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Time Post-Hatch Caloric Value of Artemia salina

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Post-Hatch Caloric and Nutritional Value of *Artemia salina*

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Senior Honors Project

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Abstract

In aquatic animal collections, such as those in the collection of Mystic Aquarium & Institute for Exploration's Fish & Invertebrate department, live food is an essential part of the diet of animals that are on display, used in education, and kept in reserve for exhibits. For Mystic Aquarium's Fish & Invertebrate department, newly hatched *Artemia salina*, or brine shrimp, are fed to an assortment of fishes and invertebrates. Hatch brine is an important source of fatty acids, which are essential for proper growth and development. Fertilized brine shrimp eggs, encapsulated in a cyst form, are decapsulated and finally hydrated, after which *Artemia* nauplii larvae hatch. After hatching, the nauplii larvae rely on their yolk sac for nutrition. As they use this yolk sac, the hatch brine lose nutritional value and caloric value. Preliminary experiments demonstrated that variation in percent moisture and percentage of empty cysts/ unhatched nauplii in the sample skewed caloric values. Thus, modified sample-collecting techniques were used subsequently. The purpose of this experiment was to quantify the caloric value by percent moisture, percent ash, percent lipid, and percent protein of hatch brine over six hour intervals between 24 and 48 hours post-hatch. The results from this experiment showed a decrease in caloric value of approximately 30-50% between 24 and 48 hours post hatch. The standing procedure for the Fish & Invertebrate Department is that hatch brine are fed to collection animals 48 hours post-hatch. Therefore, in order to increase caloric and overall nutritional value from the live *Artemia* that are fed to collection animals, the brine shrimp should be fed out earlier than the standard 48 hours, preferably as soon as they are hatched.

Introduction

In aquarium collections, proper nutrition is essential for the survival of all fish and invertebrate species. In order to provide adequate nutrition, live food can be cultured to augment flake and

other small food sources. It is important to determine the nutritional value of the food fed to collection animals, especially for those smaller individuals with more selective diets. This experiment sought to track the caloric value of cultured *Artemia salina* larvae in order to determine when they could provide the most nutrition to collection animals for the Fish and Invertebrate Department of Mystic Aquarium and Institute for Exploration. *Artemia* arrive at the aquarium canned, in their cyst form from Brine Shrimp Direct (Ogden, UT). In order to use them, they must be decapsulated, a process that involves hydrating the entire cyst and partially degrading the outer shell to make it easier for the brine shrimps to hatch (Treece 2000).

In the first 15-20 hours post-hydration, the nauplius emerges from its cyst and enters the Instar I phase (Hoff & Snell 1987). They remain in this stage for approximately 12 hours, before developing to the Instar II phase in which suspension feeding can begin to take place (Hoff & Snell 1987). However, if no exogenous food is available, *Artemia* can survive on their yolk reserves for up to five days post hatch, results in a loss in protein and therefore caloric value (Treece 2000). As the *Artemia* develop, their nutritional value, and their caloric value changes (Hoff & Snell 1987). This developmental decrease in caloric value can be linked to the digestion and elimination of the yolk sac provided to newly-hatched *Artemia* nauplii. The objective of this experiment was to measure the caloric and nutritional value of the brine shrimp between 24 and 48 hours after hatching in order to determine the optimal time at which the brine shrimp should be fed to collection animals.

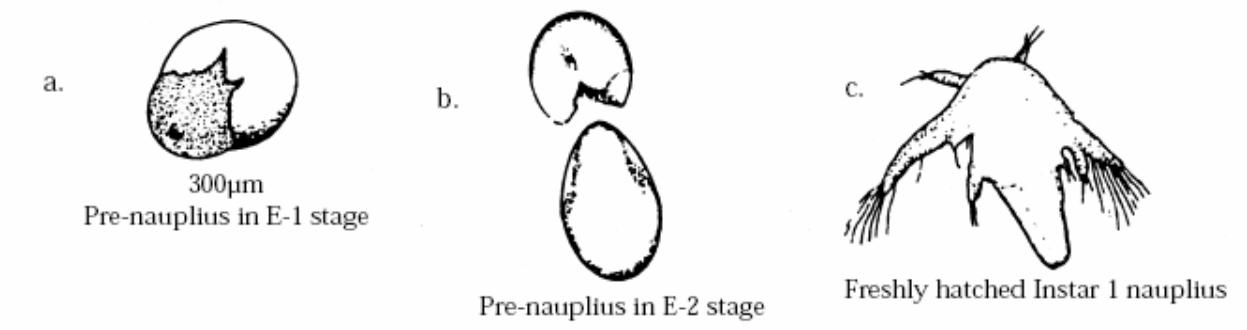


Figure 1: *Artemia* development (adapted from Treece ,2000)

Most studies in the literature focus on the different feeding options for *Artemia salina*, and its effect on growth and development (Reeve 1963; Makridis & Vadstein 1999). The current study builds upon previous knowledge of the nutritional value of hatch brine over primary growth and development. *Artemia* have also been compared to other small-feed organisms, and are usually preferred as a food source for their higher degree of unsaturated fatty acids and low cost (Lim et al 2002). Fatty acids are essential for development and growth of juvenile fishes, and a beneficial food source for many invertebrates (Narciso & Morais 2001). Different enrichment sources can be used to increase the unsaturated fatty acid content of *Artemia*, but knowing their caloric value without supplementation is very useful (Monroig et al. 2006; Woods 2003). Mystic Aquarium & Institute for Exploration's Fish & Invertebrate Department uses the lipid supplement SuperSelco to enrich its *Artemia* cultures (Brine Shrimp Direct, Ogden, UT). Unfortunately, at the time this experiment was conducted, none was available for comparative testing. This experiment aims to determine what point at what developmental point maximum nutrition can be obtained from the live feed without addition supplementation.

In the standing procedure for Mystic Aquarium's Fish and Invertebrate Department, brine shrimp cysts, after being decapsulated, are refrigerated until hatching is stimulated by the addition of saltwater. The nauplii are allowed to develop for 24 hours before being fed to collection animals.

During their development, hatch brine typically hatch from their cysts 12-15 hours after the addition of saltwater (Treece 2000). Nutrients absorbed from the yolk sac are passed on to the organisms fed the hatch brine. For this experiment, it is hypothesized that the presence of the yolk sac increases the caloric value of the younger, one day old hatch brine, than the older, two day old populations.

Methods and Materials

1) Hydration: To revive the *Artemia* from their resting state in cysts, they must first by hydrated. One can of dried Artemiz cysts from Brine Shrimp Direct (Ogden, UT) was hydrated in a 10-gallon plastic pail with freshwater and mixed with a stir bar for one and a half hours before the addition of decapsulization chemicals.

2) Decapsulation: The decapsulation process reduces the time needed in order to hatch while chemically cleaning the cysts. A 0.8M sodium hydroxide (NaOH) solution was made by dissolving 61g of NaOH in 1900 mL Instant Ocean saltwater. 2200 mL of 12.5% bleach solution was set aside. A 0.7M solution of sodium thiosulfate was made from 220g of sodium thiosulfate in 2000 mL of freshwater. All three solutions were set in the refrigerator until they were at 17-19° C.

After hydration, the cysts were filtered through 125µm screening and dried with paper towels. Dry cysts were returned to the empty hydration pail and placed under a chemical hood. The bleach and NaOH solutions were added to the cysts, which were then stirred until the mixture reached 38-40° C as the result of an exothermic reaction. Cysts were then filtered through 125µm screening set atop a drain bucket in the hood. The sodium thiosulfate solution was then added to

the cysts in order to neutralize the chloramines in the bleach solution. Distilled water was then used to rinse the to remove the degraded parts of the outer cyst. Cysts were patted dry and then scooped out of the screening into 1 L screw top Nalgene containers and refrigerated until use.

Sample Collection

PART I) Brine Shrimp samples were taken from one population consisting of 200g of decapsulated hatch brine and 38 gallons of Instant Ocean saltwater in a 44 gallon plastic garbage pail. 27 L of saltwater, containing 30-40g hatch brine samples, were taken from the population just after the addition of the saltwater (0 hours), and at 1 hour, 6 hours, 12 hours, and 24 hours after the addition of saltwater. The samples were set to air-dry in a hatch brine net for 10 minutes before being transferred to a plastic bag and frozen. Samples were defrosted in cold running water on the morning of their analysis. The samples tested on 8/25/07 leaked fresh water and had to be re-dried for 10 minutes after a 0.2-L saltwater rinse before being analyzed. Analyses were performed following the outline established by Mystic Aquarium.

PART II) For part two, a much larger population of brine shrimp were hatched over a two day period. Brine shrimp were allowed to hatch in a 100 gallon 1'deep x 3' radius blue plastic tub placed in the coral backup area of Mystic Aquarium's Fish and Invertebrate Department. The tub was exposed to 12 hours of light and minimal to no external disturbances). Approximately 90 gallons of saltwater was added to the tub that was heated to 82° F using two 300-watt heaters. Air was provided via three airlines were attached to three large airstones and placed in a triangular formation in the center of the tub, about 1' away from each other. The contents of 1-2 cans of decapsulated hatch brine cysts (approximately 2000g) were added to the water and the airlines open fully. The population remained undisturbed for the first 24 hours when the airlines

were turned off and the heaters removed. The heaters were only used for the first 24 hours after adding saltwater. 20 minutes after the air was turned off, an approximately 30-gram wet sample of brine shrimp was strained from the hatch brine solution into a salad spinner modified with the addition of 125μm mesh screening. Samples were scraped from the screening into sandwich-size, zip-top bags and frozen until thawed for analysis.

Analysis of Hatch Brine Samples for Moisture, Lipids, Ash & Calories

Each part of this experiment was repeated in triplicate, therefore, both parts had a total of 15 samples. For analysis, each sample was divided into three replicates, with room for only 4-5 samples to be analyzed at one time. Once thawed, samples were crushed using a 4" rubber, beaker cap rolled along the outside of the plastic bag containing the sample. The resulting paste was used in collecting moisture, ash and lipid values.

For moisture content, three crucibles per sample were weighed, recorded, and numbered. 3-4g of sample were added to the three crucibles per bagged sample. The crucibles were dried in a 110° F oven until dry, then reweighed and set aside for determining ash composition. The crucibles containing the hatch brine were placed in a 550° C oven for 4-5 hours until samples were white and completely turned to ash. Crucibles were reweighed once again and recorded.

Lipid extractions were carried out using chloroform and methanol mixed with the crushed hatch brine samples. For each sample, the following procedure was repeated in triplicate. Approximately 3g of hatch brine were transferred to an Eberbach blender. Traces of sample stuck to the weight paper and weigh paper itself were weighed and recorded. Then 30 mL of 1:1 chloroform:methanol mixture were added to the blender with the hatch brine sample and blended

on medium speed for 90 seconds. The mixture was filtered into a 100 mL graduated cylinder. Once all of the liquid had passed through into the cylinder, 12 mL of 0.5% NaCl were added to the cylinder and then sealed with a glass stopper and inverted seven times to thoroughly mixed. Remaining filter paper with chloroform and hatch brine was set to dry in the chemical hood in order for proper disposal. The alcohol and chloroform layers were allowed to separate for approximately 20 minutes. 3 mL of the lower lipid layer were extracted by pipette and placed in a pre-weighed 10 mL beaker. The beakers were dried at 110° F in a drying oven for 15-20 minutes and allowed to cool and until being reweighed.

In order to determine caloric value, percent lipid was multiplied by a factor of nine, and percent protein, obtained by subtracting percent lipid percent ash from percent solid, multiplied by a factor of four (Mazzaro).

Results

PART I) The results of the analysis of the 0 hours, 1 hour, 6 hours, 12 hours, and 24 hours are as follows. The three trials were conducted on August 8, 2007, August 15, 2007, and August 17, 2007. Graphs are divided between calories/100g of sample, percent lipid, percent protein, percent moisture, and percent ash. For this experiment, calories/100g and percent lipid are the most significant since caloric value is the main data for this experiment based upon the lipid-rich yolk sac.

Figure 2: Calories/100g Sample between 0-24 hours post saltwater addition

Figure 3: Percent moisture between 0 and 24 hours post saltwater addition

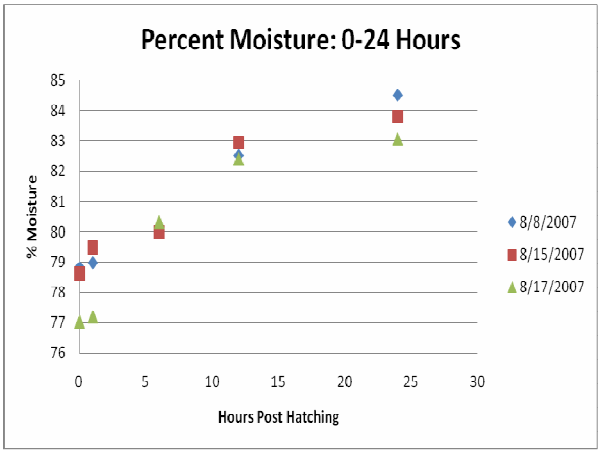


Figure 4: Percent ash between 0 and 24 hours post saltwater addition

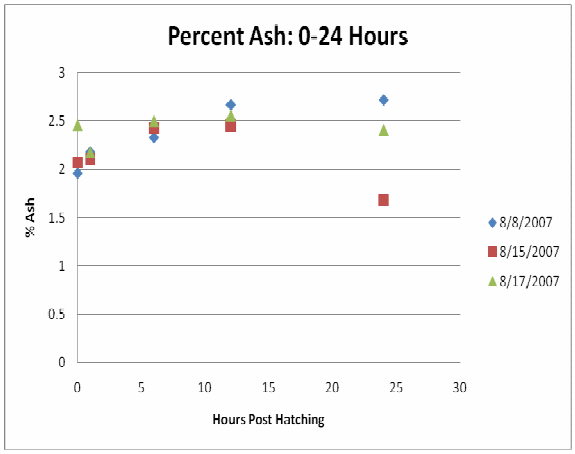


Figure 5: Percent lipid between 0 and 24 hours post saltwater addition

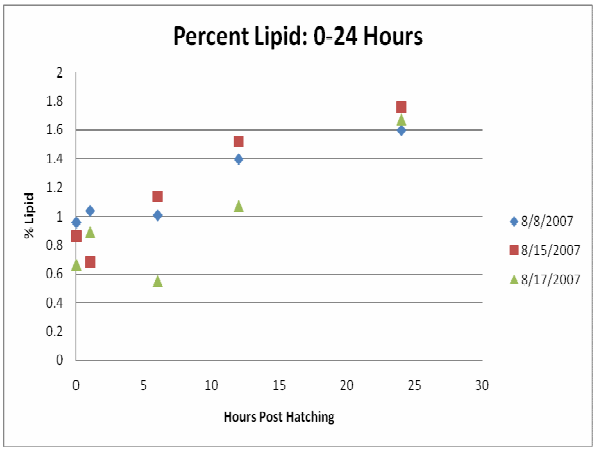
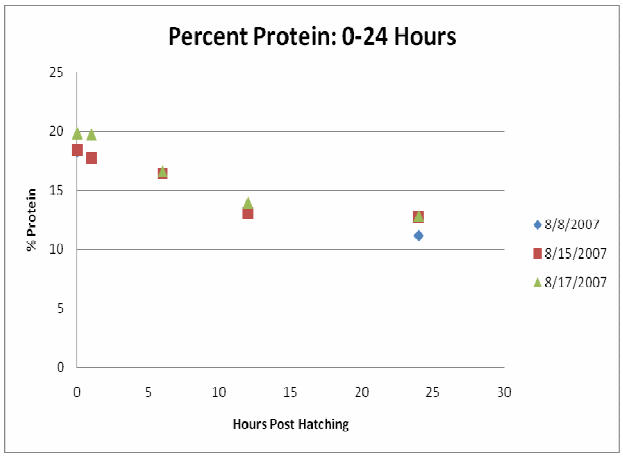


Figure 6: Percent protein between 0 and 24 hours post saltwater addition



Calories/100g of sample are shown to decrease over time, but this is not supported by a decrease in the yolk sac, since the percent lipid increases (Fig 2 & 5). Percent moisture and ash are highly variable due to the later samples containing more water due to the presence of empty cysts in the sample (Fig 3 & 4). In order to correct these issues, procedural changes were made as stated in Discussion.

PART II) Samples were collected at six-hour intervals between 24 and 48 hours in three trials. In the third trial, conducted on December 27, 2007, the last two samples were comprised of primarily unhatched cysts, due to the cysts being left out to dry too long after decapsulation. These had a higher protein percentage, resulting in a higher caloric value (Fig 7 & 11) .

Figure 7: Calories/100g of sample between 24 and 48 hours post saltwater addition

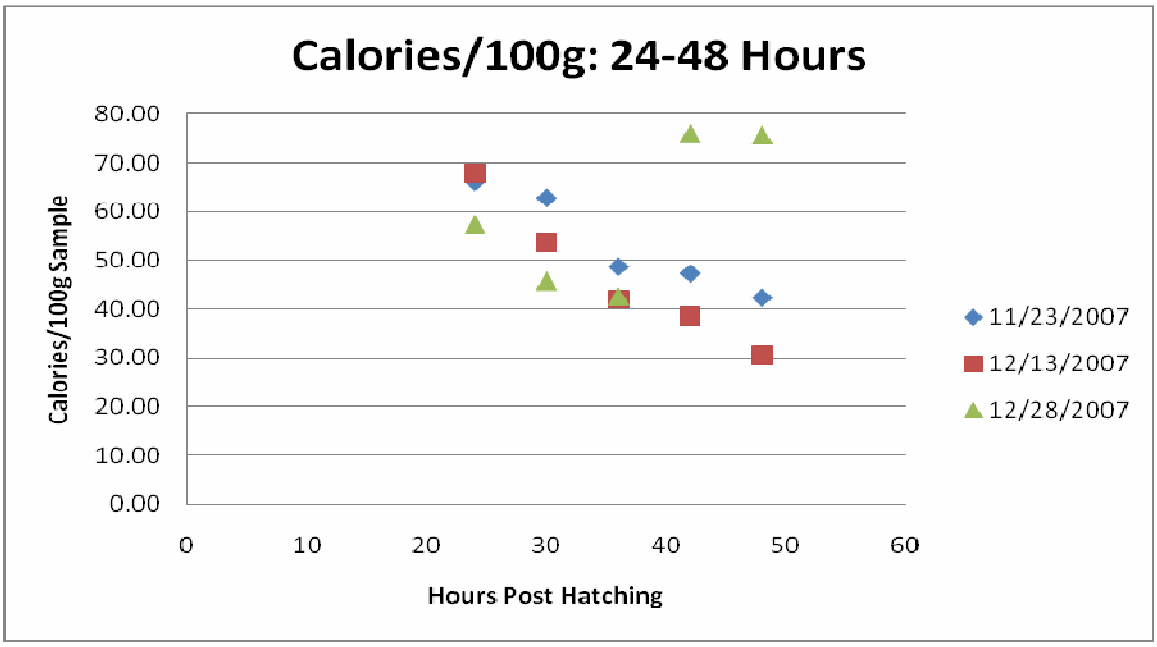


Figure 8: Percent moisture between 24 and 48 hours post saltwater addition

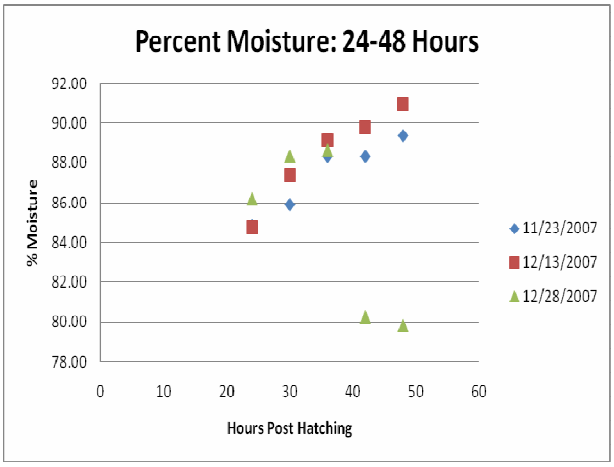


Figure 9: Percent ash between 24 and 48 hours post saltwater addition

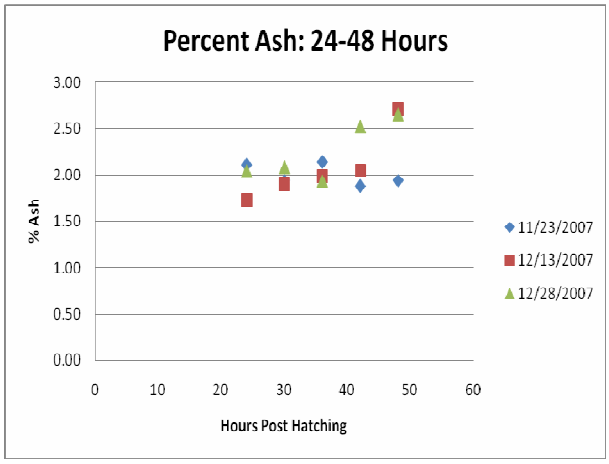


Figure 10: Percent lipid between 24 and 48 hours post saltwater addition

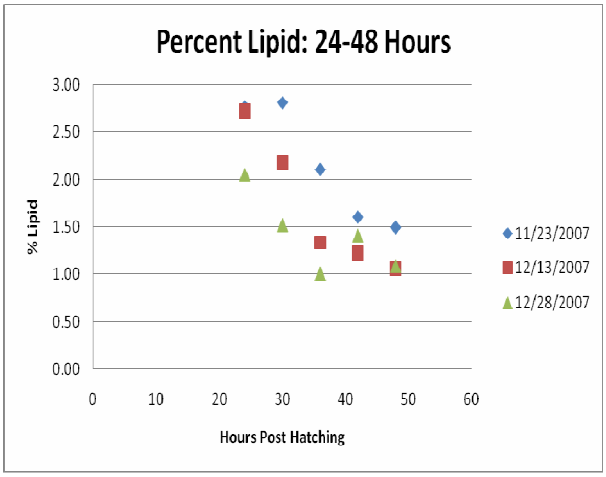
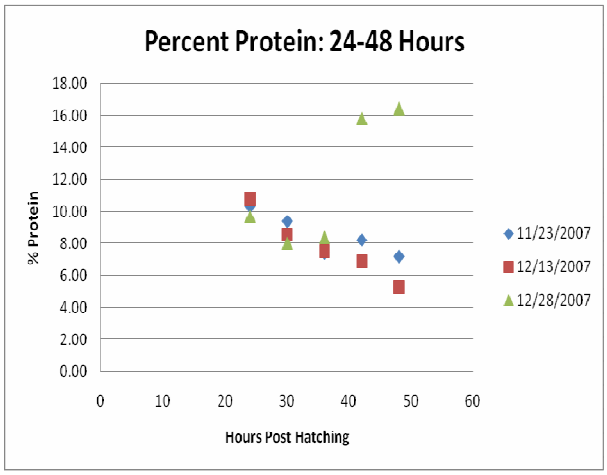


Figure 11: Percent protein between 24 and 48 hours post saltwater addition



The first trial showed a 36% decrease in caloric value between 24 and 48 hours (Fig 6). The second trial had a more noticeable decrease of 55% in caloric value. For the third trial, the first two trials had, respectively, a decrease in caloric value of 26% and 38% between 24 and 36 hours, therefore giving comparable results for the third trial at a 25.5% decrease in caloric value. As expected, in using the yolk sac, the percent lipid decreases (Fig 10), which in turn decreases caloric value. Excluding the samples comprised primarily of unhatched *Artemia* cysts, it can clearly be seen that caloric value decreases over time post-hatch.

Discussion

PART 1-

The main source of nutrients for developing *Artemia* is the yolk sac, a biological fuel source that allows the hatch brine to develop from their cyst form. As the brine shrimp emerge from their cysts, the yolk sac is slowly depleted, which is correlated with the brine shrimp's caloric value over time. The brine shrimp first emerge from their cysts at around 15-20 hours after the addition saltwater to decapsulated cysts (Hoff & Snell 1987). Molting occurs at the 15-20 hour mark sampling (Hoff & Snell 1987), and the cysts were left in solution containing the hatched brine

shrimp during. The use of an airstone to keep the brine shrimp in suspension also keeps the empty cysts mixed in with the nauplii. These cysts combined with the live brine shrimp would result in a lower caloric value than the hatch brine alone.

From this first part of the experiment, it was found that discrepancies in the percent moisture between hourly samples, collected between 0 and 24 hours, may have affected the calculated caloric values. Even though caloric value decreased over the 24-hour period, empty and unhatched cysts were also included in the analysis and therefore impacted the resulting caloric values. Before analysis, all samples were allowed to air-dry in a net for 10 minutes before being sealed in a bag and put in the refrigerator. However, due to the extra biomass from empty molts in the 24, and possibly 12-hour samples, less water was allowed to drain from the samples than in the 0, 1, and 6-hour trials. This likely resulted in excess moisture and thereby less solids in the sample. Percent protein is found by subtracting percent lipids and percent ash from the percent solids. If fewer solids were in the sample, a lower caloric value would be observed. This could be the reason why the caloric value of the later samples dropped instead of the actual caloric value of the hatch brine

In addition to the inconsistency in moisture composition among samples, the container used for the larger population was at least 8 times that used for hatch brine cultures. Due to the culture's increased size, it could not be housed in the zooplankton room where the other cultures reside. Placed in a more open space, next to holding pools for jellyfish, sea nettles, and large crabs, a cover had to be used to protect the cultures from contamination. This differs from the normal cultures where no lids are present and the cysts are exposed to light, whereas, with the cover,

very little light was able to reach the hatch brine in the large population tub. The presence of a lid would have affected the hatch brine development, since their metabolism is enhanced by the presence of light (Hoff & Snell 1987). This would result in slower development of the hatch brine, thereby altering the timing of their emergence from their cyst shells. In turn, there would be fewer empty cysts per sample, but also would result in a greater yolk reserve per younger individual. Both factors would affect the caloric value of the sampled hatch brine.

Due to the timing of hatch brine emerging from their cysts, a 24 hour sample may be the only true representative of a larva with a depleted yolk reserve prior to exogenous feeding. In addition to the 24 hour sample, a 36 hour sample may be able to indicate the nauplii's further use of their yolk reserve. After 27-35 hours, at the beginning of the Instar II phase of development, nauplii have developed enough to begin feeding (Hoff & Snell 1987). At this point, the yolk sac has been used up and no longer influence the caloric value during analysis. The addition of a 36 hour sample to the analysis would illustrate the point at which the yolk sac has been used up by the nauplii. To best follow the depletion of the yolk sac, samples taken once the embryo emerges from the cyst (15-20 hours) up until the point where the nauplii can feed on its own, about every 2 hours, could be analyzed. This sampling pattern would focus on the embryo's development during the Instar I phase, where the yolk sac is primarily utilized.

From the sampling issues explained above, procedural changes were made for Part II that would allow all the samples to dry evenly with minimal empty cyst collection and minimal external contamination with more light exposure.

PART II-

As expected, in reducing their yolk reserves, the *Artemia* nauplii lost caloric value between 24 and 48 hours post-hatch. In the first two trials there was a notable decreases in caloric value, whereas the third trial tracked the first three data points of the other two trials. Due to the final two samples in the third trial being comprised of primarily unhatched *Artemia* cysts, there was a noted change in caloric value due to the elevated protein of the cysts (De Graff et al. 1990). Therefore, these data points could not be used in determining the trend of nauplii caloric value.

From these trials, it was found that caloric value did decrease slightly between 0 and 24 hours. However, due to the variation in percent moisture and the presence of empty and unhatched cysts, the experiment was repeated. Moisture was kept consistent among samples by spinning all samples through a salad spinner lined with 125µm screening for 30 seconds. Empty and unhatched cysts were excluded from sampling in all of the test samples except the last two by allowing the culture to settle without airflow for 20 minutes. Due to the fact that *Artemia* nauplii do not emerge from their cysts until 15-20 hours post-hydration, the hourly sample was moved to 24-48 hours in order to better measure decrease in the yolk sac and be more applicable to the current standard operating procedure of the Fish & Invertebrate department.

Conclusions

The modified procedures described above resulted in a more appropriate evaluation of the caloric value of *Artemia salina* over time between 24 and 48 hours. It can be concluded that in order for aquarium collection animals to receive the most caloric value, *Artemia* should be fed just after reaching the Instar I phase when their yolk sac is mainly intact and not depleted. Thus, it is strongly recommended that the standard operating procedure for *Artemia* culturing within Mystic

Aquarium & Institute for Exploration's Fish & Invertebrate department be changed. The standing waiting period of 48 hours before feeding out hatched *Artemia* should be moved to 24 hours, just after the nauplii hatch from their cysts.

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