Larval substrate preference and the effects of food availability in the invasive tunicate *Styela clava*

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Abstract

Styela clava is a highly invasive solidary tunicate originating from the Northwestern Pacific Ocean. It has found its way due to inadvertent transport by humans around the globe where it can now be found in temperate waters everywhere. *S.clava*, while being invasive, is of economic importance in some Asian cultures where it eaten by many and considered to be a delicacy. It is estimated to fetch a retail price between $8 and $12 a pound. This has led to a need for aquaculture methods to be produced for economic growth in this untapped market. This experiment focused on the larval substrate preference on manmade materials as well as finding the optimal level of food to maximize growth in juveniles. To run this experiment 30 adult *Styela clava* were brought back to the lab and stripped of their gametes. The gametes were then mixed and checked for fertilization. Once fertilized the larvae were placed into three experimental tanks. In each 3L tank there was presented rope, tile, wood, or aluminum for the larvae to settle upon. When the larvae had settled out of the water column the second part of the experiment began. At his point the newly settled juveniles were fed 12mL, 24mL, or 48mL of the phytoplankton *Tisochrysis lutea* daily. The results of this experiment were inconclusive due to the death of the settled juveniles early on in the experiment. It was hypothesized that the deaths were due to high ammonia levels in the tanks. More studies will need to be conducted with refined methods to answer the questions set forth in this experiment.

Introduction

Styela clava, a native off the Northwestern Pacific Ocean, is a highly invasive ascidian species that is now found in all corners of the globe (Davis, 2007). It is commonly referred to as the "Clubbed Tunicate" due to its long stalk, brownish color, and overall club-like shape. The mature adult can reach a max length of 200 mm and is commonly found in waters under 25 m deep (McClary, 2008). It was first documented in Connecticut waters in the 1990's and is currently found all over Long Island Sound (Brunetti & Cuomo, 2014). *Styela clava* grows with ease on manmade materials leading to its accidental transportation on boat hulls and lines all across the world (Darbyson, 2009). *Styela clava* is an efficient filter feeder and can outcompete native economically important filter-feeders such as shellfish (Peterson, 2007). While most of the world finds this filter feeder to be a nuisance due to its fouling ability there is, perhaps surprisingly, a demand for this species within the Asian food market.

In South Korea *Styela clava* is considered to be a seafood delicacy and also an aphrodisiac (Karney 2009). In the local markets frozen *Styela clava* fetches a retail price of $8 - $12 per pound giving rise to much higher estimates of price for freshly aquacultured ones (Karney 2009). This has led to a call for the development of methods to raise *Styela clava* in captivity which would provide a higher quality, cleaner product than one harvested from potentially polluted waters. Yet almost all of the research available is geared towards the concept of invasive species eradication rather than towards the aquaculture of *Styela clava*.

In this study the Settlement preference of larval *S.Clava* is going to be explored on manmade materials (rope, aluminum, wood, and ceramic tile) in a closed system. After larvae settlement has occurred another study will be conducted to find the food abundance level for optimum growth in newly settled juveniles.

Objectives

The objectives of this study are:

- To determine the larval settlement substrate preference (line, aluminum, wood, or ceramic tile) of *Styela clava* in a closed system.
- To determine the level of food (phytoplankton/zooplankton) needed to bring about optimal growth in juvenile *S. clava*

Methods

Thirty adult *Styela clava* were collected from floating dock lines in Darien CT. Immediately after removal from the line, each tunicate was placed in a 5 gallon bucket filled with seawater from the same area. All collected tunicates were transported to the laboratory at UNH where their gametes were stripped following the protocols of Bullard & Whitlatch (2004). The stripped gametes were placed in a 4L container filled with artificial seawater and an airstone for 48 hours. Aliquots of seawater were examined under a microscope for the presence of *S.clava* larvae over the next 48 hours. At the end of the 48 hour period, an abundance of *Styela clava* larvae (~ 400 per mL) were observed and the experiment was initiated. The water from the larvae container was divided equally into the 3 containers (3 L). Artificial saltwater (27 ppt) was added to each container raising the water level to 3 L per container. Into each container was placed a length of dock line, a

wooden dowel, an aluminum tube, and a ceramic tile to serve as a settlement surface. All of these materials had been allowed to soak in seawater for 1 month prior to the start in order to develop a biofilm. Water from each container was sampled every 24 hours to determine if and when larval settlement occurred. By 48 hours the water was clear of larvae indicating settlement had taken occurred.

Figure.1 The Collection site for the adult *S.clava* was on private owned land in Darien CT off of ropes on an oyster hatchery.

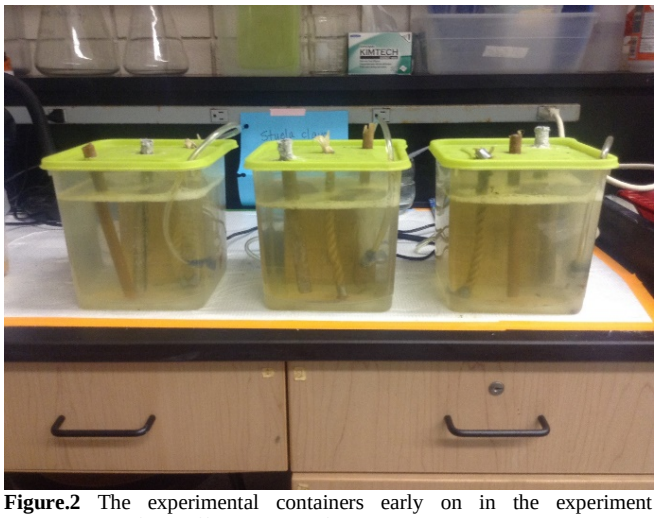

Figure.2 The experimental containers early on in the experiment containing the four different types of manmade substrate. The containers starting on the left were fed 12ml, 24ml, and 48mL of Tisochrysis lutea

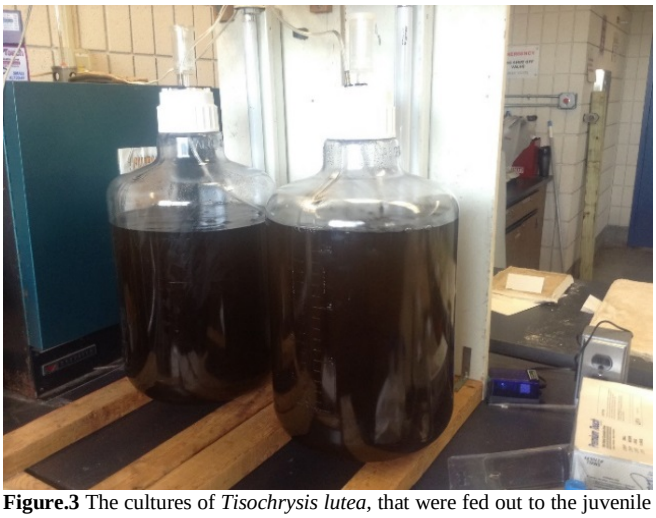

Figure.3 The cultures of *Tisochrysis lutea*, that were fed out to the juvenile *S.clava*, thriving in the 20gal Carboys.

At the conclusion of the settlement portion of the experiment, an airstone was placed into each of the 3 L containers and the feeding experiment commenced. Each container was fed a different amount (12mL, 24mL, 48mL) of the phytoplankton *Tisochrysis lutea* daily, which was grown in culture in the lab. *T.iso* feedings were supplemented by the addition (12ml, 24 ml, and 48 ml) of a commercial phytoplankton and zooplankton mixture in order to provide adequate nutrition to the settled organisms. The cultures of *T.iso* were obtained from NOAA at the National Marine Fisheries Milford branch.

Results

Upon evenly dividing the active *S. clava* larvae into the three containers for settlement, the larvae successfully settled onto the various substrates. However by the time the settled juveniles reached a size that could be accurately quantified, their presence diminished until there were none left.

All of the materials in the experimental tanks upon conclusion of the experiment showed no signs of juvenile *S.clava.* In figure.4 it can be seen that a small layer of detritus formed over the surface of the tile and in figure.5 the amount of detritus formed on the substrate was lower.

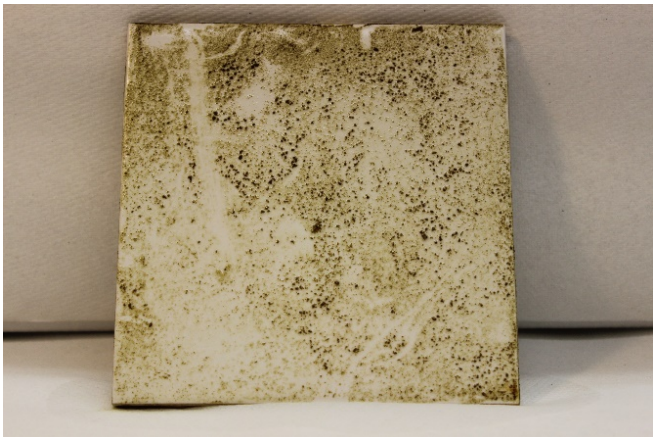

Figure.4 The ceramic plate in the tank being fed 48mL of *Tisochrysis lutea*, showing no signs of juvenile *S.clava* upon conclusion of the experiment

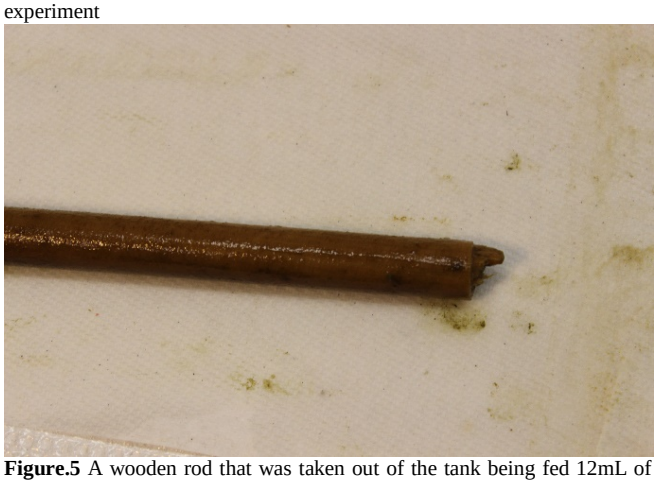

Figure.5 A wooden rod that was taken out of the tank being fed 12mL of *Tisochrysis lutea* showing no signs of *S.clava* juveniles.

Discussion

When the *S. clava* larvae were poured into the 3 L containers, aliquots were taken out and observed under a microscope to observe the presence of larvae. Within 48 hours all larvae had successfully settled and none remained in the water column. At one day old the juveniles are a size of 0.29mm (Bullard & Whitlatch 2004). Given the size and colors of the substrate choices, this becomes problematic for counting under a Dissecting Microscope. After the juveniles were given 1 month to continue to grow, the containers were checked to find that there had been significant mortalities (Figure.4 and Figure.5) and the experiment was concluded.

It has been postulated that the cause of the mortalities in the containers were due to hyperammonaemia. Ammonia is continuously created in aquatic organisms and can be reduced by frequent water changes. In this experiment the water was changed twice a week to prevent ammonia levels from becoming toxic. It is possible that this was not frequent enough and the levels became toxic to the sensitive juveniles. In future studies the frequency of water changes should be increased along with continuous monitoring. On top of monitoring ammonia in future studies the experiment should be scaled down and conducted in smaller vessels with strips of the manmade substrates. This will make quantifying settled juveniles substantially easier and observable under a dissecting microscope.

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Biography

Matt Bodnar Is currently a senior at the University of New Haven where he is finishing up his ungraduated degree in Marine Biology. He plans on going to graduate school and pursuing an interest in the life strategies of fish. He also served in the United States Marine Corps where he did a tour overseas in Afghanistan as a wrecker and mechanic.