

# **Conditioning the spiny scallop (*Chlamys hastate*) using microalgae**

FISH 491 Research Project

Submitted to Dr. Helen Gurney-Smith

Written by Daniel McNeill

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Research Advisor: Helen Gurney-Smith, CSR, Vancouver Island University

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**Abstract:** It is well documented in the scientific literature that manipulation of diets can affect gonadal development in bivalve shellfish. This process can be used in aquaculture to enable more than one reproductive event throughout a year. In our study, we examined the effects of microalgal diet ration on spiny scallop (*Chlamys hastata*) conditioning, as shown by gonad, somatic, and whole-body tissue wet and dry weights. Three diet rations were fed to scallops held at a constant temperature of 12°C over an eight-week period and were compared to wild scallops of the same population (the wild individuals were collected at the start and end of the experiment and used as controls in comparison to the cultured scallops in the laboratory). *Isochrysis* sp. (Tahitian strain: T-iso) and *Chaetoceros muelleri* (Cm) were fed at rations of 0.5%, 1.5%, and 2.5% dry weight of algae per day as a proportion of the dry tissue scallop weight. We found that the dietary ration of micro algae fed to *Chlamys hastata* during reproductive conditioning directly affected the rate of gonadal development. The 2.5% ration produced the greatest gain in average gonad dry weight ( $0.7769 \pm 0.2631$  g) of the three tested diets, and was higher than the scallops from the wild ( $0.4555 \pm 0.120$  g) at the end of the experiment (week eight). The 1.5% ration treatment showed an intermediate increase in average gonad dry weight ( $0.5519 \pm 0.1829$  g) when compared to the wild scallops. The 0.5% ration produced a lighter mean gonad dry weight ( $0.3986 \pm 0.1207$  g) when compared to the wild scallops at the end of the experiment, but this value increased from the original wild scallop gonad dry weight ( $0.2949 \pm 0.128$  g) sampled at the start of the experiment, indicating that un-manipulated rations in the wild either exceeded a 0.5% ration, or contained preferential food for gonad development. We found that the rate of gonad development of the spiny scallop was directly correlated to the quantity of micro algae

proffered, indicating that this methodology may be suitable for conditioning this species to increase fecundity for aquaculture purposes.

## **Introduction:**

The spiny scallop (*Chlamys hastata*) is a native species to the coast of British Columbia. It is found as far north as Kodiak Island, Alaska and as far south as San Diego, California in areas with high currents, commonly on gravel or rock substrates (Bernard 1983). It lives in depths from 2 to 150 m and can survive in temperatures ranging from 0 to 23°C (Bernard 1983). *Chlamys hastata* lives to an age of six years and rarely exceeds a shell height of 80 mm (MacDonald *et al.* 1991), reaching maturity by 35 mm shell height at three years old (Lauzier and Parker 1999). The commercial fishery has a minimum size limit of 55 mm shell height, allowing the species to spawn at least once before recruitment to the fishery (Lauzier and Parker 1999). Spawning occurs once during the year, usually between July and August (MacDonald *et al.* 1991). Gamete production in *C. hastata* is greater than in any other *Chlamys* species (MacDonald *et al.* 1991). This production does not exceed somatic growth, however, until the last year of life (six years old) when reproductive effort is between 60 and 70% (MacDonald *et al.* 1991). Mean somatic weight and shell height after two years of growth were found to be significantly greater in *C. hastata* than in the naturally co-occurring *C. rubida* (MacDonald *et al.* 1991), making *C. hastata* a better candidate for aquaculture of the two native *Chlamys* genus.

Broodstock conditioning consists of placing adult animals in a controlled environment and manipulating different parameters to encourage gamete development. The purpose is to maximize fecundity and improve egg and sperm quality for spawning, and therefore increase the potential success of the production of viable offspring. For bivalves, the most important factors affecting gonadal development are temperature and diet (Liu *et al.* 2008). Microalgae plays a critical role in conditioning of scallop broodstock, as they influences reproductive performance and improves the quality of the eggs and larvae (Uriarte *et al.* 2004). The quality of micro-algae has been found to significantly affect the rate of gonad development and mixed diets of high lipid quantity have been shown to give better results than single algal species diets (Utting and Millican, 1997).

Microalgae vary considerably in their biochemical composition and nutritional properties (Brown *et al.* 1997). Essential polyunsaturated fatty acids (PUFAs) are important for bivalve growth and development (Liu *et al.* 2009). Essential fatty acids like eicosapentaenoic acid (EPA, 20:4n – 3), docosahexaenoic acid (DHA, 22:6n – 3) and arachadonic acid (20:4n – 6) are critical in production and viability of scallop eggs and embryos (Utting and Millican, 1997). High levels of DHA have been found in the flagellate *Isochrysis* sp. (Tahitian strain: T-iso) (Utting and Millican, 1997). Soudant *et al.* (1996a) reported that T-iso improved the maturity of *Pecten maximus* broodstock and increased the percentage of eggs that developed into D-larvae. The diatom, *Chaetoceros muelleri* contains high levels of both carbohydrates and EPA (Volkman *et al.* 1989). Fecundity was greatest in the scallop *Mimachlamys asperrima* when a *Chaetoceros*

species was used in a conditioning study by O'Connor *et al.* (2000). Fearman *et al.* (2009) showed a significant increase in glycogen levels when using a *Chaetoceros* species to condition the mussel *Mytilus galloprovincialis*.

Algal ration may also affect shellfish. Utting and Millican (1997) reported that a 3% diet ration was sufficient for *P. maximus* when reared at a maximum of 16°C. Liu *et al.* (2008) showed that the best absorption rate for adult *Clinocardium nuttallii* was 5% food ration of their dry weight when rearing them at 14 degrees. Fearman *et al.* (2009) was successful in spawning *Mytilus galloprovincialis* when conditioning at a fed rate of 2% dry algae weight to dry mussel weight.

This study was designed to evaluate the effects of three dietary rations on gonad and somatic growth in the scallop *C. hastata*. The main objective was to examine if this scallop species can be conditioned outside its normal spawning cycle in a controlled environment and, if so, how effective are these rations in increasing the rate of gonadal development in comparison to the wild population.

## **Materials and Methods:**

A total of 600 spiny scallops, *C. hastata*, were collected by SCUBA divers at a depth of 30 m on December 13, 2009 and February 25, 2010 near Hammond Bay, Nanaimo, British Columbia (49 14.036'N, 123 56.789'W) (Figure 1). The scallops were placed in Vexar mesh bags, held in a cooler with paper towels soaked in ambient sea water and transferred to the Centre for Shellfish Research (CSR) at the Nanaimo campus

of Vancouver Island University (VIU). At the lab, sponges (*Mycale adhaerens*) growing on the shells of the scallops were gently removed using a firm-bristled brush.

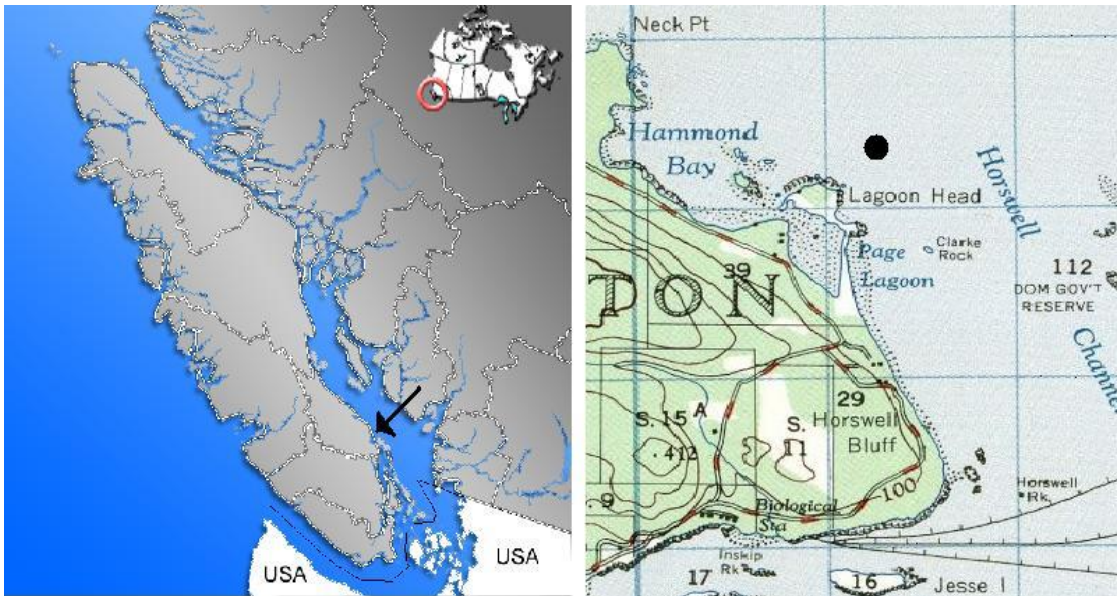


Figure 1. Left: General map view showing location of Vancouver Island and Nanaimo, B.C. (←). Right: Detailed map showing exact location of the collection site off Piper's Lagoon (●).

Total wet weight, shell height, gonad wet weight, and somatic tissue wet weight were measured from 15 randomly-chosen at the start of the experiment. Tools for sampling the scallops included tinfoil crucibles, scalpels, dissection trays, standard calipers and a four digit electronic balance (Figure 2). Dry tissue weights were recorded after the tissue was placed in a drying oven for 48 h at 85°C, when the tissue reached a constant dry weight. Dry weight values of these scallops were used in combination with dry weight of micro algae to determine the amount of algae to feed.

Additionally, a total of nine scallops per treatment (three scallops per tank) were sampled at weeks 4 and 8 for the measurements noted above. The volume of algae fed was adjusted after each sampling period to account for removal of scallops and for any

increases or decreases in body weights in order to maintain the proper rationing ratios. Ten histological tissue samples were fixed in Davidson's solution at weeks 0 and 8 of the experiment. The histological procedures followed those outlined in the "Histological Techniques for Marine Bivalve Mollusks and Crustaceans" by Howard *et al.* (2004). These samples were never analyzed because of time restrictions.



Figure 2. Sampling equipment.

The scallops were randomly selected and divided up among nine oval tanks, each containing 20 scallops per tank. Each tank contained 100 L of re-circulating sea water, sourced from the Pacific Biological Station, Department of Fisheries and Oceans Canada, Nanaimo, B.C. Feeding buckets were placed above the tanks to constantly drip microalgae into the scallop tanks (Figure 3). A continuous flow of 0.4 L min was recirculated through each tank at ambient (12 °C) temperature that fluctuated up and

down by 1 °C throughout the experiment. Lights were turned off at the end of each day in the room where the scallops were held, the lights were on from 8:30 am to 4:30 pm.

The scallops were fed daily. Three ration treatments of microalgae were tested in randomly distributed triplicate tanks. *Isochrysis* sp. (Tahitian strain: T-iso) and *Chaetoceros muelleri* (Cm) were fed at rations of 0.5%, 1.5%, and 2.5% (dry weight of algae per dry weight of scallop tissues per day). The formula used was

$$\text{Volume of Algae to feed per tank (L)} = \frac{((\text{Desired Ration (\%BW/D)} \times (\text{Total Dry Meat of Biomass (g)} \times 1000) / 100) / \text{Dry Organic Weight of 1 million algal cells (mg)})}{\text{Cell count of Algae Colony Feeding from (million cells per mL) taken from (Helm *et al.* 2004)}}$$

Algae counts were made using a hemocytometer, and this allowed us to calculate algae cells per mL. The micro-algae were reared in 250-mL flasks, 1-L flasks, 18-L carboys, 220-L columns, and 350-L bags. Inoculations of the micro algae occurred on every Monday, Wednesday, and Friday. Algae culture procedures followed those outlined in the “Standard Operating Procedures for Culturing Microalgae at the Centre for Shellfish Research at Vancouver Island University” by Vandenberg (2008).

Scallop husbandry methods included weekly cleaning of the scallop tanks, siphoning waste and removing byssal threads attached to the sides of the tanks. Cleaning the algae buckets was also performed twice a week. Flow rates and temperature were

monitored throughout the experiment and were adjusted accordingly. Dead scallops were recorded and removed from the tank (but not replaced).



Figure 3. Tank set up and algae buckets used in the experiment.

Current with this diet-ration experiment a separate temperature experiment was performed. The experimental set-up was essentially the same time in the two experiments, the temperature experiment testing the effects of three temperatures 8, 12, and 16 °C at a feeding ration of 1.5% on gonad development *C. hastate* (Halper. 2010). Reference to this separate experiment will be made in the current paper.

For statistical data analysis, Microsoft Excel was used for storing raw data and producing graphical results. The software NCSS (2007 edition) was used for conducting one-way ANOVA tests and also for Tukey-Kramer analyses.

## Results

The effect of diet on scallop tissue production was assessed over an eight-week period. Dry weights of the dissected gonad, somatic, and whole-body tissues were taken at the start of the experiment and at weeks 4 and 8. Three scallops from each tank were sampled for dry weights, so therefore there was a total of nine scallops per treatment. Histology samples were taken at weeks 0 and 8 from both wild and cultured animals; although, we didn't have time to examine these samples.

### *(a) Effect of diet rations on whole-body tissue mass*

The average whole body dry tissue weight at the start of the experiment was  $2.805 \pm 0.513$  g. By week 4, the cultured scallops' average whole-body dry tissue weights were  $2.9205 \pm 0.6208$  g for the 0.5% ration,  $2.7034 \pm 0.8276$  g for the 1.5% ration, and  $3.084 \pm 0.4453$  g for the 2.5% ration. By this time, scallops in both the 0.5 and the 2.5% rations showed an increase in weight compared to wild scallops sampled at the start of the experiment, while scallops in the 1.5% ration decreased in weight. Cultured scallops at week 8 had mean whole body-dry tissue weights of  $2.3549 \pm 0.4713$  g in the 0.5% ration,  $2.8798 \pm 0.5057$  g in the 1.5% ration, and  $3.1959 \pm 0.5287$  g in the 2.5% ration. Wild scallops sampled at week 8 had an average whole-body dry tissue weight of  $2.689 \pm 0.327$  g. Comparing the cultured scallops to wild scallops in week 8 showed that individuals in the 1.5 and 2.5% rations had greater whole-body dry tissue weights than the wild scallops, while those in the 0.5% ration had lower weight than the wild scallops (Figure 4). The 2.5% ration diet was the only treatment where scallops continued to increase in whole-body dry tissue weight throughout the experiment. A one-way

ANOVA on week 8 data showed that there was a significant difference among the different diets and Tukey-Kramer analysis revealed that this difference was between the 0.5% treatment and the 2.5% treatment (Table1).

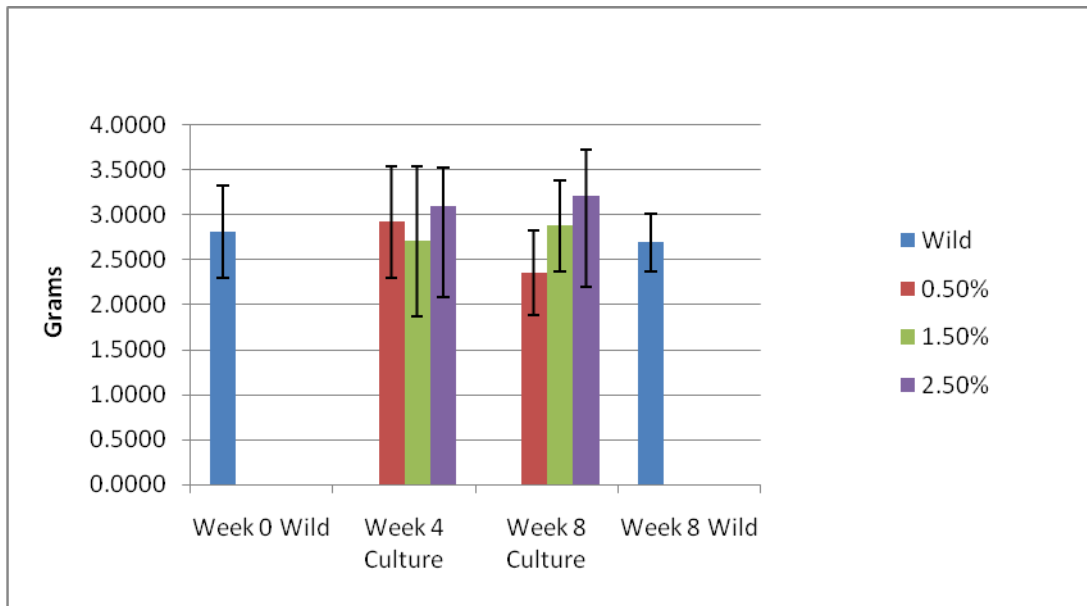
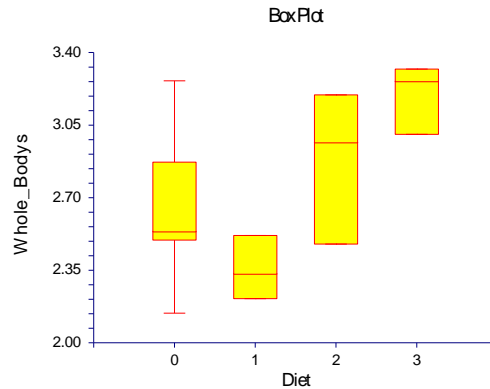


Figure 4. Average whole-body dry tissue weights throughout the experiment.

Table 1. ANOVA table of whole-body dry tissue weights at week 8. Below the table is the associated box plot with; 0 = wild, 1 = 0.5% ration, 2 = 1.5% ration, and 3 = 2.5% ration.

Analysis of Variance Table						
Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power ( $\alpha = 0.05$ )
A: Diet	3	1.165665	0.388555	4.16	0.019167*	0.772823
S(A)	20	1.866902	9.334511E-02			
Total (Adjusted)	23	3.032567				
Total	24					

\* Term significant at alpha = 0.05



*(b) Effect of diet rations on somatic tissue mass*

Throughout the experiment, the somatic tissue (primarily the adductor muscle) showed a steady decrease in dry weights for all of the cultured and wild scallops, suggesting that the scallops were taking energy (glycogen) from the muscle and transferring it to the gonads. Week 0 had the heaviest recorded average dry weights for the somatic tissue throughout the entire experiment which was  $1.287 \pm 0.247$  g. Cultured scallops at week 4 had average somatic tissue dry weights of  $1.1792 \pm 0.3100$  g in the 0.5% ration,  $1.1186 \pm 0.3479$  g in the 1.5% ration, and  $1.2848 \pm 0.2780$  g in the 2.5% ration. At week 8, the wild scallops' mean somatic tissue dry weight was  $1.089 \pm 0.140$  g. Also during week 8, the cultured scallop average dry weight for the somatic tissue was  $0.9010 \pm 0.2344$  g for the 0.5% ration,  $1.0560 \pm 0.2469$  g for the 1.5% ration, and  $1.1021 \pm 0.2579$  g for the 2.5% ration (Figure 5). According to a one-way ANOVA, there was no significant difference at week 8 between treatments and the wild scallops for the somatic dry weight (Table 2).

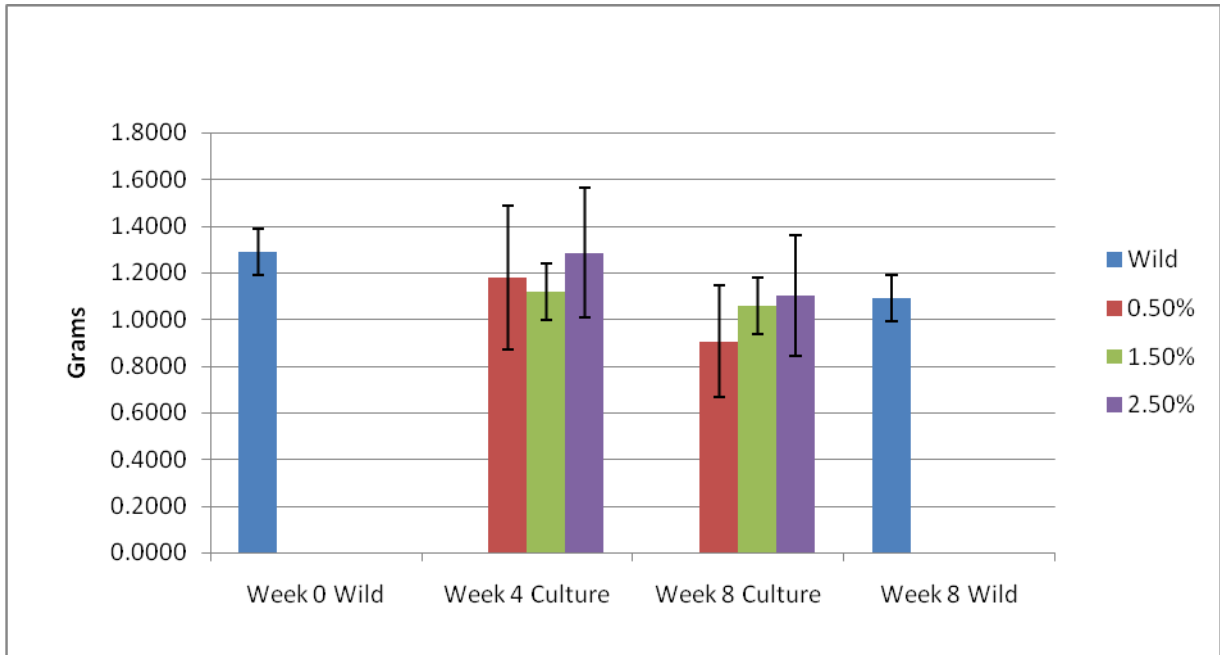


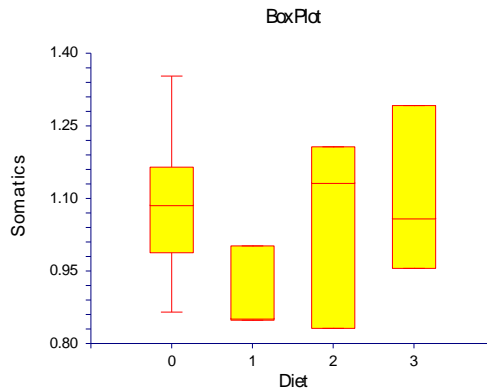
Figure 5. Average somatic dry tissue weights throughout the experiment.

Table 2. ANOVA table of somatic tissue dry weights at week 8. Below the table is the associated box plot with; 0 = wild, 1 = 0.5% ration, 2 = 1.5% ration, and 3 = 2.5% ration.

**Analysis of Variance Table**

Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power ( $\alpha=0.05$ )
A: Diet	3	9.419486E-02	3.139829E-02	1.46	0.254751	0.327135
S(A)	20	0.4293056	2.146528E-02			
Total (Adjusted)	23	0.5235004				
Total	24					

\* Term significant at alpha = 0.05



(c) *Effect of diet rations on gonadal tissue mass*

Scallops in the 2.5% ration had the heaviest gonadal dry weights on average, with those in the 1.5% ration showing intermediate weights, and scallops in the 0.5% ration with the lowest dry weight. Comparing the 12 °C experiment to the 1.5% treatment showed a decrease in average gonadal dry weight for ration 1.5% from week 0 to week 4. From week 4 to week 8 treatment 1.5% had increased and past the 12 degree experiment in average gonadal dry weight. This is the result of receiving more *C. mulleri* towards the end of the experiment, confirming the ration of algae has a direct affect on gonadal development.

Mean gonadal dry weights at the start of the experiment were  $0.2940 \pm 0.128$  g for wild scallops. Cultured scallops at week 4 had average gonadal dry weights of  $0.2946 \pm 0.1231$  g in the 0.5% ration,  $0.3737 \pm 0.1212$  g in the 1.5% ration, and  $0.4257 \pm 0.1815$  g in the 2.5% ration. At week 8 the wild scallops' dry gonadal weight was  $0.4555 \pm 0.120$  g. Cultured scallops gonadal dry weights at week 8 were  $0.3986 \pm 0.1207$  g for the 0.5% ration,  $0.5519 \pm 0.1829$  g for the 1.5% ration, and  $0.7769 \pm 0.2631$  g for the 2.5% ration. Animals fed the 2.5% ration had the greatest gonadal dry weights by the end of the experimental period, indicating that this diet was the best for encouraging gonad development. A one-way ANOVA for gonad dry weights showed there was a significant difference among ration treatments and wild scallops at week 8. Tukey Kramer analysis showed the 2.5% and 1.5% ration treatments were different from the 0.5% treatment and the wild scallops (Table 3).

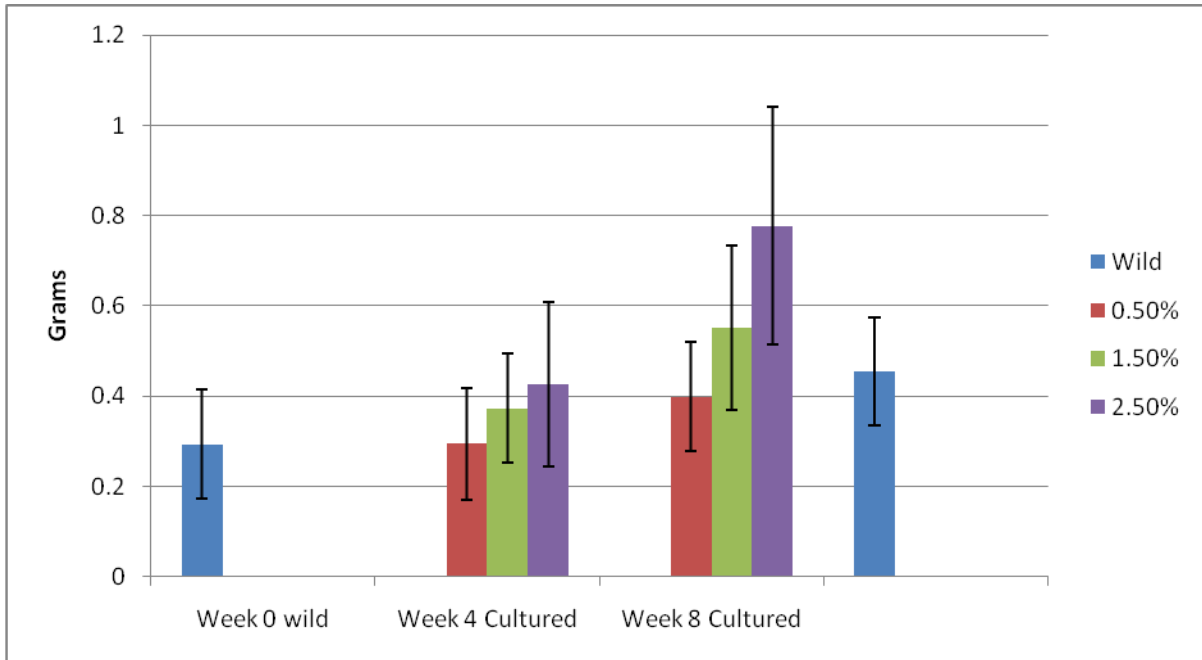


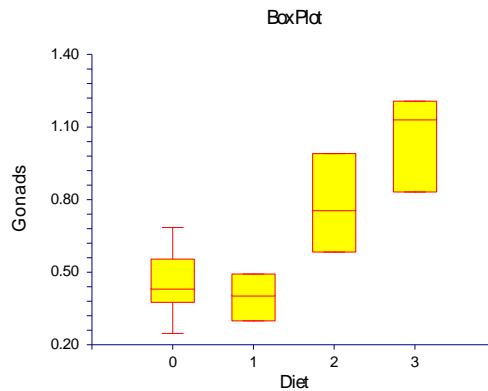
Figure 6. Average gonadal dry weights throughout the experiment.

Table 3. ANOVA table of gonad tissue dry weights at week 8. Below the table is the associated box plot with; 0 = wild, 1 = 0.5% ration, 2 = 1.5% ration, and 3 = 2.5% ration.

**Analysis of Variance Table**

Source Term	DF	Sum of Squares (Alpha=0.05)	Mean Square	F-Ratio	Prob Level	Power ( $\alpha=0.05$ )
A: Diet	3	1.123352	0.3744508	19.55	0.000004*	0.999992
S(A)	20	0.3829871	1.914936E-02			
Total (Adjusted)	23	1.50634				
Total	24					

- Term significant at alpha = 0.05



An analysis of average gonadal dry weights among tanks within treatments indicated that there were tank differences between the triplicates at week 8 (Figure 7). The greatest variation in gonadal dry weight was observed with the 2.5% ration, with tank 6 showing the greatest average gonadal dry weight ( $0.9900 \pm 0.2156$  g). It should be noted that only three scallops from each tank were sampled for dry weights, due to space and resource restrictions.

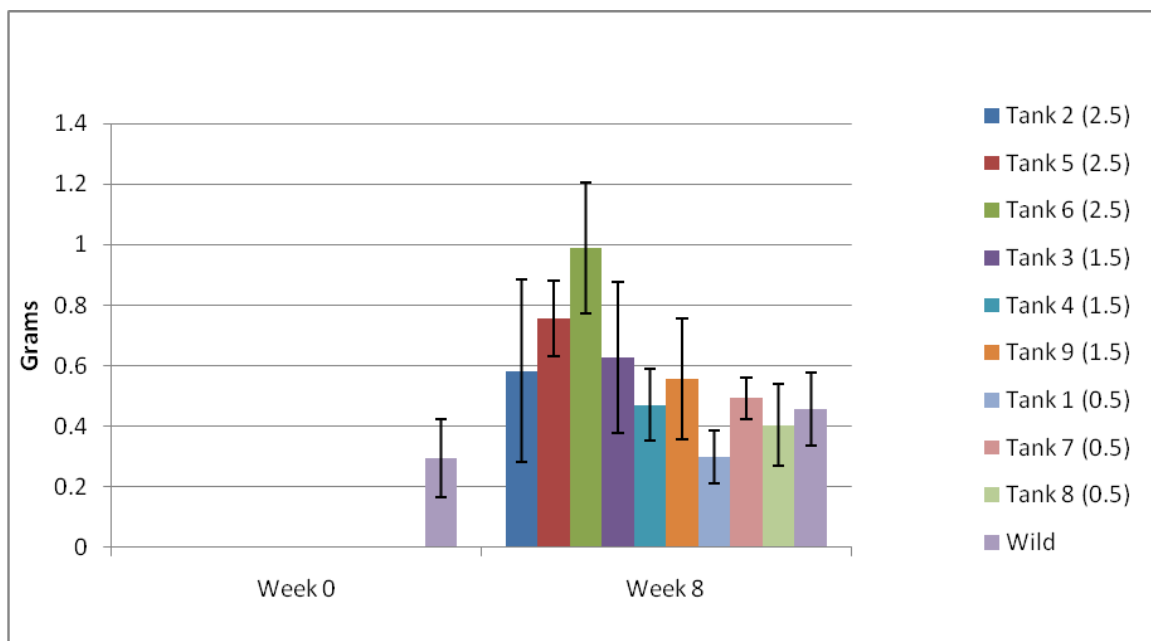


Figure 7. Comparison of Average Gonadal Dry Weights between tanks within treatments and wild scallops.

Comparing the 1.5% ration treatment in the current study and the 12°C treatment in the temperature experiment to the wild scallops collected at the start of the experiment showed that the average gonadal dry weights had increased for both experiments by week 4 (Figure 8). Initially, the 12°C treatment in the temperature experiment had increased past the 1.5% ration treatment in the current study by week 4, but was not a significant

difference. Scallops treatments in both experiments had greater mean gonadal dry weights by the end of the experiment than wild scallops. The 1.5% ration treatment in the current study did increase slightly past the 12°C treatment in the temperature experiment in average gonadal dry weight by week 8, but was also not a significant difference.

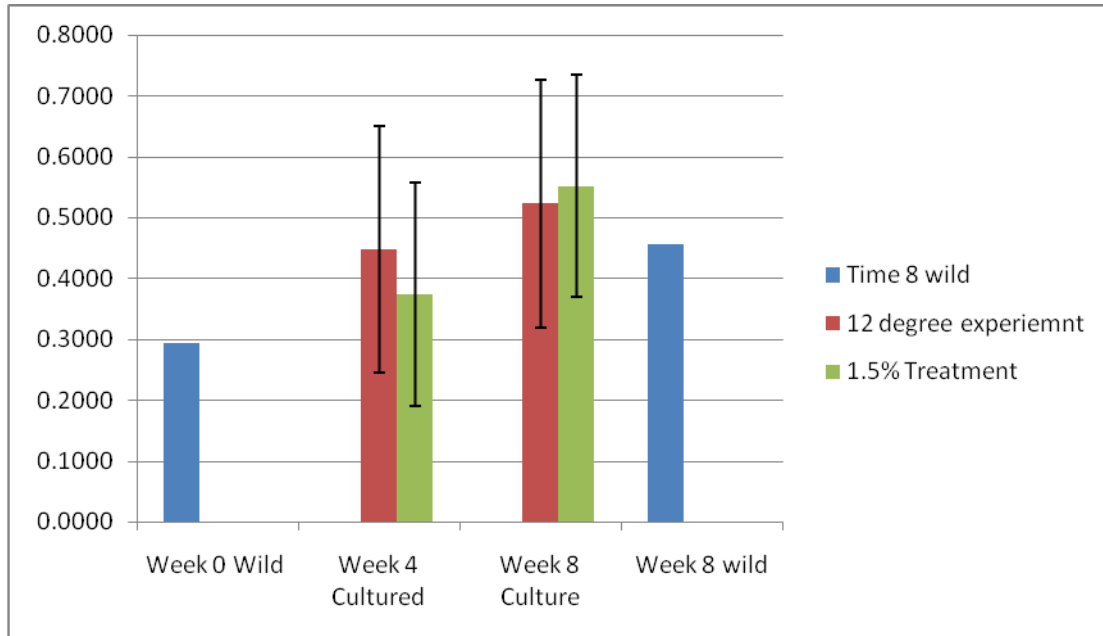


Figure 8. Comparison of average gonadal dry weight among the wild scallops, the 1.5% treatment in the current study, and the 12°C treatment in the temperature experiment.

## Discussion:

During the experimental period, the gonad size of *C. hastata* increased with increasing ration of microalgae. The amount of microalgae fed in the three regimes was determined through a formula provided by Helm *et al.* (2004). This formula incorporates the dry weights of the scallops and dry weight of microalgae to calculate the necessary

rations of feed for the day. The size of the micro algae used also played a factor; Cm is considerably bigger in size when compared to T-iso and did not reach the same high densities as T-iso. This resulted in feeding larger volumes of Cm. When sufficient amounts of Cm were not available, T-iso rations were doubled to ensure that rations were met. High culture density counts of T-iso often meant that fed volumes were small when T-iso rations were doubled. Culturing Cm throughout most of the experiment was inconsistent due to factors such as strain of Cm, temperature fluctuations in the algal room, and lack of algae culture experience. Of the total 54 days of conditioning the scallops, Cm was inconsistently fed out a total of 29 days. For the last 17 consecutive days the animals received Cm. T-iso was consistently cultured throughout the entire experiment, and densities of T-iso were an average 5.844 million cells/mL while the mean density for Cm was 2.617 million cells/mL (Appendix 1). In times of short supply of Cm the ration experiment was given priority over the temperature experiment.

Gonadal growth increased for all experimental regimes when compared to the week 0 gonadal dry weights. Scallops in the 2.5 and 1.5% rations had significantly heavier gonads than the 0.5% ration and wild scallops at the end of the experiment. Individuals in the 0.5% ration did increase slightly in gonad dry weight over time, but it was not greater than the wild scallops at the end of the experiment. Increasing the rations to 1%, 3%, and 5% may be more suitable for attaining heavier gonads and examination of the upper limits of feed percentage is recommended. This experiment was originally set at these feed rations for the first two days, but we found that the

animals were not clearing all the microalgae from the water. Therefore in order to ensure that the algae rations fed were being incorporated, the rations were halved.

Generally there was an overall decrease in somatic tissue weight for both cultured and wild animals during the experimental period. Muscle tissue was greatest at the start of the experiment and an inverse relationship was seen where the muscle tissue decreased in dry weight as the gonads increased in dry weight. This follows previous trends found in other bivalves like the razor clam (*Ensis arcuatus*), where during times of low productivity and with the onset of gametogenesis in the winter, the clams would begin to take triacylglycerols and glycogen from their digestive gland and muscle tissue and put this energy in to developing gonads (Darriba *et al.* 2005). Barber and Blake (1981) defined the gametogenic energy metabolism of *Argopecten irradians concentricus*. They found that when the adductor muscle reaches maximum weight its glycogen reserves begin to be utilized; these stores of energy are depleted in conjunction with gamete development. The Results from this experiment followed the same trend; this indicates that these processes can be used for promoting conditioning to extend the period at which spawning events can occur. For culture of B.C spiny scallops, these results indicate that harvesting scallops in autumn would be preferential, as this is the period when the muscle is largest and the gonad is smallest for the year. Further research should examine the affects of increased ration and, extending the conditioning period on gonad development and, test the ability to spawn.

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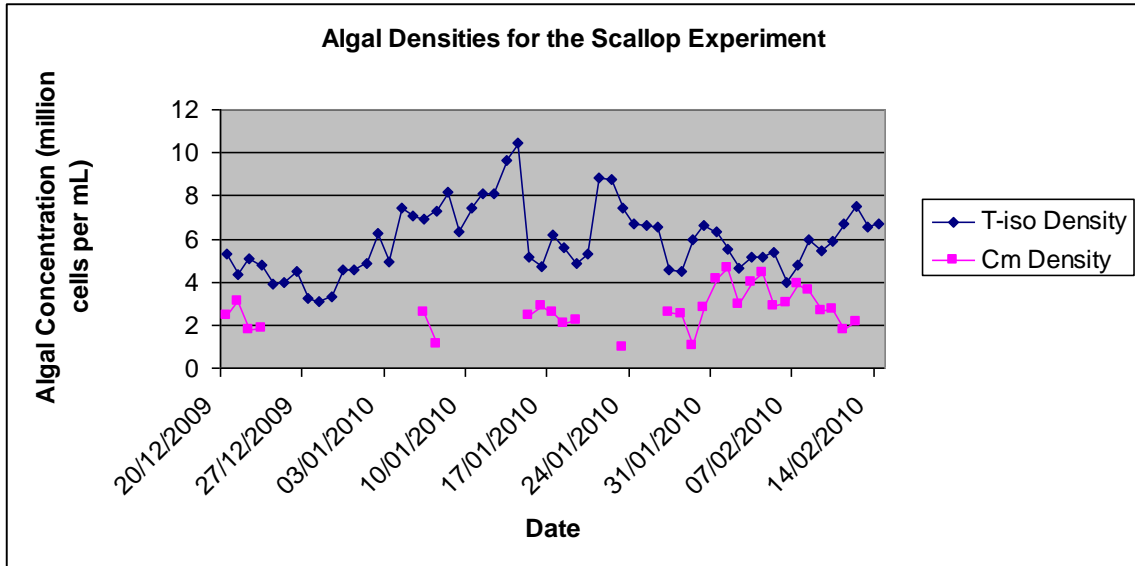
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## Appendices:



Appendix 1. Algae counts throughout the experiment