Culture of the Sea Cucumbers *Holothuria Mexicana, Holothuria floridana* and Hybrids and *Isostichopus Badionotus* in Former Shrimp Ponds: A Belize Case Study

Cultivo de Pepino de Mar *Holothuria floridana* y *Isostichopus badionotus* en Antiguos Estanques de Camarones: Un Estudio de Caso de Belice

Holothuria floridana et *Isostichopus badionotus* Culture d'Holothuries dans d'Anciens Étangs à Crevettes: Une Étude de Cas Réalisée au Belize

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ABSTRACT

The culture of *Holothuria mexicana*, *Holothuria floridana* and their hybrids and *Isostichopus badiontus* sea cucumbers was tested in former shrimp ponds at Bel-Euro Aquaculture Limited in southern Belize. Broodstock were collected from the wild, acclimated and introduced in two former shrimp ponds; for survival and spawning purposes. Juveniles were also collected from the wild, acclimated and introduced in two former ponds for growth observations. *I. badionotus* broodstock, and juveniles did not survive pond conditions. *H. mexicana*, *H. floridana* and their hybrids broodstock were observed spawning naturally in the ponds in the months of July-September, 2017 resulting in naturally pond raised juveniles. Juveniles collected from the wild obtained total growth of 96% in three months. Pond conditions fluctuated with temperature ranges of 26°C - 34°C, salinity ranges of 14-32 psu and 2 - 7mg/L dissolved oxygen; both broodstock and juveniles survived at all temperature and dissolved oxygen conditions but not at < 20 psu in salinity. The June - November rainy season and October - February rainy cold fronts in 2017 - 2018 decreased pond salinity; a shortcoming can be minimized by doing more water exchange and by directly abstracting water from the sea than from a nearby natural canal. *H. mexicana*, *H. floridana* and their hybrids culture is viable; the culture of *I. badionotus* in former shrimp ponds requires more studies.

KEYWORDS: *Holothuria floridana, Isostichopus badionotus*, aquaculture

INTRODUCTION

The culture of sea cucumbers have been tried in a few countries, including the culture of *Holothuria leucospilota* (Yu et al. 2012), *Stichopus janponicus* (Zhou et al. 2006) and *Apostichopus japonicus* (Chang et al. 2004) in China; the former for about 30 years (Sui 2004)*, Holothuria scabra* in Vietnam (Pitt and Quang Duy 2004), in Australia (Giraspy and Ivy 2005)*,* in Solomon Islands (Battaglene et al. 1999), in India (James et al. 1994) and in Thailand (Sithisak et al. 2013) among other countries; *Holothuria spinifera* in India (Asha and Muthiah 2005)*; Parastichopus californicus* in Canada (Paltzat et al. 2008); *Isostichopus fuscus* in Equador and Mexico (Mercier et al. 2014); *Isostichopus badionotus* in Mexico (Zacarias-Soto et al. 2013), and in Bermuda (Sarkis 2015) and *Holothuria floridana* in Mexico (Aquacultura Dos Mil, personal communication, December 2017), among other species. On-going sea cucumber studies look at diets (Seo and Lee 2011), temperature effects on growth (An et al. 2007), stocking density (Qin et al. 2009), co-culture e.g. with Red Tilapia (Sithisak et al. 2013) among other studies. The results of studies that look at sea cucumber co-culture with juvenile blue shrimp (Purcell et al. 2006) and polyculture with the green-lipped mussel (Slater and Carton 2007) seem promising. The experiences shared by these countries may prove useful to countries interested in sea cucumber culture and for aquaculture farms that are interested in bio-remediation efforts or alternative species to culture.

Some of the problems found in sea cucumber culture however are the shortage of good quality broodstock (especially if catches have declined due to overexploitation) and, consequently, the production of poor quality fertilized oocytes, the inability of hatcheries to provide proper feed due to the lack of appropriate equipment and techniques, good water quality due to poor design and inappropriate selection of the farming site and the emergence of diseases such as “stomach atrophy”, “stomach ulcer” and “body ulcer” (Chang et al. 2004)*,* among others*.* Purcell et al. (2012) completed a comprehensive review of sea cucumber culture problems, including sociological issues such as governance; economics such as economic viability; technical and biological concerns such as genetic risks, spawning, diets, larval and juvenile culture, diseases and parasites, grow-out and others.

The culture of sea cucumbers is a work in progress and can help decrease wild harvests. Sea cucumber culture may also involve re-establishing commercial species where they are locally extinct due to overfishing, or release of juveniles to help restore endangered or threatened species (Bell et al. 2008). This may be useful for Belize where the two most common species *Isostichopus badionotus* and *Holothuria mexicana* declined due to overfishing and subsequent closure of the fishery (Rogers et al. 2018). Since the outbreak of shrimp disease in Belize in 2015 (McKenzie 2015) there was interest in exploring an alternative aquaculture species, in this case sea cucumbers. This study was therefore designed as a partnership with the private sector to explore the potential of sea cucumber culture in former shrimp ponds.

METHODS

Note: The sea cucumber *Holothuria mexicana, Holothuria floridana* and their hybrids are described to exist and interbreed together (Engstrom 1980, Fuente-Betancourt et al. 2001).

I. badionotus (~ 2 juveniles and ~ 60 broodstock; Figure 1A) and *H. Mexicana, H. floridana* & hybrids (~ 60 juveniles and ~ 60 broodstock) (Figure 1B) were collected from nearby Placencia Lagoon and nearby coastal waters in October 2015. Between October 2015 and March 2016, all broodstock were kept in experimental ponds (Figure 1C); Pond 1 and Pond 2 at Bel-Euro Aquaculture Limited, Toledo District, Belize. From March 2016 - July, 2018, some broodstock were kept in spawning tanks at the Bel-Euro hatchery. Ponds and hatchery tanks were monitored for natural spawning for both species. Temperature, dissolved oxygen and salinity in ponds and hatchery tanks were monitored daily. Total length and weight measurements of juveniles in ponds were taken every 2 weeks. Juveniles that resulted from natural spawning were also measured.

The ponds are located ~ 2 km from the sea. Seawater supply to the ponds flows through a ~2 km natural canal to a ~ 0.2 km dredged canal (Figure 1D) then to a reservoir which feeds all the ponds. Depending on the rainy or dry season, the water in the reservoir ranges from 18 - 34 psu. Two weeks prior to broodstock and juvenile collection, two experimental ponds were prepared (with calcium oxide to kill most microorganisms, especially parasites and to raise the pH of acidic water to a neutral or slightly alkaline value; and urea added for algae production). Three cages at different depths (4, 6 and 8 feet; Fig. 1C) were placed inside each of the ponds for easier broodstock and juvenile growth observations. Cages were cleaned weekly from barnacles and algae that grew on them. Pond 1 had mesh at the water inlet to filter sediments from going into the pond. Pond 2 had no mesh at the inlet as we assumed the larger amounts of sediments would provide feed for the sea cucumbers. All broodstock were acclimated during transport to Bel-Euro by doing sea water exchange every 20 minutes. Dried and crushed mangrove detritus (Figure 1E) was used to feed the broodstock and juveniles, along with the algae created from pond fertilization.

RESULTS AND DISCUSSION

Broodstock Survival in Former Shrimp Ponds

Overall *H. Mexicana, H. floridana & hybrids* survived better than *I. badionotus.* In pond 1, *H. mexicana* were able to tolerate slow temperature changes (26°C – 34°C), salinity fluctuations (20 psu – 35 psu) and dissolved oxygen (DO) at 4 - 8 mg/L but not at 2 - 3 conditions. *I. badionotus* acclimated well in the ponds, survived slow temperature changes (26 °C – 34°C) and DO at 4 - 8 mg/L but only survived at >29 psu salinity. Prolonged low salinity conditions (20 - 26 psu) and high temperatures (34° C) caused skin ulcerations in *H. Mexicana, H. mexicana, floridana* and hybrids (Figure 2A & B) but these recovered (Figure 2C) when water was pumped from the canal and salinity increased and temperature decreased.

From October 2015 to June 2016, no changes were observed among broodstock of both species in cages at different depths in pond 1 as temperature was kept at 30 - 32ºC, salinity at 30-34 psu and DO at 6-7 mg/L. Drastic declines in DO (if water wasn't pumped when required), may have been the primary cause for broodstock that were outside the cages to migrate towards the inlet canal and many times were found outside the pond when the low tides occurred (they weren't able to find their way back into the pond; Figures 2 D & E); and sometimes perished if not found in time. Prolonged low DO, extreme high temperatures and prolonged low salinities caused broodstock to perish (Figures 2F & G). These concerns can be addressed by redesigning the pond by having a deeper inlet side with steeper slope and by doing water exchange more often in order to maintain constant temperature and salinity i.e. once or twice a week or when required. The rainy season from June 2016 to November 2016 decreased salinity (28 psu) and temperature (30 ºC) but didn't affect the broodstock. The cold fronts from November 2016 to January 2017 decreased tempeture (28 ºC) but didn't affect the broodstock either. Broodstock weren't affected unless the pond conditions weren't favorable (if no water exchange was done). However, the unusual cold and rainy weather conditions from October 2017 – February 2018 lowered the temperature (26 - 28°C) and salinity in the ponds (14 – 20) psu. No broodstock survived these conditions. Although salinity was low in the ponds, it was only slightly higher in the inlet canal (28 psu). A few crabs and fish were observed in pond 1. The crabs were observed biting the broodstock but no negative impacts on the broodstock were proven.

Pond 2 had conditions slightly different than pond 1. Temperatures ranged from 25°C – 35°C), salinity fluctuations (20 psu – 35 psu) and dissolved oxygen (DO) at 1.5 - 9.5 mg/L. *I badionotus* and *H. mexicana*, *H. floridana* and their hybrids survived and behaved similar as in pond 1.

Figure 1 A. I. badionotus broodstock; **B**. *H. mexicana, H. floridana* and their hybrids broodstock; **C**. Pond 1 with cages containing broodstock; **D**. dredged canal that brings water from the natural sea canal to be pumped into ponds ; **E**. Mangrove detritus (contains sand) was used to feed broodstock in ponds

They also migrated towards the inlet canal in low dissolved oxygen and low tides. Initially, the water was clear and many more species of fish, crabs and other organisms were seen inside the pond (than in pond 1). After the first month, the visibility became extremely low due to the accumulated sediments from the inlet canal (since the inlet canal didn't have a mesh to filter sediments). The accumulation of excess sediments and extreme low DO caused all the broodstock to perish after 6 months. This concern can be addressed by having filters at the water inlet to filter sediments and large organisms. The filter however must be brushed every hour during water exchange otherwise it becomes clogged by sediments (Figure 2H).

Broodstock Natural Spawning in Former Shrimp Ponds

H. mexicana, H. floridana & hybrids were observed spawning naturally in pond 1 in May, 2017 (Figure 2I) and in July, August and September 2017. These natural spawning observations correspond with spawning months

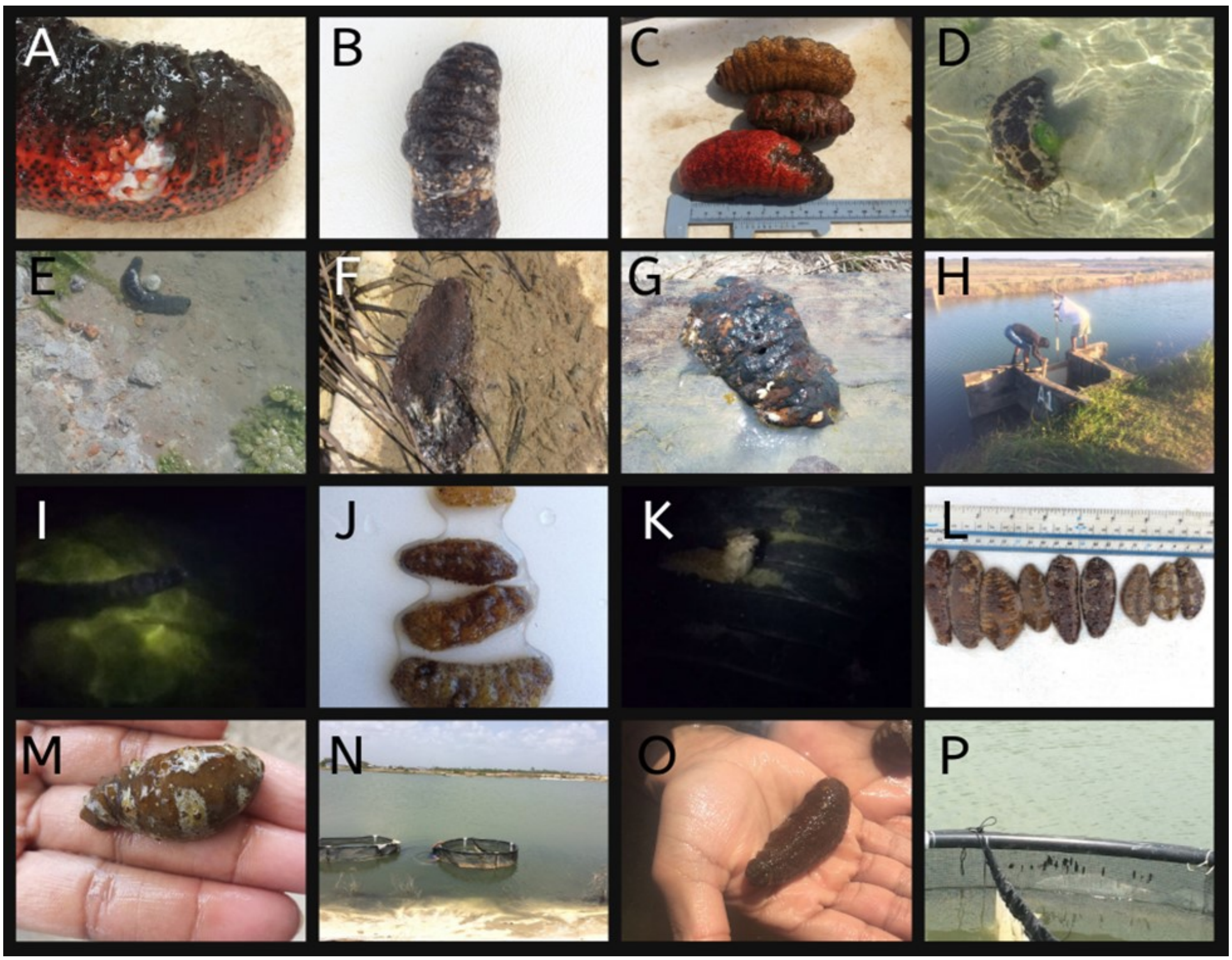

Figure 2. A. & B. Skin ulcers on *H. floridana* and *H, mexicana*; **C.** *H. floridana* and *H, mexicana* recovered from skin ulcers; **D.** I. badionotus near water inlet in pond 1; **E.** *H. mexicana* near water inlet in pond 1; **F.** Desiccated *I. badionotus* near water inlet in pond 1 in low tide; **G.** *H. mexicana* didn't surivive low salinity and DO in ponds; **H.** workers cleaning mesh at pond water inlet (water is pumped from the reservoir seen on the background); **I.** *H. mexicana* spawning naturally in ponds; **J.** *H. mexicana, H. floridana* and hybrids juveniles resulting from natural spawning in ponds; **K.** *I. badionotus* spawning naturally in pond 1; **L. & M.** *H. mexicana, H. floridana* and hybrids from the wild; **N.** Cages near inlet canal where *H. mexicana, H. floridana* and hybrids from the wild were raised; **O.** Larger *H. mexicana* after 3 months growth in cages in ponds; **P.** *Dessicated H. mexicana, H. floridana* and hybrids on cage walls.

documented for *H. mexicana* in 2014 – 2015 in Belize (Rogers et al. 2018). About 86 juvenile *H. Mexicana, H. floridana* & *hybrids* (Figure 2J) that resulted from these and from other possible spawning were found around the broodstock cages in pond 1. When first found in July 2017, these juvenile were on average 4 cm in length and weighed 0.1g – 4 g; by the end of September, they were on average 6 cm in length and weighted on average 8 g; suggesting a possible growth rate of 2 g/ind/month. This growth rate was much smaller than the growth rate of juvenile *H. scabra* raised in earthen ponds in New Caledonia which had a growth rate of 30 - 35 g/ind/mo(Purcell, 2004). No additional feed or urea was added to the ponds; these juveniles fed from the sediment in the ponds and mangrove detritus remains used to feed juveniles obtained from the wild. Similar to the adults, these juveniles did not survive low salinity that resulted from the unusual cold and rainy weather in October 2017. Placing broodstock in ponds to spawn naturally in ponds may be a consideration for raising juveniles and maintaining broodstock; this minimize hatchery expenses in maintaining broostock and raising juveniles. A consideration for maintaining adequate pond conditions is to abstract water directly from the sea instead of the natural canal. Seawater has higher salinity but investments in abstracting seawater 2 km away may be high, and even higher when unusual weather decreases salinity and seawater must be pumped immediately. The income to be generated however seems to outweigh the expenses (Gregoire 2017). *I. badionotus* were observed spawning (Figure 2K) in pond 1 in July 2017. No *I. badionotus* larvae or juvenile was found.

Wild Juvenile Survival and Growth in Former Shrimp Ponds

The two *I. badionotus* collected from the wild didn't survive the acclimation stage. *H. Mexicana, H. floridana* & *hybrids* juveniles (Figures 2L and M) that were collected from the wild were monitored for growth in shallow cages near the inlet canal (Figure 2N) in pond 1 and in pond 2. They were fed with mangrove detritus and sand; however they also fed from the algae produced in the pond from urea added to the ponds. They all survived the beginning of the dry season and had a growth rate of 96.6% for the first three months (Figure 2O); had an initial average length of 8 cm average weight of 4 g. As the water level in the ponds decreased (due to evaporation, low tides), and dissolved oxygen (DO) decreased, juveniles surfaced on the walls of the cage to possibly obtain oxygen. The prolonged low DO and continued evaporation due to the heat, the juveniles desiccated on the walls of the cages (Figure 2P). They survived low salinities of 20 psu but not any less. Optimum pond conditions can be maintained by abstracting water directly from the sea and by doing water exchange more often.

CONCLUSION AND RECOMMENDATIONS

The culture of *H. mexicana, H. floridana* x hybrids in ponds is viable. The culture of *I. badionotus* in ponds requires more experimentation and may require more investment. Overall, the major challenge for broodstock survival in ponds was the expense of doing water exchange and the low salinities and dissolved oxygen. Investments in abstracting water directly from the sea may be considered and weekly water exchange or when necessary e.g. after heavy rains must be done. Having mesh the water inlet and brushing it hourly while doing water exchange is necessary to prevent sediment accumulation in the ponds and to filter out large organisms. Other water quality parameters should also be monitored e.g. ammonia, pH, total suspended solids (TSS), biological oxygen demand (BOD), nitrite, nitrate, turbidity, orthophosphate. Additional studies on natural spawning in ponds and studies that look at the growth of the resulting juveniles is recommended.

ACKNOWLEDGEMENTS

Thanks to the Belize Fisheries Department, the Ministry of Economic Development, Beltraide, John Sansone, Benjamin Jardim, Zachary Jardim, Luis Mejia, Eric Pixabaj and Kwame Raynolds of Bel-Euro Aquaculture Limited. Thanks to the UBERI student volunteers: Lenin Carias, Kevin Novelo, Sherman Cawich, Leanna Aranda & Janel McNab.

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