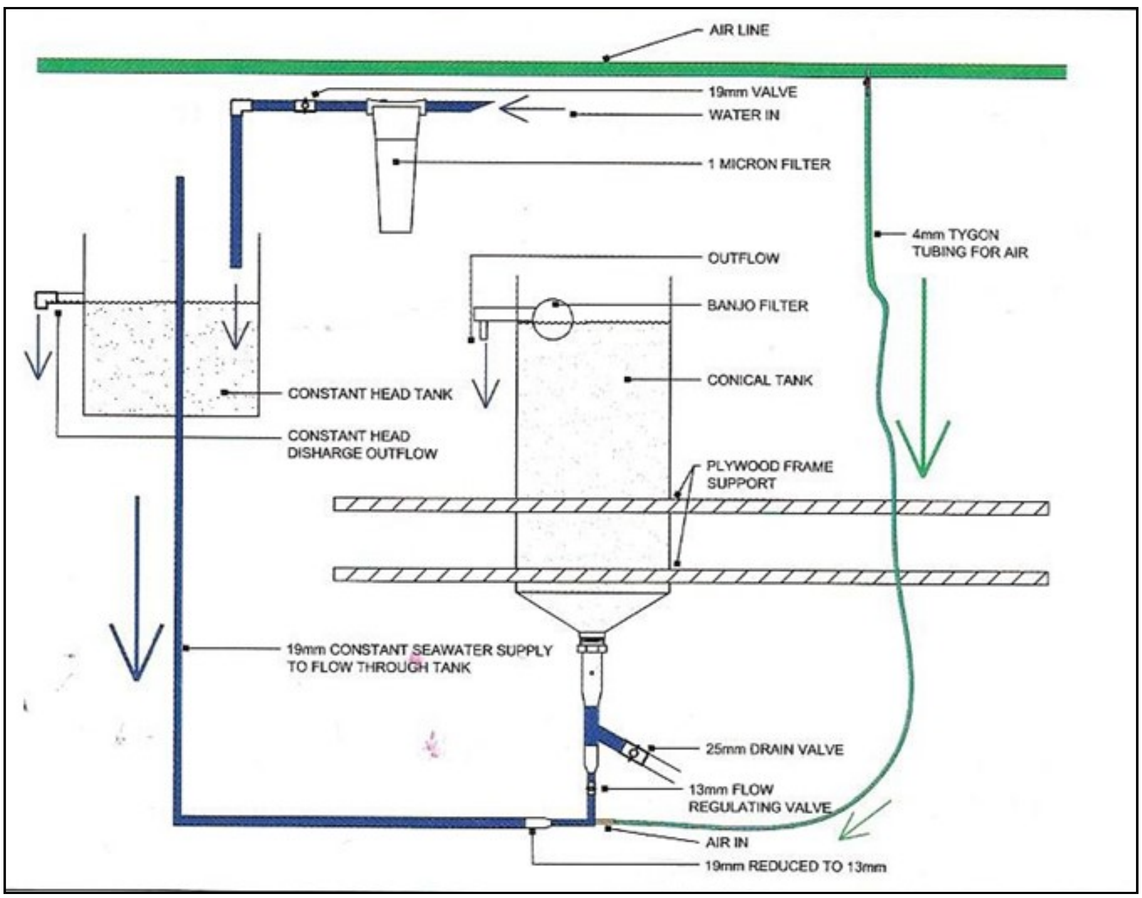

Figure1. Continuous flow-through system used for *I. badionotus* larval rearing. Diagram taken from (Sarkis et al. 2006).

markedly higher to Day 2 in flow-through systems, where no bacterial contamination was seen. Thereafter, survival rate to Late Auricularia by Day 8 was comparable in both systems (64% and 68% in static and flow-through respectively); similarly percent survival to Day 15 was comparable between the two systems 27% and 30% for static and flow-through, respectively.

The main difference between the two culture systems lay in the duration of the larval life and the time period necessary for the majority of the culture to metamorphose to the Pentactula stage; using the continuous flow system, 65% of the culture had metamorphosed to the Pentactula stage by Day 13, compared to the static system where at Day 15 < 20% of the culture was counted as Pentactula. The second difference lay in the labour involved in rearing using continuous flow; only one water change was conducted at Day 8 for the flow-through tanks, as opposed to the standard 3X a week change necessary for static tanks. Finally, heavy copepod infestation was recorded in some of the static culture tanks towards the end of larval life; whereas none were recorded in the continuous flow system.

CONCLUSIONS

The production of metamorphosed larvae for the Atlantic sea cucumber, *Isostichopus badionotus*, can be achieved using continuous flow systems in 13 days after fertilization, with one water change during this period. Duration of larval life is shorter than that obtained when culturing in static systems, with comparable percent survival rates. Further studies on food ration and composition should enable higher levels of production of metamorphosed larvae. Given these results, settlement and grow-out for the species in the Wider Caribbean Region should be further assessed to complete the culture cycle for this native species. Based on the little information available for natural sea cucumber stocks in the Wider Caribbean Region and the lack of understanding of its ecosystem function, the development of aquaculture may prove to be a more sustainable and reliable alternative supply of this species to the Asian market.

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