Development of micro-culturing techniques for freshwater copepods

Julia Betz
Mentored by Dr. Kathy Baker-Brosh and Ms. Joanne Mattson

Introduction

Copepods are crustaceans that transition through 10-14 molts including the nauplius and copepodite stages before becoming mature adults. These microorganisms are classified as a type of zooplankton and are an important component of the food web. They consume phytoplankton, bacteria, and smaller zooplankton and, in turn, are a food source for larval fish and other larger zooplankton. A micro-culture protocol was developed for the locally collected copepods Microcyclops rubellus and Acanthocyclops robustus using a study by Park et al. (2005) as a guide. The newly developed protocol for culturing a single freshwater copepod involved addressing issues with capture, isolation, food selection, and maintenance.

Application

The purpose of this project was to create a successful protocol for micro-culturing freshwater copepods allowing other scientists to focus on morphology, life history, documentation of development and environmental factors, as well as perform parasitological and behavioral studies. Previously, a protocol for maintaining them in an easily monitored and isolated environment was not established. Many studies subjected fast-swimming plankton to carbonated water to slow them for analysis. This method carried the risk of stressing or killing the copepods. Micro-cultures were used to attempt to mitigate these harmful risks, while allowing the species to be closely monitored throughout a full life cycle.

Methods and Materials

Isolation
A collection of live mesozooplankton was taken from Otter Point Creek, a tributary of the Bush River, Chesapeake Bay, Maryland. Using a disposable pipet, 2 ml of the live sample were extracted. A series of droplets were made on a petri dish and placed under the dissecting microscope. Each individual droplet was inspected for copepods and marked. Each copepod was removed from the droplet with a pipette and placed on another petri dish. If other organisms were still present, filtered water was added to the new droplet to separate the organisms. These steps were repeated until each copepod was isolated.

Building slides
The micro-culture slides were created following Park et al. (2005). These slides consisted of a microscope slide, a Sedgewick-Rafter cover slip, silicone aquarium sealant, and small strips of hard plastic (approximately 0.5 mm x 2.5 mm) from a petri dish cut using a Dremel® tool. The hard plastic strip was glued to both ends of the microscope slide creating a space with 1 mm walls on each side (approximately 1.5 ml of water was held). The slide was then overlaid by a coverslip, shown in Figure 1a.

Incubation chambers
The slides were then placed in incubation chambers which were created following Park et al. (2005). These consisted of a petri dish lined with 3 cut out circles of a paper towel. To prevent water evaporation, 3 ml of spring water were placed in the chamber. Plastic coffee stirrers were cut to size to keep the slides elevated from the paper towels. A single copepod was placed on each slide with either culture medium containing Nannochloropsis or Chilomonas as a food source. The lid was placed back onto the petri dish. The slides were exposed to 820 lumens of fluorescent lighting on a timer for 12 hours each day. The finished setup is shown in Figure 1b above.

Methods and Materials (cont.)

Maintenance
The culture chamber lid was removed and the slide was placed under a dissecting microscope. The copepod’s health was first checked by analyzing the copepods movement and checking for survivorship before maintenance. Then approximately 1 ml of the culture medium which contained waste products was removed from the slide. Spring water with either Nannochloropsis or Chilomonas media was added back onto the slide in approximately 1 ml increments. The concentration of food was observed under the microscope; if the food was Chilomonas and the concentration was overly abundant some of the medium was removed and spring water was added to dilute the Chilomonas. The paper towels were moistened as needed. The slide with the copepod and new food was placed back into the chamber. The lid was closed and all of the steps were repeated for each slide and chamber.

Figure 1: On the left (1a.) is the slide and coverslip apparatus built in the lab used as the micro-culture. To the right (1b.) is the petri dish used as a humidifying chamber.

Figure 2: Above are the stages showing the transformations of a copepod’s life cycle. To the right the percentage of copepods surviving through each stage is displayed. Colors represent stages of the copepod’s life cycle. The nauplius stage was deemed the most vulnerable due to the large drop within the first few days. Still, 42% of the copepods cultured continued on to become egg-bearing copepods.

Results and Discussion

Aided by the work of Park et al. (2005), this study developed a working protocol for isolating and culturing local freshwater copepods in a micro-environment. A silicone based sealant in the previous study had not been specified. In this study, a home improvement sealant was tried and found unsuccessful, probably due to fungicidal properties. A silicone aquarium sealant was then used. Its aquatic-friendly characteristics worked well when building the micro-culture slides. Wooden sticks which were used to elevate the slides to keep them from touching the paper towels grew mold from the constant moisture and were replaced with plastic coffee stirrers. Not specified in earlier studies, a pipet size of 1 ml was deemed best for removing waste between the coverslip and the slide. Maintenance every two days proved adequate in keeping a healthy environment which countered the daily schedule previously suggested. Temperature shocking the copepods by transporting samples during winter months into the warmer lab resulted in an increase in the copulation rates and, therefore, more egg clutches. This also resulted in spermatophores storing sperm for future egg clutches. Nannochloropsis, a green algae, was a viable food source for some copepods (Kumazawa, 2000) as was Chilomonas paramecium, a flagellate protist (Park, Chang, & Shin, 2005). Both copepod species could survive their entire lives on either food source, but observations proved that higher concentrations of Chilomonas caused them to swarm and consume the copepods. Chilomonas was easier to maintain and not as easily contaminated as Nannochloropsis. The copepods were sustained for a maximum of 72 days with 81% of the newly hatched nauplii surviving to the copepodite stage, 54% of the copepods continuing to the copepodite stage, and 46% of the copepods reaching sexual maturity, as seen in Figures 2. This new protocol proved a viable option for further experimentation involving copepods.

References