Lab and Field Study of Glycogen, Percent Tissue, and Tissue Density of *Quadrula metanevra*

by

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ABSTRACT

Conservation of freshwater mussel biodiversity requires health data from both stressed sites and reference sites. This study explored differences in energetics and health indices of *Quadrula metanevra* mussels from a reference site in the laboratory, TWRA's Cumberland River Aquatic Center (C-RAC), and in the field in the Tennessee River. Over one year, mussels were measured for life history measurements and glycogen concentrations. Health indices calculated were percent tissue and tissue density. No difference was found between basket-dwelling and sediment-dwelling mussels in the C-RAC, which provided evidence in support of using caged mussels in field studies. On average, Tennessee River mussels showed consistently higher values in foot and mantle glycogen concentrations, percent tissue, and tissue density, which could be from a consistent difference in water temperatures. Glycogen estimations from foot tissues seemed to show the least variability while doing the least harm to the individuals. Between glycogen and health indices, the most consistent similar results were found between foot and gill glycogen concentrations and tissue densities.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Biodiversity

Animal, plant, and microbe diversity is essential to sustainability of food, medicines, and water (Allan and Flecker 1993, Pimentel et al. 1997, Tscharntke et al. 2012). Freshwater biodiversity, in particular, is essential to maintaining clean water supplies, something humans cannot live without (Dudgeon et al. 2006).

Freshwater mussels, or Unionids, occupy an important niche in freshwater ecosystems and are useful in many ways. They are effective bioindicators of toxic metals (Otter et al. 2015, Soto et al. 1995,) and of general ecological health with retardation of growth measurements (Gray and Kreeger 2014, Widdows et al., 2002). They do this by absorbing harmful molecules from the water and sediment as a byproduct of cycling nutrients through the ecosystem as they filter feed (Christian et al. 2004, Nichols and Garling 2000, Vaughn and Hakenkamp 2001). Research by Nielsen and Vismann (2014) shows that an individual mussel could filter up to three liters of water per hour, depending on the species, community assemblage, and water velocity. Furthermore, they are a food source to higher trophic levels (Neves and Odom 1989, Wilson et al. 2012) and provide habitat for other organisms (Chowdhury 2016, Ragnarsson and Raffaelli 1999, Wagner 1976), and overall stability for the ecosystem (Jungerstam et al. 2014). In addition to ecological importance, Neves (1999) reports the global pearl

industry has been worth nearly three billion dollars and generated over 200,000 jobs in the past.

Threats

Despite the value of mussels and their diversity, many factors threaten their habitats. A study by Brainwood (2008) links waterway impoundments, such as weirs and dams, to decreased population densities by preventing the migration of host fish needed for reproduction. Likewise, research by Dean (2002) shows reduced species richness from habitat alterations of dams. Construction and agriculture near riparian zones increase runoff and lead to increased sedimentation in streams, which many mussel species are sensitive to (Box and Mossa 1999, Gangloff and Feminella 2007). Sedimentation and metals can lead to decreased growth and reproduction rates (Gascho 2012, Keller and Zam 1991). *Dreissena polymorpha*, or zebra mussels, are a common invasive that negatively impact ecosystems (Drake and Bossenbroek 2004). They fill the same niche and out-compete native species in many areas (Nalepa and Schloesser 2014), because they are less sensitive to pesticides (Moulton et al. 1996) and have a shorter reproductive cycle (Higgins and Zanden 2010). In addition, a study by Bouchard (2009) showed that wastewater treatment plant effluent could significantly increase mortality in freshwater mussel populations. Furthermore, climate change could also potentially threaten Unionid diversity (Hastie et al. 2003, Xenopoulos et al. 2005, Golladay et al. 2004).

Although threats to biodiversity occur globally, the diversity of freshwater mussels occurs unevenly worldwide (Brooks 2006, Schulze and Mooney 1994). Graf and Cummings (2007) report that the highest freshwater mussel biodiversity in the world is the southeast United States. Within this region, Neves (1999) states that Tennessee ranked second highest in number of endangered or threatened species, and the Tennessee River diversity of Unionids is especially high (Neves 2004). In order to preserve North America's most endangered freshwater fauna, nonlethal indicators are needed to identify areas with the highest environmental stress. To find sensitive health estimations with low variability, mussels from threatened sites must be compared with their controls from reference sites. While some research has focused on mussel health from reference sites, the number of studies is insufficient. Many species have not been extensively studied, and better understanding of surrogate species is necessary to prevent further population declines.

Health Estimates

Previous research by Widdows (1995) shows that energy estimations are a reliable way to predict mussel health. Glycogen is an essential source of energy in bivalves and fluctuates seasonally (Hummel et al. 1988, Berthelin et al. 2000) with the highest concentrations expected in autumn months to prepare reserves to use through the winter months (Gray and Kreeger 2014). It can be sampled nonlethally from the foot (Naimo et al. 1998), mantle (Berg et al. 1995),

and adductor muscle hemolymph (Fyhn and Costlow 1975, Ford 1986, Gustafson 2005). De Zwaan and Zandee (1972) compared glycogen concentrations in tissues of the marine mussel *Mytilus edulis* over 14 months, and they report that mantle tissues had the greatest variation and gills have the least. All tissues in their experiment had the highest glycogen concentrations in summer from greater food availability and growth and the lowest concentration in winter because of decreased activity. Few studies, if any, have explored similar experiments in freshwater mussels.

In addition to energy estimations, many studies have used combinations of body measurements as estimates of bivalve health (Fisher et al. 1996, Hopkins 1949), but the combinations are inconsistent. Davenport and Chen (1987) compared variability of weights and volumes across individuals, and De Zwaan and Zandee (1972) compared glycogen concentrations across tissues, but studies rarely, if ever, combine body measurements with glycogen concentrations. The relationship of growth measurements to glycogen concentrations requires further investigation.

Laboratory and Field

Many studies have determined bivalve health in either a field setting (Davenport and Chen 1987, Okumuş and Stirling 1998, Payne and Miller 2000, Widdows 2002) or a controlled lab setting (Gustafson 2005, Stuart et al. 1982). However, few studies have explored the direct real-time relationship between the

two settings. Likewise, cages, or baskets, are commonly used in field sites (Buddensiek 1995, Fisher et al. 1996), assuming the same results will be gained as if they were in sediment, the natural location. More evidence of this substituted location reliability is needed.

Mussels Used

Freshwater mussels in the family Unionidae have a unique life cycle. After sperm are released into the water column, eggs are internally fertilized and glochidia are formed. These glochidia attach to the gills or fins of a fish while they transform into juvenile mussels. Most mussel species have adapted to use only a few fish species (Dodd et al. 2005). In order to attract host fish, some mussel species have adapted lures, an extension of the mantle tissue, to appear as a food source for the fish. Once the fish is attracted, the gravid mussel can thrust glochidia onto the fish (Strayer et al. 2004). After the transformation, the young mussels detach and become benthic animals for their remaining lifetime (Dodd et al. 2005).

Quadrula metanevra, or Monkeyface, mussels are used in this experiment due to their abundance and coexistence with threatened and endangered species in the Tennessee River. They are distributed in the Upper Mississippi and Ohio River drainages: north to Minnesota, East to West Virginia, South to Louisiana, and West to Kansas. As adults, they grow up to 10.2cm in length (Cordeiro et al. 2009). This is a tachytictic species, meaning that gametogenesis

starts in fall, gametes are released the following spring, and parasitic glochidia are released before winter (Garner 1999, Graf and Foighil 2000). Sietman et al. (2012) reports that females remain mostly buried in sediment when displaying lures for their host fish: spotfin shiner (*Cyprinella spiloptera*), bluntnose minnow (*Pimephales notatus*), eastern blacknose dace (*Rhinichthys atratulus*), creek chub (*Semotilus atromaculatus*), and sauger (*Sanders canadense*) (Cordeiro et al. 2009).

Objectives

The purpose of this study was to explore differences in energetics and health indices of *Q. metanevra* mussels in the laboratory and field taken from a reference site over one year: September 2015 to September 2016. The specific objectives were to 1) compare sediment-dwelling mussels in the Tennessee River to sediment-dwelling mussels in TWRA's Cumberland River Aquatic Center (C-RAC), 2) compare sediment-dwelling to basket-dwelling mussels in the C-RAC, and 3) compare repeated measures of total weight change through the year.

METHODS

Mussels

Approximately four weeks prior to test initiation, 118 *Q. metanevra* (Monkeyface) mussels were removed from the Tennessee River at Diamond Island (35.16606^{o} N, 88.31782^{o} W), approximately Tennessee River Mile (TRM) 196 (Tennessee 2013), and transported to the Cumberland River Aquatic Center, managed by Tennessee Wildlife Resources Agency (C-RAC, TWRA) in Gallatin, Tennessee, approximately Cumberland River Mile 242 (Cumberland 2013). At the C-RAC, all mussels were maintained in 120.7cm diameter reservoirs and supplied with water from the Cumberland River at Old Hickory Lake with a turnover rate of approximately 12.3 minutes.

Test Setup

Prior to test initiation, three replicate tanks were setup (120.7cm diameter). These tanks were 58.4 cm deep with an overflow drainpipe in the center (7.6 cm in diameter and 36.8 cm height). Each tank was prepared by brush scrubbing while Cumberland River (Old Hickory Lake) water ran through it and allowing it to flush for five minutes. Incoming water to the tank went through an approximately 30.5cm pipe with 13 holes in it to reduce bubbles on the water surface (figure 1). To provide substrate, one cubic foot of large gravel (Southern Sunset Rock) and one cubic foot of medium gravel (River Rock) were added.

Then, one cubic foot of small gravel (Pea Gravel) was added. Lastly, after the water flushed for ten minutes, one cubic foot of sand was added. After each layer was added, the substrate was mixed and smoothed. The formulated substrate was allowed to flush with river water for one week before mussels were placed in the tank. The water was 30-33cm deep to the top of the sand layer.

Floating baskets were made out of 2.5 cm diameter PVC pipes cut with a miter saw, pool noodles cut with a pocket knife, and 2 cm^2 plastic netting cut with scissors. For each basket, seven pipes were cut 27.9 cm long. This made up the top square, with connectors, two parallel sides of the bottom square, and one supporting piece in the bottom middle. Four holes were drilled in the bottom middle support pipe to allow water to fill the pipes and sink the basket. Four pipes were cut to 12.7 cm for the other parallel sides of the bottom square. These were connected with the middle support pipe, with the connector adding 2.5 cm. Four pipes were cut 16.5 cm long to give the basket vertical depth and connect the corners of the top and bottom squares. Four pieces of pool noodles were cut approximately 16 cm length and placed over the top square pipes before connecting them. The plastic netting was cut to cover the inner basket to approximately 7 cm deep and attached with zip ties (figure 2). Each replicate tank had three floating baskets: one with blue pool noodles, one with green, and one with orange (figure 3).

Test Initiation

At test initiation, six mussels were taken from the holding tank and immediately dissected and used for baseline measurements for the study. All other mussels were randomly distributed between the three tanks to either floating baskets or placed into the gravel. All test mussels used in the experiment had the following dimensions: total weight mean 127.3g ± 3.1 SE (min=44.6, max=234.1), shell length mean 68.2mm ± 0.6 SE (min=51.1, max=82.9), shell height mean 56.6 ± 0.5 SE (min=43.5, max=67.4), shell width mean 42.9mm ± 0.4 SE (min=29.6, max=54.7), total volume mean 62mL ± 1.7 SE (min=13, max=120).

Schedule for Mussel Processing

In September 2015, baseline growth measurements were taken on all test mussels in the C-RAC (above), and six were randomly selected for dissection from the holding tank. In November 2015 and February, May, July, and September 2016, growth measurements were repeated with all remaining mussels in the C-RAC, and 18 were randomly selected for dissection: six from each of three replicate tanks (one from each of three floating baskets and three from sediment). Growth measurements were taken within seven days of dissections in the C-RAC.

Water in C-RAC

Procedure

At each processing time, temperature (°C), pH, DO (mg/L), conductivity (µs/cm), and flow rate (seconds/L converted to cfs) were measured in each tank on site. Then, five replicate water samples were taken in the C-RAC in 1-liter clear plastic field sample bottles: two replicate samples from two of the three tanks, randomly selected, and one sample from the third tank. These samples were immediately placed in ice until arrival at the processing destination, Middle Tennessee State University (MTSU). Then, they were placed in a 5°C refrigerator. Water tests performed at MTSU were the following: nitrate (mg/L), nitrite (mg/L), ortho-phosphate (mg/L), alkalinity (mg/L $CaCO_3$), total suspended solids (mg/L), and ash free dry weight (AFDW) (mg/L).

Five independent water samples were taken from matching locations of first samples for AFDW in 1-Liter Nalgene wide-mouth opaque amber High Density Polyethylene bottles. This was necessary to keep light out of the sample and prevent breakdown in organic material. These bottles were immediately placed in ice until processing destination arrival. Then, they were placed in a -20°C freezer and thawed before processing.

Equipment and Process

Temperature was measured with HOBO Water Temp Pro V2 onset logger every half hour through the experiment year and averaged for each day. It was

attached to the base of the green floating basket in Tank One. An additional temperature measurement was taken simultaneously with pH with an ORION STAR A321 field meter at each sample time. Concurrently, dissolved oxygen and conductivity were measured with YSI Pro 2030 DO/COND field meter. C-RAC flow rate was estimated by timing how many seconds it took to fill a 1-liter graduated pitcher from two to three spout holes from each tank and multiplying to account for 13 holes. Nitrate, nitrite, and orthophosphate were measured on HACH DR 3900 within 48 hours of sampling. Alkalinity was measured using HACH method 8221 burette titration method within 24 hours of sampling. Total suspended solids (TSS) were measured using the method outlined in Rice et. al. (2012) within ten days of sampling. AFDW was measured within ten days of sampling following TSS protocol, then burning the samples at 500^{o}C for one hour in a muffle furnace (Lindberg tube furnace). Temperature in this furnace was monitored every ten minutes, but was unable to be precisely controlled because of an analog dial.

Processing Procedure in C-RAC

At each processing time, including the baseline mussels, all mussels were measured for growth in the C-RAC by measuring total weight (g) after pat drying; shell length, height, and width (mm); and total volume by water displacement in a one-liter graduated pitcher (mL). Within seven days of growth measurements, 18 mussels were randomly selected for dissection: six from each tank. In each tank,

three were taken from sediment, and one was taken from each of the three baskets. This was the dissection process: hemolymph was extracted from the anterior adductor mussel (0.44 to 1.25 milliliter for whole study) with a BD 1-mL Sub-Q slip-tip syringe (26 guage, 5/8 inch needle); the foot, gills, and mantle were individually removed with scissors or scalpel and forceps, weighed, placed into a two-milliliter cryovial, flash frozen in liquid nitrogen, and stored at -80^{o}C until processed for glycogen (Naimo, et al 1998). The rest of the wet tissue was drained, weighed, placed in a double-layered aluminum foil pouch, and also flash frozen in liquid nitrogen and stored at -80 ^{o}C. Cavity volume was measured by filling each empty shell with water and pouring into a graduated cylinder (mL), and shell weight was also measured (g). Post-hemolymph extraction tissue weight was calculated by adding foot, gill, and mantle weight to remaining tissue weight. Dissecting tools were cleaned between each use with soap and water and with 70% isopropyl alcohol swabs. Tools were allowed to air-dry to avoid cellulose contamination from paper towels in glycogen processing.

From each of the individual frozen tissues, a subsample was weighed for glycogen concentration. Subsample averages for all experiment mussels were as follows: foot (7.6mg ± 0.03 SE), gills (8.3mg ± 0.04 SE), and mantle (5.5mg ± 0.09 SE). 12 total mussels were processed for glycogen in September 2015: six from the C-RAC and six from the Tennessee River. Twenty-four total mussels were processed in all other sample time points: 18 from the C-RAC and six from the river. Twenty-three total were processed in September 2016: 17 from the C-

RAC (two instead of three from sediment in tank two) and six from the river by following Naimo et al. (1998) spectrophotometric method. Final absorptions were recorded using Spectronic Genesys 5. The maximum instrument limit of quantification was 0.3 ppm glycogen.

Alterations to Spectrophotometric Method

Mantle final digest was diluted if the concentration was above quantification limit with ultrapure water: either by using one milliliter sample from graduated cylinder with one milliliter ultrapure water into the final test tube or by using two milliliter sample, as the protocol directed, and diluting final test tube digest resulting in more than 7.1mL total in the tube.

Water in Tennessee River

Replicate water samples in the Tennessee River were taken at the surface from the boat stern, port, and starboard sides in five 1-Liter clear field sample bottles and five 1-Liter Nalgene wide-mouth opaque amber HDPE bottles for AFDW. All water tests and procedures for the Tennessee River were identical to those done for water from the Cumberland River in the C-RAC. The only exception being that temperature was measured with ORION STAR A321 field meter instead of an automatic logger. Flow rates were estimated by USGS gage height for Tennessee River at Savannah, TN.

Processing Procedure at Tennessee River

Each time dissections were performed in the C-RAC, including test initiation in September 2015, six mussels were taken from the Tennessee River at Diamond Island (35.16606º N, 88.31782º W) at approximately TRM 196 (Tennessee 2013) by divers utilizing surface-supplied air and kept in a five-gallon bucket of river water for a maximum of four hours. These mussels were measured for one-time growth measurements and dissected on site: at a table on the bank, in a parked TWRA boat, or in a minivan. The processing at the Tennessee River followed the same procedures as in the C-RAC. Tennessee River growth and dissections occurred within three days of the C-RAC dissections. In February, the flow was too high to safely dive.

Statistics

All statistics were run in JMP Pro 9 software (Cary, NC). Significant differences were defined at $\alpha = 0.05$.

Non-repeated Measures

The health estimates analyzed were glycogen concentrations in foot, gills, and mantles; percent tissue (tissue weight / total weight); and tissue density (tissue weight / cavity volume) (g/mL).

Two-way parametric ANOVAs, assuming equal variances, were performed on 59 total individuals to examine the interaction between location and time

between the Tennessee River (*n* = 6 per sample time) and C-RAC sediment mussels (*n* = 9 per sample time). These samples included November 2015 and May and July 2016. Sample sizes were identical in September 2016 with the exception of eight from C-RAC sediment instead of nine. Since no mussels were collected from the Tennessee River in February 2016, this month was not included in these two-way ANOVAs. If a significant interaction or a significant main effect was observed, a Tukey's Post Hoc test was used to see where or when the differences occurred.

Within the C-RAC, two-way parametric ANOVAs, assuming equal variances, were performed on 89 total individuals to examine the interaction between deployment and time between the sediment mussels (*n* = 9 per sample time) and basket mussels (*n* = 9 per sample time). These samples included November 2015 and February, May, and July 2016. Sample sizes were identical in September 2016 with the exception of eight from the sediment instead of nine. If a significant interaction or a significant main effect was observed, a Tukey's Post Hoc test was used to see where or when the differences occurred.

Repeated Measures

A two-way parametric repeated measures ANOVA, assuming equal variances, was performed on 32 individuals kept in the C-RAC to examine the interaction between deployment and time from September 2015 to September 2016 in total weight growth. If a significant interaction or a significant main effect

was observed, a Tukey's Post Hoc test was used to see where or when the differences occurred. Statistical analysis was performed using total weight measurements, however graphical representation of the data used percent weight change from test initiation (September 2015) for ease of interpretation.

RESULTS

Means and standard errors for each energetic and health index endpoint from November 2015 to September 2016 are shown in figures 4-14. The results of all other endpoints are shown in table 1. Glycogen concentrations from hemolymph were below the detection limit of the equipment used and therefore not reported in this document.

Comparison Between Tennessee River Sediment and C-RAC Sediment

Foot Tissue Glycogen

Analysis of foot tissue glycogen concentrations showed a significant interaction effect between location and time ($F_{(3,51)} = 4.56$, $p = 0.0068$) (figure 4). Main effects of location ($F_{(1,51)} = 14.99$, $p = 0.0003$) and time ($F_{(3,51)} = 4.27$, $p = 0.0002$) were also significant. In September 2016, mussels in the Tennessee River (22.66 mg/g ± 2.98 SE) had significantly higher glycogen concentrations in foot tissue than mussels in the C-RAC (8.96 mg/g ± 0.61 SE) while all other sample times were not statistically different. Comparing concentrations at the same location across time revealed statistically higher concentrations in November 2015 (17.75 mg/g ± 2.37 SE) than September 2016 (8.96 mg/g ± 0.61 SE) in the C-RAC and no statistically different concentrations in mussels from the Tennessee River.

Gill Tissue Glycogen

Analysis of gill tissue glycogen concentrations showed a significant interaction effect between location and time ($F_{(3,51)} = 6.78$, $p = 0.0006$) (figure 5). Main effects showed a significant effect of time ($F_{(3, 51)} = 4.92$, $p = 0.0045$), but not location ($F_{(1,51)} = 2.67$, $p = 0.1081$). However, a Tukey's Post Hoc test was performed to see when or where differences occurred and revealed lower concentrations in C-RAC mussels (5.04 mg/g ± 0.41 SE) than in Tennessee River mussels (8.83 mg/g ± 0.45 SE) in September 2016. Comparing concentrations at the same location across time revealed lower concentrations in May 2016 (4.88 mg/g ± 0.50 SE) than in November 2015 (8.50 mg/g ± 0.72 SE) and September 2016 (8.83 mg/g ± 0.45 SE) in the Tennessee River.

Mantle Tissue Glycogen

Significant effects of location ($F_{(1,51)} = 11.33$, $p = 0.0015$) and time ($F_{(3,51)} = 3.54$, $p = 0.0209$) in mantle tissue glycogen concentration were observed, but not the interaction between them ($F_{(3,51)} = 0.70$, $p = 0.5571$) (figure 6). The Tukey's Post Hoc analysis revealed neither significant differences at any single time point between the C-RAC and Tennessee River mussels nor significant differences across time within each location. In these samples, concentrations were highest in November 2015 in the Tennessee River (82.88 mg/g ± 20.67 SE) and lowest in September 2016 in the C-RAC (6.70 mg/g ± 0.90 SE).

Percent Tissue

Analysis of percent tissue showed a significant interaction effect between location and time ($F_{(3,51)} = 2.88$, $p = 0.0448$) (figure 7). Main effects of location ($F_{(1,51)} = 42.88$, $p < 0.0001$) and time ($F_{(3,51)} = 13.15$, $p < 0.0001$) were both significant. In November 2015, Tennessee River mussels (12.69% ± 1.09 SE) had significantly higher values than C-RAC mussels (8.54% ± 0.47 SE). Comparing percent tissues at the same location across time revealed higher values in the Tennessee River in November 2015 (above) compared to July (8.07% ± 0.47 SE) and September 2016 (9.64% ± 0.70 SE).

Tissue Density

Analysis of tissue density showed a significant interaction effect between location and time ($F_{(3,51)} = 3.80$, $p = 0.0156$) (figure 8). Main effects showed that both location ($F_{(1,51)} = 33.20$, $p < 0.0001$) and time ($F_{(3,51)} = 16.09$, $p < 0.0001$) were significant. Tennessee River mussels (0.46 g/mL ± 0.03 SE) had significantly higher tissue density values than those in the C-RAC (0.32 g/mL ± 0.02 SE) in September 2016. Comparing tissue densities at the same location across time revealed a significant decrease in the Tennessee River in July 2016 (0.36 g/mL ± 0.02 SE). In the C-RAC, July (0.32 g/mL ± 0.01 SE) and September 2016 (0.32 g/mL ± 0.02 SE) had significantly lower values than November 2015 (0.44 g/mL ± 0.02 SE) and May 2016 (0.40 g/mL ± 0.01 SE).

Comparison Between C-RAC Basket and C-RAC Sediment

Foot Tissue Glycogen

Analysis of foot tissue glycogen concentrations showed no significant interaction effect between deployment and time ($F_{(4,79)} = 1.24$, $p = 0.2997$) (figure 9). Therefore, main effects were analyzed separately and showed a significant effect of time ($F_{(4,79)} = 6.78$, $p < 0.0001$), but not deployment ($F_{(1,79)} = 0.92$, $p = 0.3411$). Comparisons of concentrations in the same deployment across time revealed significantly lower concentrations in September 2016 (8.96 mg/g ± 0.61 SE) than in November 2015 (17.75 mg/g ± 2.37 SE) in sediment mussels.

Gill Tissue Glycogen

Analysis of gill tissue glycogen concentrations showed no significant interaction effect between deployment and time ($F_{(4,79)} = 0.15$, $p = 0.9641$) (figure 10). Main effects analyzed separately showed a significant effect of time ($F_{(4,79)} = 8.54$, $p < 0.0001$), but not deployment ($F_{(1,79)} = 0.13$, $p = 0.7178$). Comparing concentrations in the same deployment across time revealed a significant decrease in concentration in floating basket-dwelling mussels between November 2015 (7.79 mg/g ± 0.63 SE) and September 2016 (4.85 mg/g ± 0.29 SE).

Mantle Tissue Glycogen

Analysis of mantle tissue glycogen concentrations showed no significant interaction effect between deployment and time ($F_{(4,79)} = 0.34$, $p = 0.8485$) (figure 11). Main effects analyzed separately showed a significant effect of time ($F_{(4,79)} = 10.66$, $p < 0.0001$), but not deployment ($F_{(1,79)} = 0.01$, $p = 0.9063$). Comparing concentrations in the same deployment across time revealed significantly higher concentrations in both treatments in November 2015 (54.71 mg/g ± 10.38 SE sediment, 58.56 mg/g ± 11.84 SE baskets) than in July (13.30 mg/g ± 4.24 SE sediment, 17.22 mg/g ± 7.26 SE baskets) and September 2016 (6.70 mg/g ± 0.90 SE sediment, 9.41 mg/g ± 1.75 SE baskets).

Percent Tissue

Analysis of percent tissue showed no significant interaction effect between deployment and time ($F_{(4,79)} = 0.76$, $p = 0.5541$) (figure 12). Main effects analyzed separately showed a significant effect of time ($F_{(4,79)} = 16.00$, $p < 0.0001$), but not deployment ($F_{(1,79)} = 0.07$, $p = 0.7896$). Comparing percent tissues in the same deployment across time revealed significantly higher values in both treatments in February 2016 (9.62% ± 0.45 SE sediment, 9.74% ± 0.54 SE baskets) compared to July (7.16% ± 0.35 SE sediment, 7.79% ± 0.32 SE baskets) and September 2016 (7.07% ± 0.26 SE sediment, 6.62% ± 0.31 SE baskets).

Tissue Density

Analysis of tissue density showed no significant interaction effect between deployment and time ($F_{(4,79)} = 0.76$, $p = 0.5527$) (figure 13). Main effects analyzed separately showed a significant effect of time ($F_{(4,79)} = 20.52$, $p < 0.0001$), but not deployment ($F_{(1,79)} = 0.00$, $p = 0.9836$). Comparing densities in the same deployment across time revealed significantly higher values in November 2015 (0.44 g/mL ± 0.02 SE) and February 2016 (0.42 g/mL ± 0.02 SE) than in July (0.32 g/mL ± 0.01 SE) and September 2016 (0.32 g/mL ± 0.02 SE) in sediment-dwelling mussels. Values were significantly higher in November 2015 (0.41 g/mL ± 0.02 SE) and February 2016 (0.44 g/mL ± 0.01 SE) than in September 2016 (0.30 g/mL ± 0.01 SE) in basket-dwelling mussels.

Total Weight Growth: Repeated Measures

Analysis of total weight growth showed no significant interaction effect between deployment and time ($F_{(5,147)} = 1.08$, $p = 0.3763$) (figure 14). Main effects analyzed separately showed no effect of deployment (basket-dwelling mussels were not different from sediment-dwelling mussels ($F_{(1,30)} = 0.06$, $p = 0.8106$)) and a significant effect of time ($F_{(5,147)} = 25.28$, $p < 0.0001$). Overall, for both deployments, significant decreased weights were observed in July and September 2016.

Water

Daily temperature means from the HOBO logger in the C-RAC are shown in figure 15. Many rapid temperature spikes occurred between September 2015 and February 2016.

Tennessee River gage height values were lowest in fall months, between 1.7 and 2.1 meters: September 2015 and 2016 and November 2015. They were highest in spring and summer months, between 2.7 and 2.9 meters: May and July 2016. Flow rates in the Cumberland River tanks, in cubic feet per second, were much more variable: the lowest was in May and September 2016 and the highest in September 2015 and November 2015 (figure 16). Although direct comparison between the different scales was impossible, the C-RAC flow was more consistent with the tanks being much smaller bodies of water.

Total suspended solids (TSS) fell within a 2 to 8 mg/L range for both locations, except February and May 2016 in the C-RAC (figure 17). In February, the mean was 11.2 mg/L, and in May, the mean was 15.1 mg/L.

Ash free dry weight samples were taken independently of TSS samples, and the results were highly variable between sites and across sample times (figure 18). In the Tennessee River, these values were between 3.5 and 6 mg/L over the year. In the C-RAC, they were between 2.0 and 6.0 mg/L over the year.

All other water endpoints are provided in table 2a and 2b.

DISCUSSION

Biodiversity is extremely important globally, especially of freshwater mussels (Vaughn and Hakenkamp 2001). Unionids occupy an important niche in freshwater ecosystems and are useful in many ways, but despite their value and diversity, many factors threaten their habitats (Grillo and Venora 2011). In order to preserve biodiversity, sensitive health estimations with low variability are needed to efficiently compare mussel communities in healthy sites to communities in stressed ones. Then, areas with the highest conservation needs can be identified.

Water Influence

In the current study, foot and gill glycogen concentrations and tissue density were significantly lower in the C-RAC than the Tennessee River in September 2016 (figures 4, 5, and 8). Although these health estimations showed a significant interaction between location and time, which could be a confounding factor, no statistical difference was found between the two sites in all other months. The uniformity between locations was expected, since all mussels used in this experiment originated from the same population. A previous study by Gabbott and Walker (1971) compared glycogen concentrations of lab oysters to field oysters: values of oysters in the lab decreased while those in the field increased. Their reason for this was higher temperature and lower food

availability in their laboratory than in their field site. This interaction has been shown to influence mussels' physiology (Galbraith et al. 2009). In the current study, while food availability in the C-RAC did not appear problematic, temperature spikes did occur (figure 15) and consistent differences between water temperatures the Tennessee River and the Cumberland River (which supplies the C-RAC) may have affected mussel physiology. This may potentially explain the lower health estimations observed in September 2016. An earlier study by Tsuchiya (1983) on marine mussels showed that rapid water temperature increases can be fatal to half a population after only one hour.

In the present study, an overall decrease in glycogen concentrations in all three tissues was seen over the experiment year in all C-RAC mussels (figures 9-11). This was statistically apparent between November 2015 and September 2016. The same was true for percent tissue and tissue density in all C-RAC mussels (figures 12-13), but these health indices were highest in February 2016 rather than November 2015. This would not be alarming, as fluctuations are natural (Hummel et al. 1988, Berthelin et al. 2000). However, the difference in September 2016 between sites, mentioned above, made it noteworthy.

In the current study, on average, Tennessee River mussels showed consistently higher values in foot and mantle glycogen concentrations, percent tissue, and tissue density, as seen in significant main effects. These health estimations, excluding mantle glycogen, also showed a significant interaction between location and time, which could be a confounding factor. Since all

mussels in this study were originally from the Tennessee River, cooler water in the Cumberland River could have contributed to this pattern (table 2a). A previous literature review by Cope and Waller (1995) showed a wide range of population recovery in North America from relocation because of conservation. They also state that more research is needed for future relocation projects.

Baskets and Sediment

In the present study, no difference was found in any tissue's glycogen concentration between basket-dwelling and sediment-dwelling mussels in the C-RAC (figures 9-11). The same was true for the total weight growth measurements (figure 14): they did not differ statistically between the two treatments at any sample time. This was expected and agreed with a previous study by Hunt and Slone (2010). Their experiment showed no difference in chemical contamination between caged and sediment-dwelling mussels in Boston Harbor.

Reproduction

In the present experiment in the Tennessee River, percent tissue and tissue density were both lowest in July and September 2016, with the latter being slightly higher (figures 7-8). This would likely be the result of reproduction: a previous study by Garner et al. (1999) showed that *Q. metanevra* are gravid during spring and early summer and release glochidia during mid- to late-

summer. During this time, food would also be more abundant (Okumuş and Stirling 1998). However, an experiment by Lurman et al. (2014) showed that this extra energy would be spent on gametogenesis. The same experiment also showed that during winter, bivalves decrease activity and stay closed longer. Therefore, after glochidia were released, the mussels would have stored up energy to be used during the less-active winter. This would explain the slight increase in September in the present study.

A combination of reproduction and water temperature inconsistencies in the C-RAC may explain why the total weight change of all mussels in the C-RAC was significantly higher in May than in July and September 2016 (figure 14). If these mussels had equal water quality to the Tennessee River, the total weight may have increased between July and September 2016, but it only decreased further.

Measurements

Since mantle glycogen concentration was the most variable within each sample of the three tissues, it would not be the best choice for population health estimations. Gill tissue required the most amount of tissue per individual for glycogen estimation with the spectrophotometric method. Also, gills are necessary for basic survival functions: respiration and reproduction. Therefore, gills would not be the best tissue choice for glycogen estimations either.

Glycogen estimations from foot tissues seemed to strike a balance between low variability and doing the least harm to the individuals.

The most consistent similar results between energetics and health indices were found between foot and gill glycogen concentrations and tissue densities. This reasoning was based on the statistic similarity of these health estimations across lab and field sites, especially in September 2016, and across time in the C-RAC mussels.

Conclusions

Overall, health estimations of *Q. metanevra* held in the laboratory did not differ from those in the field. Holding mussels in baskets had no effect on glycogen concentrations, percent tissue, tissue density, or total weight growth. This provides evidence in support of using caged mussels in the field.

REFERENCES

Allan, J. D., & Flecker, A. S. (1993). Biodiversity conservation in running waters. *BioScience*, *43*(1), 32-43.

Berg, D. J., Haag, W. R., Guttman, S. I., & Sickel, J. B. (1995). Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. *Journal of the North American Benthological Society*, *14*(4), 577-581.

Berthelin, C., Kellner, K., & Mathieu, M. (2000). Histological characterization and glucose incorporation into glycogen of the Pacific oyster Crassostrea gigas storage cells. *Marine Biotechnology*, *2*(2), 136-145.

Bouchard, B., Gagné, F., Fortier, M., & Fournier, M. (2009). An in-situ study of the impacts of urban wastewater on the immune and reproductive systems of the freshwater mussel Elliptio complanata. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *150*(2), 132-140.

Box, J. B., & Mossa, J. (1999). Sediment, land use, and freshwater mussels: prospects and problems. *Journal of the North American Benthological Society*, *18*(1), 99-117.

Brainwood, M., Burgin, S., & Byrne, M. (2008). The impact of small and large impoundments on freshwater mussel distribution in the Hawkesbury-Nepean River, southeastern Australia. *River Research and Applications*, *24*(10), 1325-1342.

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A., Gerlach, J., Hoffmann, M., Lamoreux, J. F., ... & Rodrigues, A. S. (2006). Global biodiversity conservation priorities. S*cience*, *313*(5783), 58-61.

Buddensiek, V. (1995). The culture of juvenile freshwater pearl mussels Margaritifera margaritifera L. in cages: a contribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation*, *74*(1), 33-40.

Chowdhury, G. W., Zieritz, A., & Aldridge, D. C. (2016). Ecosystem engineering by mussels supports biodiversity and water clarity in a heavily polluted lake in Dhaka, Bangladesh. *Freshwater Science*, *35*(1), 188-199.

Christian, A. D., Smith, B. N., Berg, D. J., Smoot, J. C., & Findlay, R. H. (2004). Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. *Journal of the North American Benthological Society*, *23*(1), 101-113.

Cope, W. G., & Waller, D. L. (1995). Evaluation of freshwater mussel relocation as a conservation and management strategy. *River Research and Applications*, *11*(2), 147-155.

Davenport, J., & Chen, X. (1987). A comparison of methods for the assessment of condition in the mussel (Mytilus. edulis L.). *J. Moll. Stud*, *53*, 293-297.

De Zwaan, A., & Zandee, D. I. (1972). Body distribution and seasonal changes in the glycogen content of the common sea mussel Mytilus

edulis. *Comparative Biochemistry and Physiology Part A: Physiology*, *43*(1), 53-58.

Dean, J., Edds, D., Gillette, D., Howard, J., Sherraden, S., & Tiemann, J. (2002). Effects of lowhead dams on freshwater mussels in the Neosho River, Kansas. *Transactions of the Kansas Academy of Science*, *105*(3), 232-240.

Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock Jr, R. V. (2005). Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology*, *91*(5), 1064-1072.

Drake, J. M., & Bossenbroek, J. M. (2004). The potential distribution of zebra mussels in the United States. *BioScience*, *54*(10), 931-941.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, *81*(2), 163-182.

Fisher, N. S., Teyssié, J. L., Fowler, S. W., & Wang, W. X. (1996). Accumulation and retention of metals in mussels from food and water: a comparison under field and laboratory conditions. *Environmental Science & Technology*, *30*(11), 3232-3242.

Ford, S. E. (1986). Effect of repeated hemolymph sampling on growth, mortality, hemolymph protein and parasitism of oysters, Crassostrea

virginica. *Comparative Biochemistry and Physiology Part A: Physiology*, *85*(3), 465-470.

Fyhn, H. J., & Costlow, J. D. (1975). Anaerobic sampling of body fluids in bivalve molluscs. *Comparative Biochemistry and Physiology Part A: Physiology*, *52*(2), 265-268.

Gabbott, P. A., & Walker, A. J. M. (1971). Changes in the Condition Index and Biochemical Content of Adult Oysters (Ostreaedulis L.) Maintained under Hatchery Conditions. *Journal du Conseil*, *34*(1), 98-105.

Galbraith, H. S., & Vaughn, C. C. (2009). Temperature and food interact to influence gamete development in freshwater mussels. *Hydrobiologia*, *636*(1), 35-47.

Gangloff, M. M., & Feminella, J. W. (2007). Stream channel geomorphology influences mussel abundance in southern Appalachian streams, USA. *Freshwater Biology*, *52*(1), 64-74.

Garner, J. T., Haggerty, T. M., & Modlin, R. F. (1999). Reproductive cycle of Quadrula metanevra (Bivalvia: Unionidae) in the Pickwick Dam tailwater of the Tennessee River. *The American Midland Naturalist*, *141*(2), 277-283.

Gascho Landis, A. M., Haag, W. R., & Stoeckel, J. A. (2012). High suspended solids as a factor in reproductive failure of a freshwater mussel. *Freshwater Science*, *32*(1), 70-81.

Golladay, S. W., Gagnon, P., Kearns, M., Battle, J. M., & Hicks, D. W. (2004). Response of freshwater mussel assemblages (Bivalvia: Unionidae) to a

record drought in the Gulf Coastal Plain of southwestern Georgia. *Journal of the North American Benthological Society*, *23*(3), 494-506.

Graf, D. L., & Cummings, K. S. (2007). Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies*, *73*(4), 291-314.

Graf, D. L., & Foighil, D. Ó. (2000). The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. *Journal of Molluscan Studies*, *66*(2), 157-170.

Gray, M. W., & Kreeger, D. (2014). Monitoring fitness of caged mussels (Elliptio complanata) to assess and prioritize streams for restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(2), 218-230.

Grillo, O., & Gianfranco, V. (Eds.). (2011). *Biodiversity loss in a changing planet*. InTech.

Gustafson, L. L., Stoskopf, M. K., Bogan, A. E., Showers, W., Kwak, T. J., Hanlon, S., & Levine, J. F. (2005). Evaluation of a nonlethal technique for hemolymph collection in Elliptio complanata, a freshwater bivalve (Mollusca: Unionidae). *Diseases of Aquatic Organisms*, *65*(2), 159-165.

Hastie, L. C., Cosgrove, P. J., Ellis, N., & Gaywood, M. J. (2003). The threat of climate change to freshwater pearl mussel populations. *AMBIO: A Journal of the Human Environment*, *32*(1), 40-46.

Higgins, S. N., & Vander Zanden, M. J. (2010). What a difference a species makes: a meta–analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecological monographs*, *80*(2), 179-196.

Hopkins, A. E. (1949). Determination of condition of oysters. *Science*, *110*(2865), 567-568.

Hummel, H., De Wolf, L., & Fortuin, A. W. (1988). The annual cycle of glycogen in estuarine benthic animals. *Aquatic Ecology*, *22*(2), 199-202.

Hunt, C. D., & Slone, E. (2010). Long-term monitoring using resident and caged mussels in Boston Harbor yield similar spatial and temporal trends in chemical contamination. *Marine Environmental Research*, *70*(5), 343-357.

Jungerstam, J., Erlandsson, J., McQuaid, C. D., Porri, F., Westerbom, M., & Kraufvelin, P. (2014). Is habitat amount important for biodiversity in rocky shore systems? A study of South African mussel assemblages. *Marine Biology*, *161*(7), 1507-1519.

Keller, A. E., & Zam, S. G. (1991). The acute toxicity of selected metals to the freshwater mussel, Anodonta imbecilis. *Environmental Toxicology and Chemistry*, *10*(4), 539-546.

Kennedy, A. J., Lindsay, J. H., Biedenbach, J. M., & Harmon, A. R. (2017). Life stage sensitivity of the marine mussel Mytilus edulis to ammonia. *Environmental Toxicology and Chemistry*, *36*(1), 89-95.

Lurman, G. J., Walter, J., & Hoppeler, H. H. (2014). The effect of seasonal temperature variation on behaviour and metabolism in the freshwater mussel (Unio tumidus). *Journal of Thermal Biology*, *43*, 13-23.

Moulton, C. A., Fleming, W. J., & Purnell, C. E. (1996). Effects of two cholinesterase-inhibiting pesticides on freshwater mussels. *Environmental Toxicology and Chemistry*, *15*(2), 131-137.

Naimo, T. J., Damschen, E. D., Rada, R. G., & Monroe, E. M. (1998). Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. *Journal of the North American Benthological Society*, *17*(1), 121-128.

Nalepa, T. F., & Schloesser, D. W. (2014). *Quagga and zebra mussels: Biology, impacts, and control*. Boca Raton, FL: CRC Press.

NatureServe. (2017). *NatureServe Explorer: An online encyclopedia of life*. Version 7.1. NatureServe, Arlington, Virginia. Available http://explorer.natureserve.org. (Accessed: June 13, 2017).

Neves, R. (1999). Conservation and commerce: management of freshwater mussel (Bivalvia: Unionoidea) resources in the United States. *MALACOLOGIA-PHILADELPHIA-*, *41*(2), 461-474.

Neves, R. (2004). Propagation of endangered freshwater mussels in North America. *Journal of Conchology Special Publication*, *3*, 69-80.

Neves, R. J. (1999). Biological feasibility of freshwater mussel and pearl culture in Gulf Coast states. *Gulf of Mexico Science*, *17*(2), 103-108.

Neves, R. J., & Odom, M. C. (1989). Muskrat predation on endangered freshwater mussels in Virginia. *The Journal of Wildlife Management*, 934-941.

Nichols, S. J., & Garling, D. (2000). Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. *Canadian Journal of Zoology*, *78*(5), 871-882.

Nielsen, P., & Vismann, B. (2014). Clearance rate of Mytilus edulis (L.) as a function of current velocity and mussel aggregation. *Journal of Shellfish Research*, *33*(2), 457-463.

Okumuş, İ., & Stirling, H. P. (1998). Seasonal variations in the meat weight, condition index and biochemical composition of mussels (Mytilus edulis L.) in suspended culture in two Scottish sea lochs. *Aquaculture*, *159*(3), 249-261.

Otter, R. R., McKinney, D., Brown, B., Lainer, S., Monroe, W., Hubbs, D., & Read, B. (2015). Bioaccumulation of metals in three freshwater mussel species exposed in situ during and after dredging at a coal ash spill site (Tennessee Valley Authority Kingston Fossil Plant). *Environmental Monitoring and Assessment*, *187*(6), 334.

Payne, B. S., & Miller, A. C. (2000). Recruitment of Fusconaia ebena (Bivalvia: Unionidae) in relation to discharge of the lower Ohio River. *The American Midland Naturalist*, *144*(2), 328-341.

Pimentel, D., Wilson, C., McCullum, C., Huang, R., Dwen, P., Flack, J., ... & Cliff, B. (1997). Economic and environmental benefits of biodiversity. *BioScience*, *47*(11), 747-757.

Ragnarsson, S. Á., & Raffaelli, D. (1999). Effects of the mussel Mytilus edulis L. on the invertebrate fauna of sediments. *Journal of Experimental Marine Biology and Ecology*, *241*(1), 31-43.

Rice, E. W. B., Eaton, R. B., Clesceri, A. D., & Bridgewater, L. S. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). Washington, DC: American Public Health Association.

Schulze, E. D., & Mooney, H. A. (1994). Ecosystem function of biodiversity: a summary. In *Biodiversity and Ecosystem Function* (pp. 497-510). Springer Berlin Heidelberg.

Sietman, B. E., Davis, J. M., & Hove, M. C. (2012). Mantle display and glochidia release behaviors of five quadruline freshwater mussel species (Bivalvia: Unionidae). *American Malacological Bulletin*, *30*(1), 39-46.

Sietman, B. E., Whitney, S. D., Kelner, D. E., Blodgett, K. D., & Dunn, H. L. (2001). Post-extirpation recovery of the freshwater mussel (Bivalvia: Unionidae) fauna in the upper Illinois River. *Journal of Freshwater Ecology*, *16*(2), 273-281.

Soto, M., Kortabitarte, M., & Marigomez, I. (1995). Bioavailable heavy metals in estuarine waters as assessed by metal/shell-weight indices in sentinel

mussels Mytilus galloprovincialis. *Marine Ecology Progress Series*, *125*, 127-136.

Strayer, D. L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J., & Nichols, S. J. (2004). Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*, *54*(5), 429-439.

Stuart, V., Field, J. G., & Newell, R. C. (1982). Evidence for absorption of kelp detritus by the ribbed mussel Aulacomya-Ater using a new Cr-51-labeled microsphere technique. *Marine Ecology Progress Series*, *9*(3), 263-271.

Tscharntke, T., Clough, Y., Wanger, T. C., Jackson, L., Motzke, I., Perfecto, I., ... & Whitbread, A. (2012). Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, *151*(1), 53-59.

Tsuchiya, M. (1983). Mass mortality in a population of the mussel Mytilus edulis L. caused by high temperature on rocky shores. *Journal of Experimental Marine Biology and Ecology*, *66*(2), 101-111.

U.S. Geological Survey. (2016). USGS 03593500 Tennessee River at Savannah, TN. Retrieved from https://waterdata.usgs.gov/tn/nwis/uv?site_no=03593500.

US Army Corps of Engineers. (2013). [Navigation Charts of the Cumberland River: Smithland, Kentucky to Celina, Tennessee and of the Tennessee River: Paducah, Kentucky to Knoxville, Tennessee]. *USACE Digital Library*. Retrieved from http://cdm16021.contentdm.oclc.org/cdm/.

Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, *46*(11), 1431-1446.

Wagner, R. E. (1976). The effect of size and temperature on the filtration rate of the freshwater mussel, Elliptio complanatus. *Bios*, 168-178.

Widdows, J., Donkin, P., Brinsley, M. D., Evans, S. V., Salkeld, P. N., Franklin, A., ... & Waldock, M. J. (1995). Scope for growth and contaminant levels in North Sea mussels Mytilus edulis. *Marine Ecology Progress Series*, *127*, 131-148.

Widdows, J., Donkin, P., Staff, F. J., Matthiessen, P., Law, R. J., Allen, Y. T., ... & Jones, B. R. (2002). Measurement of stress effects (scope for growth) and contaminant levels in mussels (Mytilus edulis) collected from the Irish Sea. *Marine Environmental Research*, *53*(4), 327-356.

Wilson, C. D., Arnott, G., & Elwood, R. W. (2012). Freshwater pearl mussels show plasticity of responses to different predation risks but also show consistent individual differences in responsiveness. *Behavioural processes*, *89*(3), 299-303.

Xenopoulos, M. A., Lodge, D. M., Alcamo, J., Märker, M., Schulze, K., & Van Vuuren, D. P. (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, *11*(10), 1557-1564.

APPENDICES

APPENDIX A: Figures

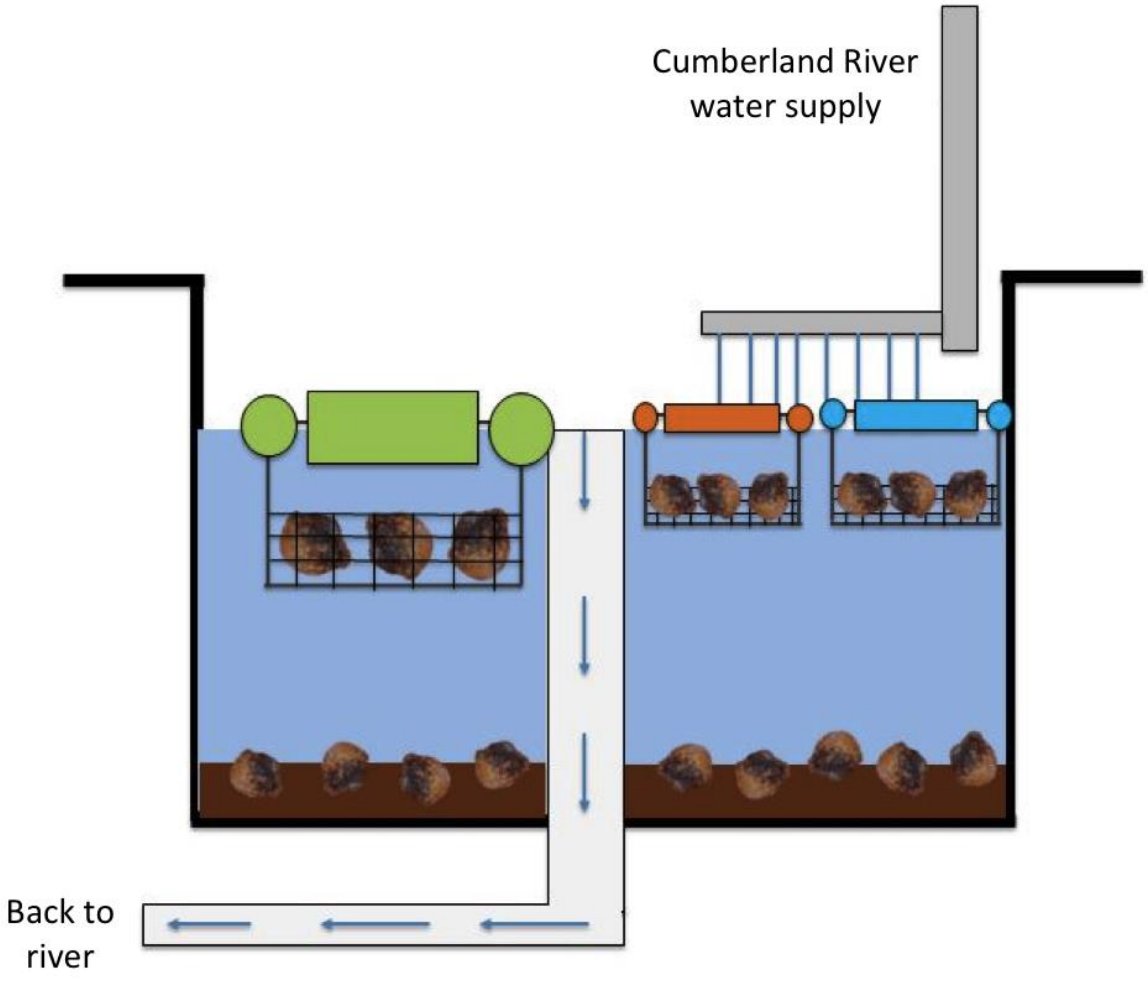

Figure 1: Tank set up in the C-RAC. Rectangles and circles represent pool noodles. The black lines underneath them represent PVC floating baskets. Water from the Cumberland River flows from the water supply into the tank from above and through 13 holes and exits through an overflow drainpipe back to the same River.

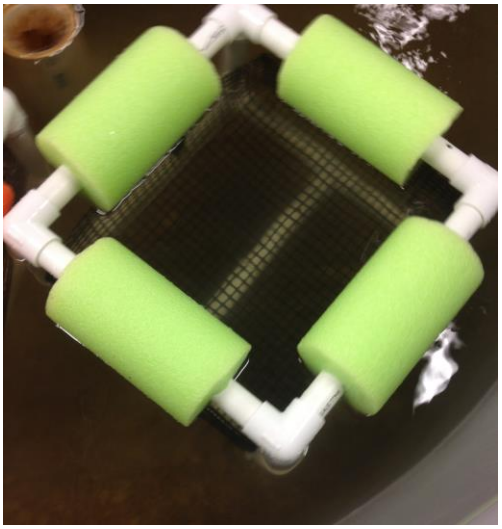

Figure 2: Top view of floating basket in the C-RAC.

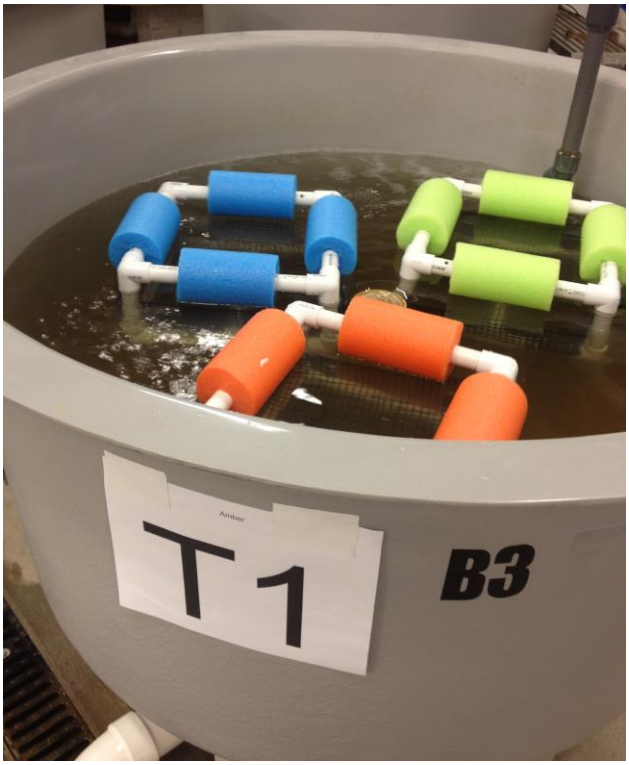

Figure 3: Three baskets in one tank in the C-RAC.

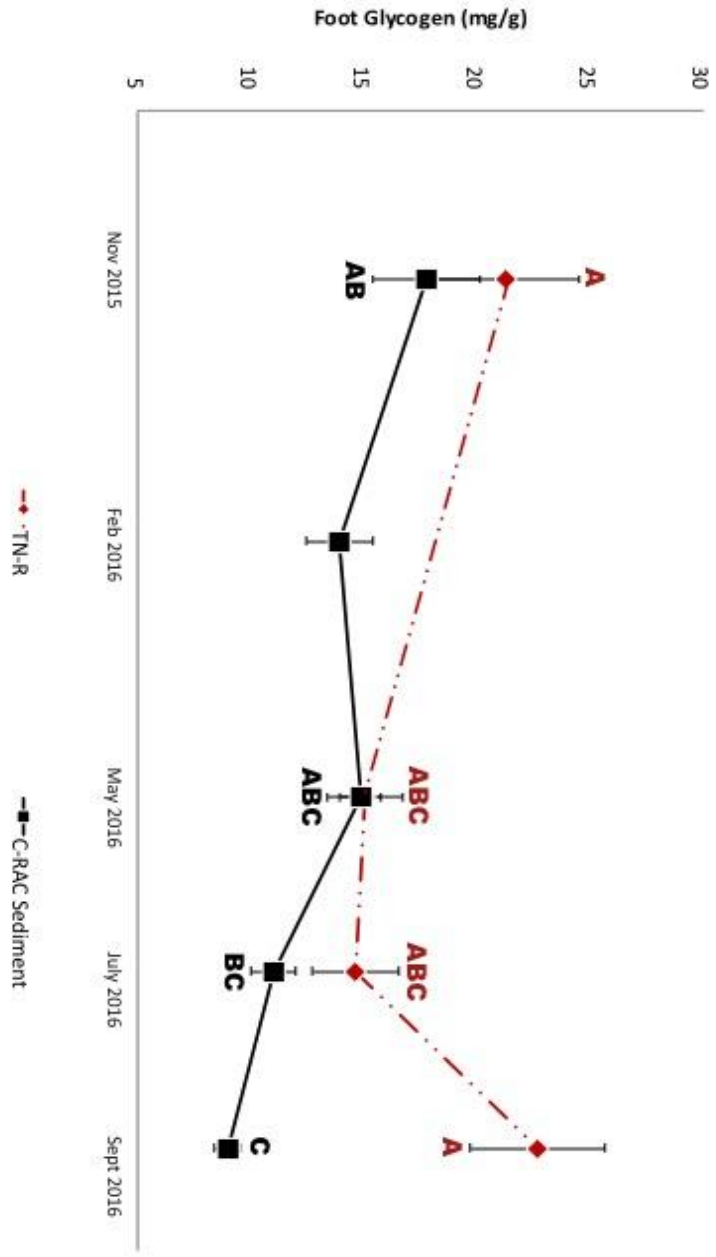

Figure 4: Glycogen concentration (mg/g) in foot tissues (mean ± SE) in the Tennessee River and C-RAC. Dashed line and diamonds represent mussels in the Tennessee River. Solid line and squares represent mussels in the Cumberland River Aquatic Center sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

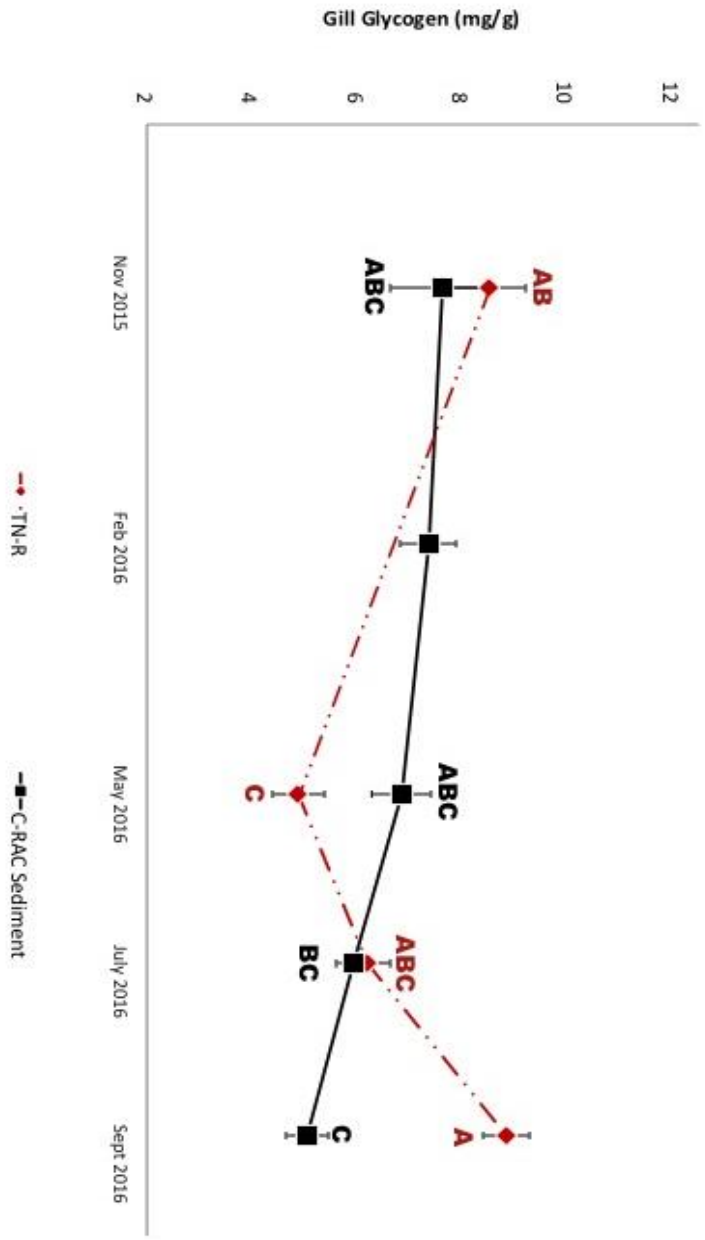

Figure 5: Glycogen concentration (mg/g) in gill tissues (mean ± SE) in the Tennessee River and C-RAC. Dashed line and diamonds represent mussels in the Tennessee River. Solid line and squares represent mussels in the Cumberland River Aquatic Center sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

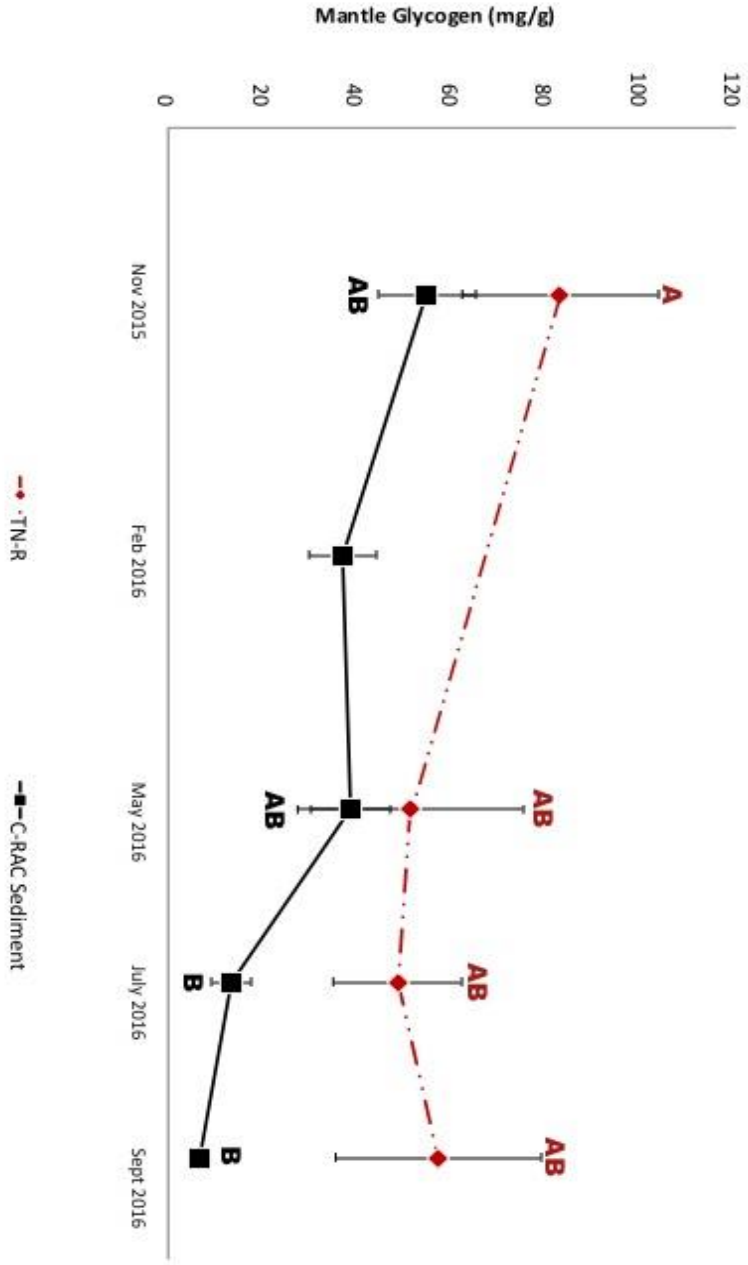

Figure 6: Glycogen concentration (mg/g) in mantle tissues (mean ± SE) in the Tennessee River and C-RAC. Dashed line and diamonds represent mussels in the Tennessee River. Solid line and squares represent mussels in the Cumberland River Aquatic Center sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

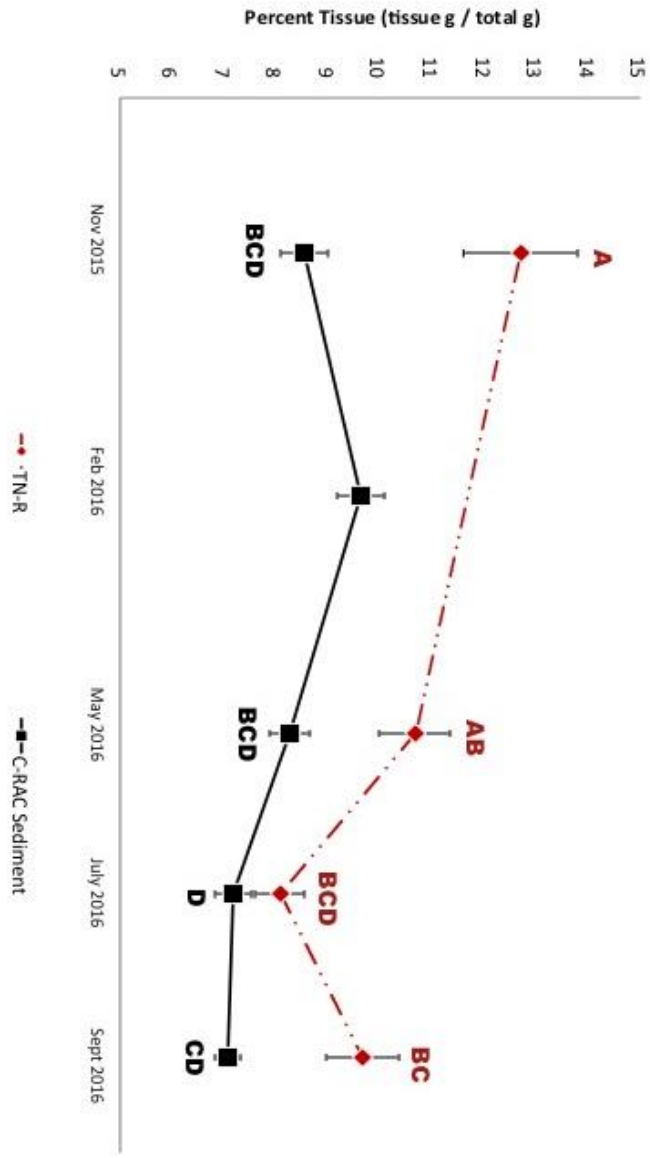

Figure 7: Percent tissue (tissue weight divided by total weight) (mean ± SE) in the Tennessee River and C-RAC. Dashed line and diamonds represent mussels in the Tennessee River. Solid line and squares represent mussels in the Cumberland River Aquatic Center sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

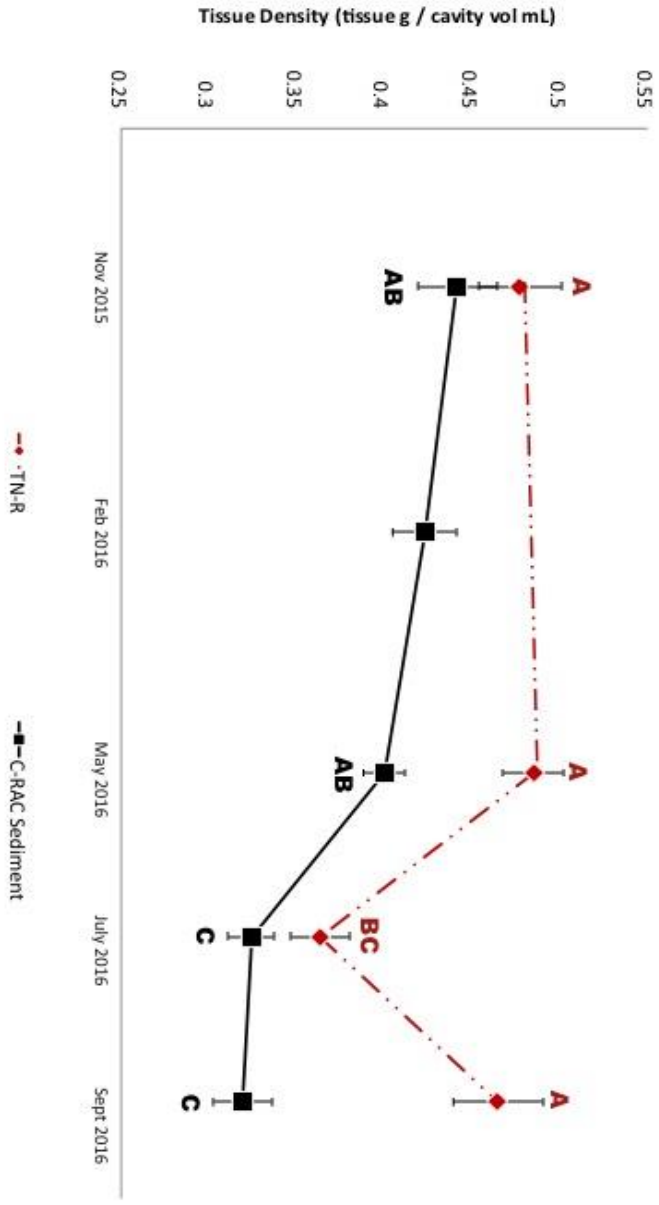

Figure 8: Tissue density (tissue weight divided by cavity volume) (mean ± SE) in the Tennessee River and C-RAC. Dashed line and diamonds represent mussels in the Tennessee River. Solid line and squares represent mussels in the Cumberland River Aquatic Center sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

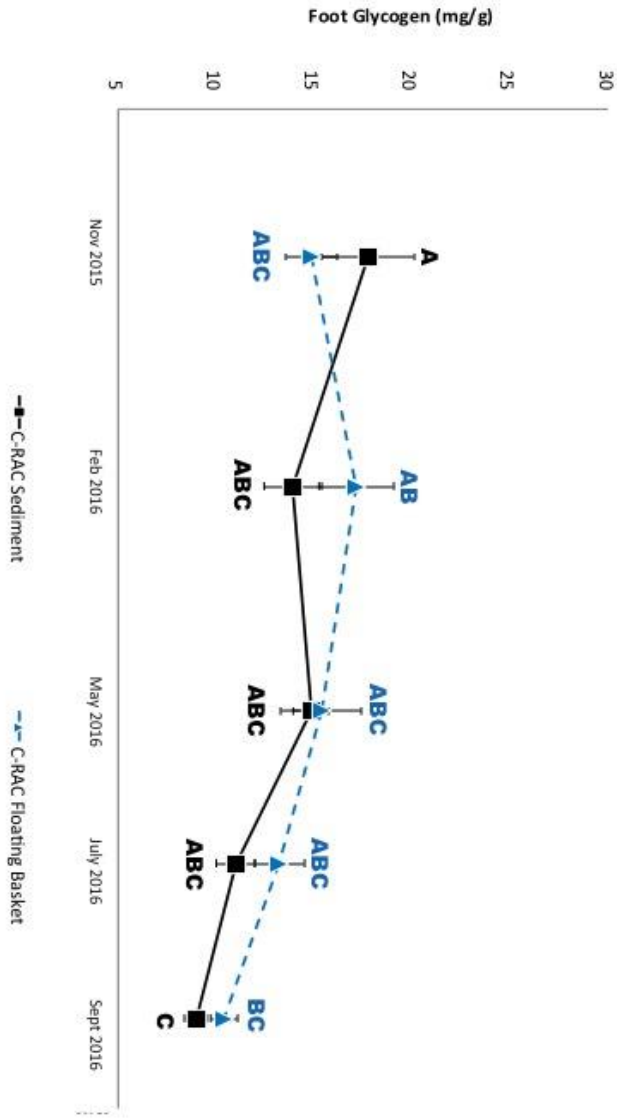

Figure 9: Glycogen concentration (mg/g) in foot tissues (mean ± SE) in C-RAC baskets and sediment. Dashed line and triangles represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

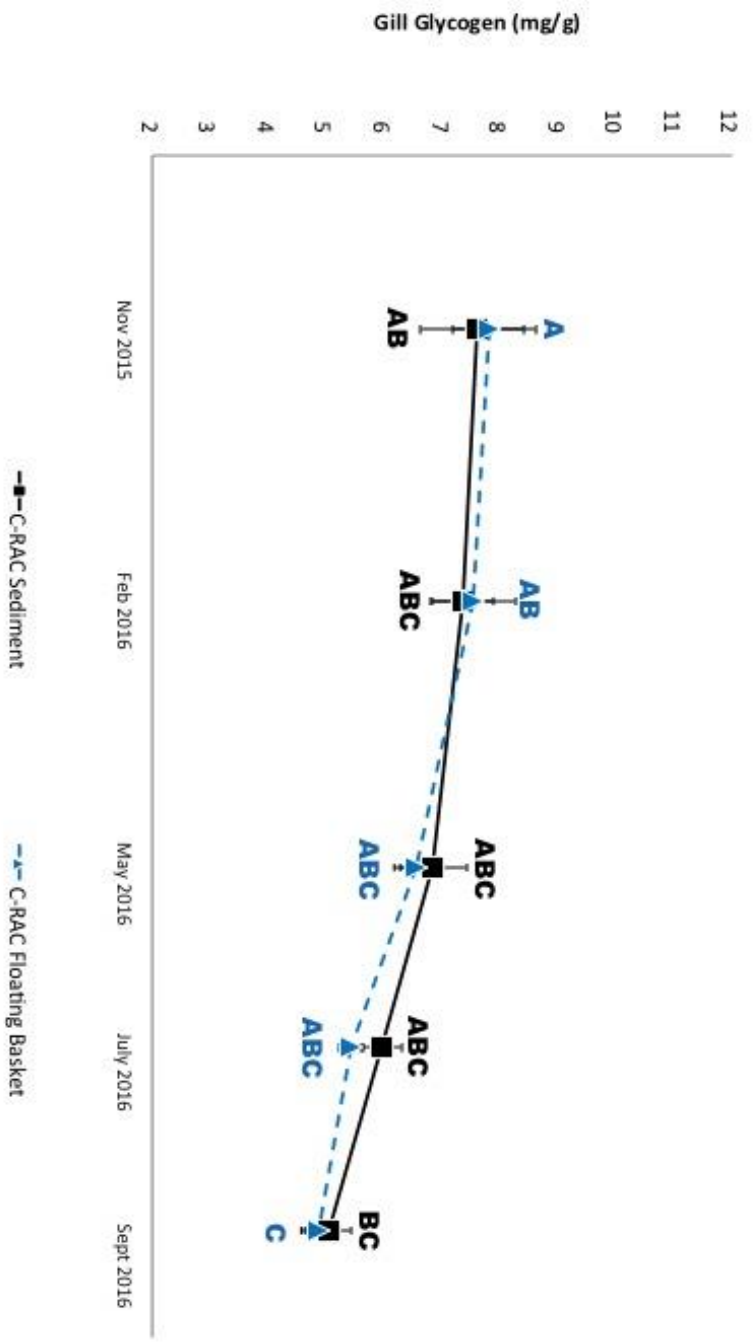

Figure 10: Glycogen concentration (mg/g) in gill tissues (mean ± SE) in C-RAC baskets and sediment. Dashed line and triangles represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

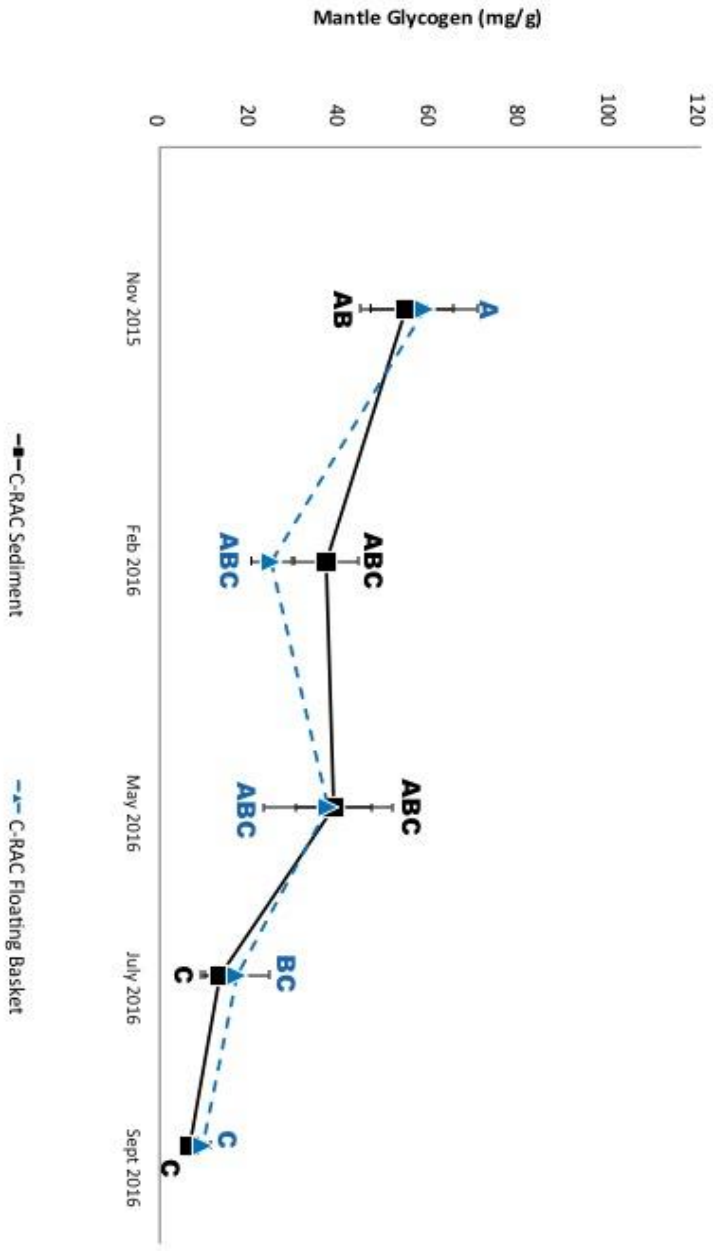

Figure 11: Glycogen concentration (mg/g) in mantle tissues (mean ± SE) in C-RAC baskets and sediment. Dashed line and triangles represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

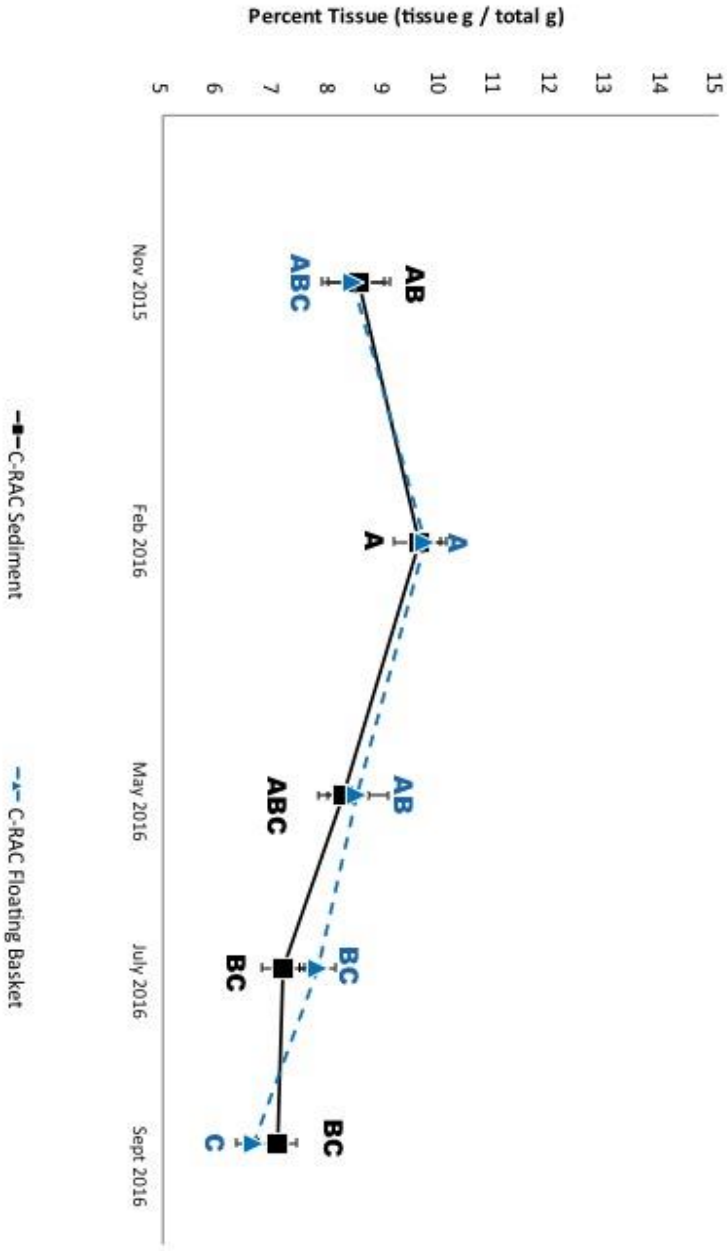

Figure 12: Percent tissue (tissue weight divided by total weight) (mean ± SE) in C-RAC baskets and sediment. Dashed line and triangles represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

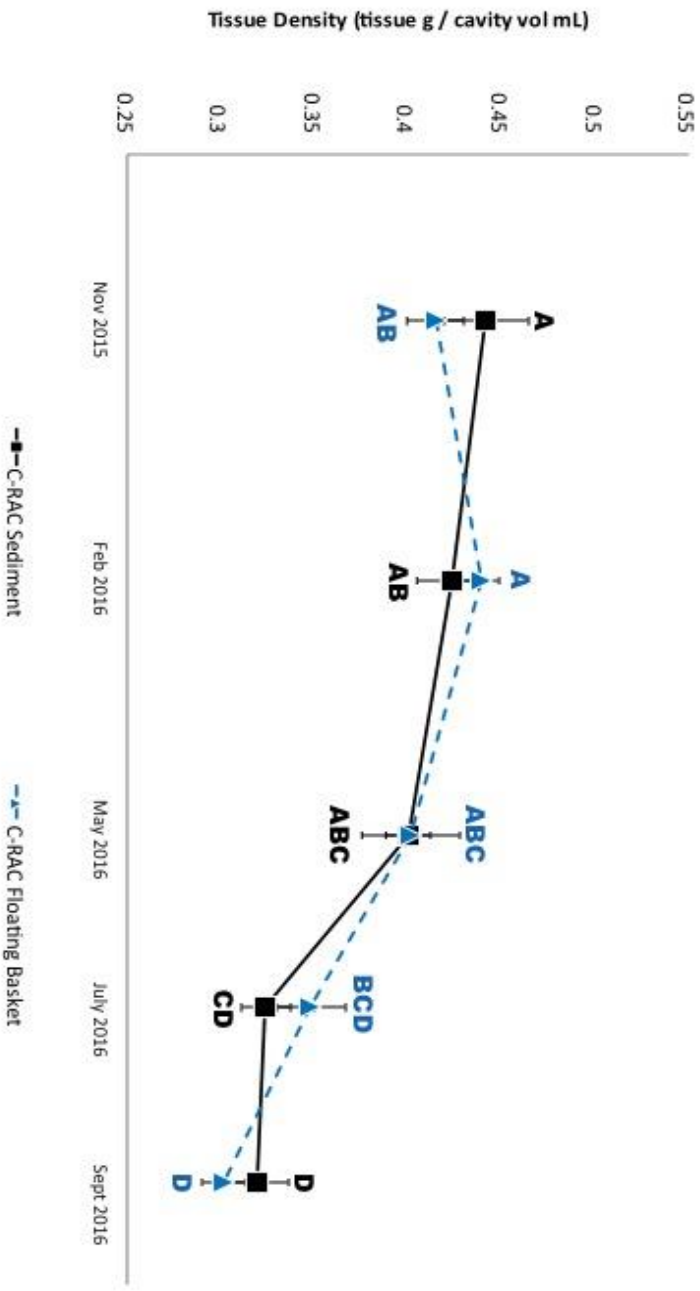

Figure 13: Tissue Density (tissue weight divided by cavity volume) (mean ± SE) in C-RAC baskets and sediment. Dashed line and triangles represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Letters represent results from the two-way ANOVA Tukey's Post Hoc test.

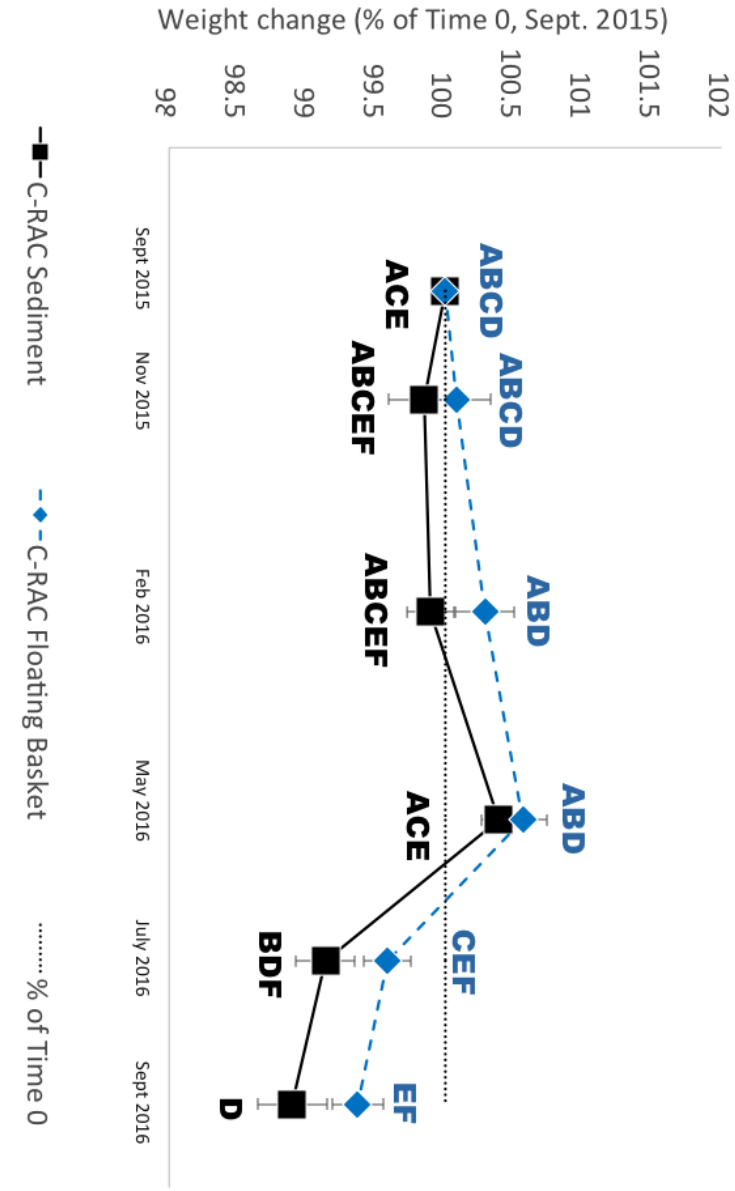

Figure 14: Repeated measures of percent total weight change (mean ± SE) in C-RAC baskets and sediment. Weights at each sample time were divided by the individual's original weight, multiplied by 100, and averaged. Dashed line and diamonds represent mussels in the C-RAC floating baskets. Solid line and squares represent mussels in the C-RAC sediment. Horizontal dashed line represents original mean total weight in September 2015. Letters represent results from the Tukey's Post Hoc test on the two-way repeated measures ANOVA on original data.

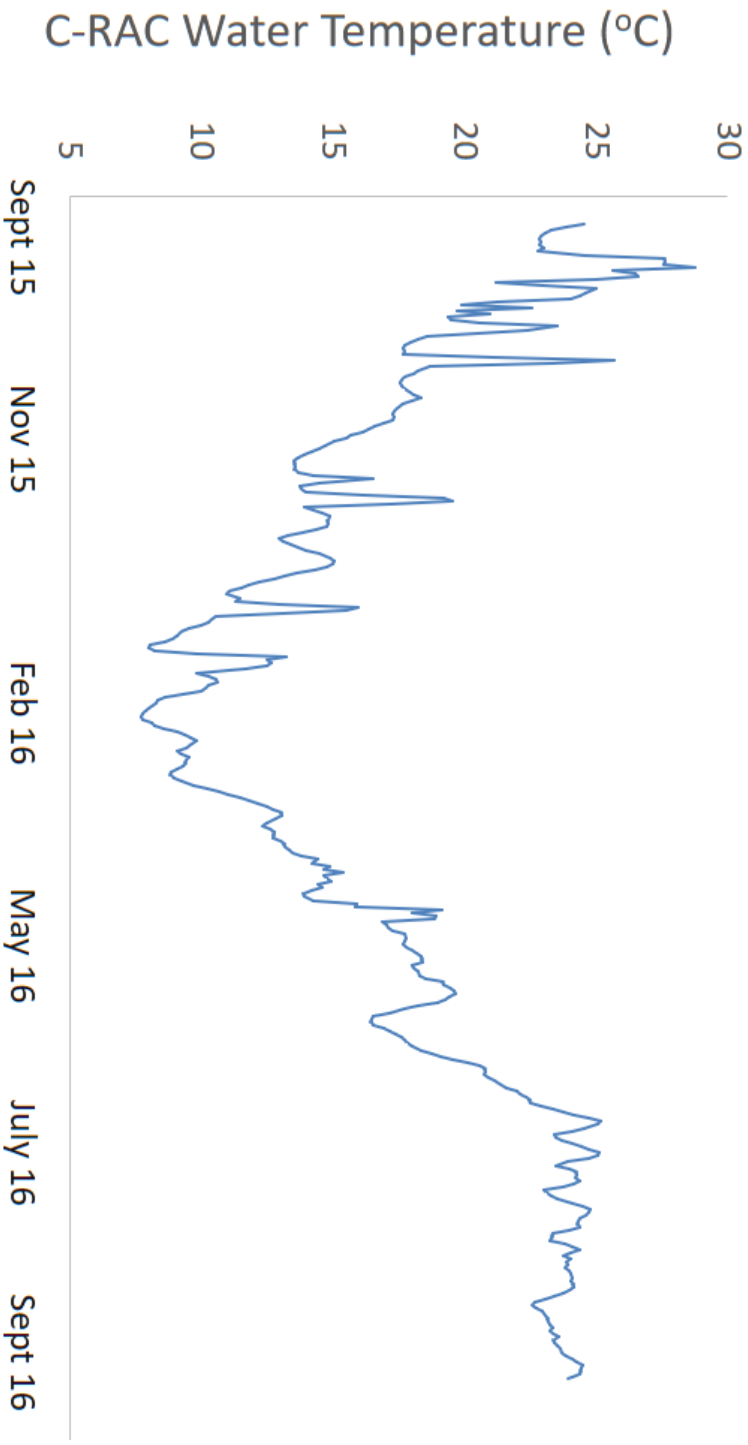

Figure 15: Daily means of water temperature in C-RAC tanks. Temperatures were logged every half hour with HOBO Water Temp Pro V2.

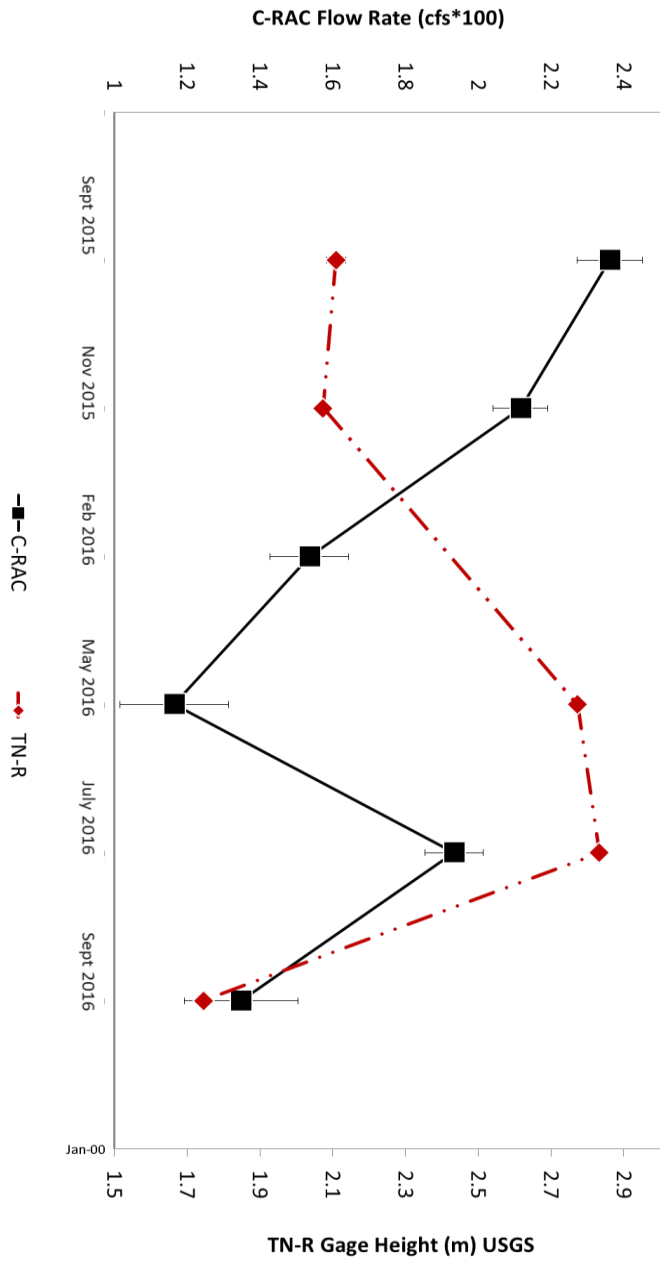

Figure 16: Flow rate in the C-RAC tanks (mean ± SE) and gage height in the Tennessee River. Flow rate in the C-RAC tanks represented by squares and solid line. Gage height from USGS in the Tennessee River at Savannah, TN represented by diamonds and dashed lines.

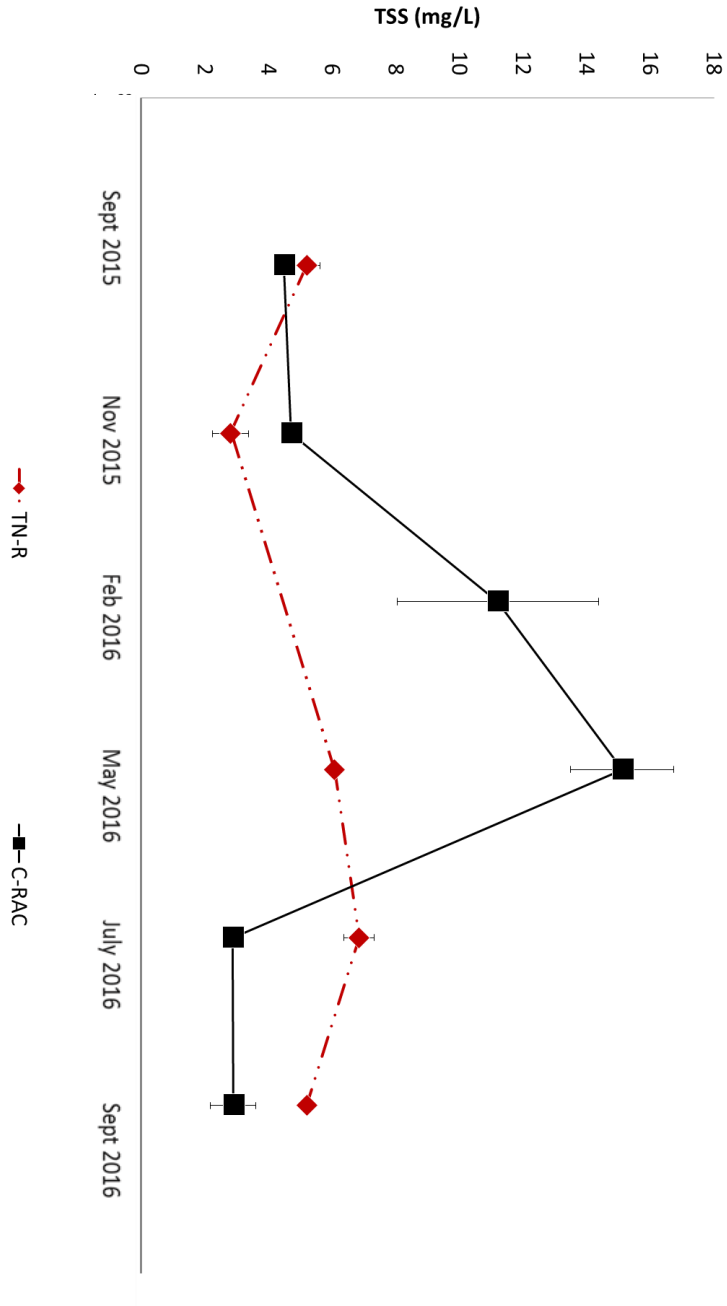

Figure 17: Total suspended solids (mean ± SE). Squares and solid line represents water in the C-RAC. Diamonds and dashed lines represent water in the Tennessee River.

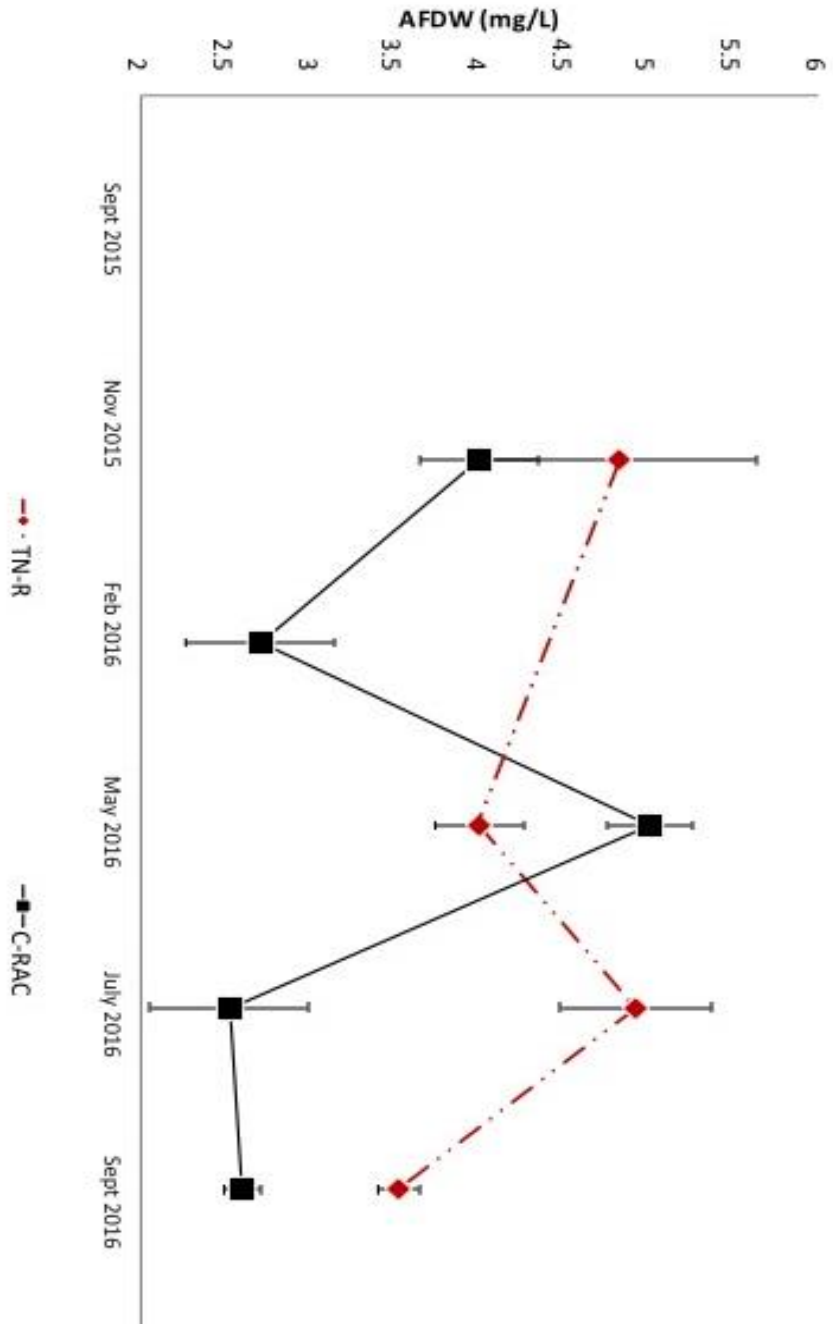

Figure 18: Ash free dry weight (mean ± SE). Squares and solid line represents water in the C-RAC. Diamonds and dashed lines represent water in the Tennessee River.

APPENDIX B: Tables

Table 1: All mussel endpoints (mean ± SE).

Time		n	Location	Total Weight (g)	Shell Length (mm)	Shell Height (mm)	Shell Width (mm)	Total Volume (mL)	Hemolym ph Volume (mL)	Tissue Weight (g)	Cavity Volume (mL)	Shell Weight (g)
0	September 2015											
		6	TN River	142.0 ± 7.8	69.4 ± 2.2	57.4 ± 1.7	44.6 ± 0.6	58.2 ± 9.2	0.8 ± 0.0	13.9 ± 1.2	28.0 ± 4.8	116.3 ± 5.8
		6	C-RAC	125.8 ± 14.8	65.9 ± 2.6	54.6 ± 2.4	43.8 ± 1.7	59.8 ± 6.1	0.9 ± 0.1	10.6 ± 0.9	25.5 ± 2.4	103.9 ± 12.9
1	November 2015											
		6	TN River	79.3 ± 12.8	61.6 ± 3.3	53.3 ± 2.4	38.0 ± 2.2	35.5 ± 7.7	0.8 ± 0.1	9.7 ± 1.2	20.3 ± 2.3	69.6 ± 9.1
		9	C-RAC-Sediment	135.1 ± 5.6	68.3 ± 1.2	57.1 ± 1.0	44.1 ± 0.9	71.1 ± 4.2	0.9 ± 0.0	11.6 ± 0.9	26.1 ± 1.1	108.6 ± 4.4
		9	C-RAC-Baskets	129.7 ± 6.6	68.1 ± 1.6	57.8 ± 1.1	43.8 ± 1.0	67.2 ± 3.2	0.9 ± 0.1	10.9 ± 0.7	26.3 ± 1.4	103.9 ± 5.5
2	February 2016											
		6	TN River	-- ± --	-- ± --	-- ± --	-- ± --	-- ± --	-- ± --	-- ± --	-- ± --	-- ± --
		9	C-RAC-Sediment	120.3 ± 9.4	66.7 ± 2.3	57.1 ± 1.4	41.3 ± 1.1	58.3 ± 4.5	0.8 ± 0.0	11.4 ± 0.8	27.1 ± 1.7	96.5 ± 7.9
		9	C-RAC-Baskets	146.2 ± 12.4	72.6 ± 2.5	60.2 ± 1.6	45.4 ± 1.3	70.0 ± 6.5	0.8 ± 0.1	13.9 ± 0.9	31.8 ± 2.2	116.9 ± 10.5
3	May 2016											
		6	TN River	155.5 ± 17.6	74.3 ± 2.9	61.4 ± 1.8	44.9 ± 1.8	83.3 ± 8.7	1.0 ± 0.0	16.1 ± 0.9	33.5 ± 2.6	123.9 ± 15.5
		9	C-RAC-Sediment	114.3 ± 15.6	64.1 ± 2.5	51.8 ± 1.7	41.1 ± 1.9	56.1 ± 8.9	0.8 ± 0.1	9.1 ± 0.7	22.6 ± 1.6	93.6 ± 13.8
		9	C-RAC-Baskets	119.2 ± 10.8	68.0 ± 2.7	53.4 ± 2.3	42.1 ± 1.3	53.9 ± 5.8	0.9 ± 0.1	10.3 ± 1.1	25.4 ± 2.2	95.9 ± 8.7
4	July 2016											
		6	TN River	146.1 ± 14.9	72.0 ± 2.1	57.2 ± 1.1	45.3 ± 2.2	73.3 ± 6.3	0.8 ± 0.1	11.5 ± 0.9	31.9 ± 2.4	120.1 ± 13.7
		9	C-RAC-Sediment	122.3 ± 9.5	67.4 ± 1.9	56.8 ± 1.4	42.5 ± 1.1	61.4 ± 4.6	0.8 ± 0.0	8.6 ± 0.4	26.7 ± 1.5	99.3 ± 8.3
		9	C-RAC-Baskets	131.0 ± 10.1	69.5 ± 2.5	57.5 ± 1.4	43.0 ± 1.4	65.0 ± 5.1	0.9 ± 0.0	10.1 ± 0.8	29.0 ± 1.8	105.1 ± 8.4
5	September 2016											
		6	TN River	150.9 ± 16.3	72.9 ± 1.9	61.2 ± 2.1	45.7 ± 2.0	71.7 ± 7.9	0.8 ± 0.0	14.0 ± 0.8	30.9 ± 2.8	121.0 ± 13.3
		8	C-RAC-Sediment	113.5 ± 10.7	66.6 ± 2.3	55.7 ± 1.9	41.3 ± 1.1	55.0 ± 5.7	0.9 ± 0.0	8.1 ± 0.9	25.0 ± 1.9	89.5 ± 8.6
		9	C-RAC-Baskets	116.3 ± 9.0	65.7 ± 1.9	55.3 ± 1.6	41.0 ± 1.2	53.9 ± 4.6	0.8 ± 0.1	7.8 ± 0.8	25.4 ± 1.8	93.3 ± 7.3

Table 2a: Water endpoints (mean ± SE). Temperature and pH were taken simultaneously with ORION STAR A321 field meter.

Time		Location	Temperature (°C)		pH		DO (mg/L)		Conductivity (µs/cm)		Flow Rate (cfs)	
			n		n		n		n		n	
0	September 2015	TN River	5	27.52 ± 0.21	5	7.26 ± 0.10	5	5.94 ± 0.02	5	180.30 ± 0.09	0	-- ± --
		C-RAC	5	23.80 ± 0.00	5	7.43 ± 0.06	5	8.38 ± 0.04	5	187.42 ± 0.04	0	2.4E-02 ± 9.0E-04
1	November 2015	TN River	5	20.00 ± 0.05	5	7.76 ± 0.04	5	8.10 ± 0.11	5	209.62 ± 0.12	na	4.8E+04 ± 0.0E+00
		C-RAC	3	18.90 ± 0.00	3	7.67 ± 0.02	4	9.45 ± 0.27	3	206.10 ± 0.12	3	2.1E-02 ± 9.6E-04
2	February 2016	TN River	0	-- --	0	-- --	0	-- --	0	-- --	0	-- --
		C-RAC	3	8.40 ± 0.06	3	7.98 ± 0.00	3	11.87 ± 0.22	3	108.20 ± 0.12	5	3.6E-04 ± 2.5E-05
3	May 2016	TN River	3	20.30 ± 0.06	3	7.45 ± 0.01	3	7.46 ± 0.11	3	120.30 ± 0.15	na	8.5E+03 ± 0.0E+00
		C-RAC	3	18.30 ± 0.06	3	7.80 ± 0.03	3	9.05 ± 0.13	3	146.47 ± 0.22	5	2.8E-04 ± 3.5E-05
4	July 2016	TN River	3	29.53 ± 0.09	3	8.16 ± 0.01	3	5.59 ± 0.08	3	158.90 ± 0.26	0	-- ± --
		C-RAC	3	24.30 ± 0.00	3	7.72 ± 0.03	3	7.33 ± 0.07	3	160.83 ± 0.12	5	1.0E-03 ± 4.3E-05
5	September 2016	TN River	4	29.23 ± 0.03	4	7.98 ± 0.05	3	6.37 ± 0.03	3	192.57 ± 0.12	0	-- ± --
		C-RAC	3	24.20 ± 0.00	3	7.59 ± 0.01	3	6.94 ± 0.15	3	164.17 ± 0.12	5	7.2E-04 ± 8.3E-05

Table 2b: Water endpoints (mean ± SE), continued. Asterisk denotes samples were processed out of hold time.

Time		Location	Alkalinity (mg/L $CaCO_3$)		Nitrate (µg/L)		Nitrite (µg/L)		Ortho-Phosphate (µg/L)		TSS (mg/L)		Ash Free Dry Weight (mg/L)	
			n		n		n		n		n		n	
0	September 2015	TN River	5	62.08 ± 0.05	3	53.33 ± 12.02	5	5.40 ± 0.51	5	166.00 ± 8.72	5	5.2 ± 0.4	0	-- ± --
		C-RAC	5	77.28 ± 5.24	5	144.00 ± 24.21	5	4.20 ± 0.37	5	68.00 ± 2.00	5	4.5 ± 0.2	0	-- ± --
1	November 2015	TN River	5	77.40 ± 1.33	5	114.00 ± 16.00	5	5.00 ± 0.00	5	202.00 ± 4.90	5	2.8 ± 0.6	5	4.8 ± 0.8
		C-RAC	5	76.60 ± 2.71	5	176.00 ± 12.88	5	4.00 ± 0.45	5	126.00 ± 9.27	5	4.7 ± 0.3	5	4.0 ± 0.4
2	February 2016	TN River	0	-- --	0	-- --	0	-- --	0	-- --	0	-- --	0	-- --
		C-RAC	5	70.20 ± 2.11	5	262.00 ± 11.14	3	4.33 ± 0.67		74.00 ± 4.00	* 8	11.2 ± 3.2	* 7	2.7 ± 0.4
3	May 2016	TN River	5	63.28 ± 0.45	5	164.00 ± 21.82	3	16.67 ± 0.88	5	118.00 ± 3.74	5	6.1 ± 0.1	5	4.0 ± 0.3
		C-RAC	5	75.72 ± 0.40	5	118.00 ± 15.94	3	5.67 ± 0.33	5	88.00 ± 3.74	5	15.1 ± 1.6	5	5.0 ± 0.3
4	July 2016	TN River	5	69.72 ± 0.55	5	26.00 ± 2.45	3	6.33 ± 0.88	5	62.00 ± 13.56	5	6.8 ± 0.5	5	4.9 ± 0.5
		C-RAC	5	67.80 ± 1.33	5	178.00 ± 21.31	3	6.00 ± 0.00	5	180.00 ± 15.81	5	2.9 ± 0.2	5	2.5 ± 0.5
5	September 2016	TN River	5	68.48 ± 0.41	4	20.00 ± 0.00	3	6.67 ± 0.88	5	156.00 ± 6.78	5	5.2 ± 0.1	5	3.5 ± 0.1
		C-RAC	5	62.76 ± 0.43	4	137.50 ± 2.50	3	4.67 ± 1.20	5	190.00 ± 39.50	5	2.9 ± 0.7	5	2.6 ± 0.1