

March 16, 1999

Dr. Richard Neves
Virginia Polytechnic Institute
Department of Fisheries and Wildlife Sciences
Blacksburg, VA 24061-0321

Dear: Dr. Neves

RE: PEER REVIEW OF RESEARCH PROPOSAL ENTITLED:

Effects of Particle Concentration and Quality on the Feeding

Physiology of Unionid Mussels

Enclosed is a blank peer review form. Please fill out the form and return to me, at the following address:

Bob Schnelle Cinergy Corp.

139 East Fourth St. Room 522-A

Cincinnati, OH 45202

Failure to respond within 30 days constitutes your approval of the proposal. If you have any questions, please call me at 513/287-2239.

Very truly yours,

ROBERT C. SCHNELLE, Chair

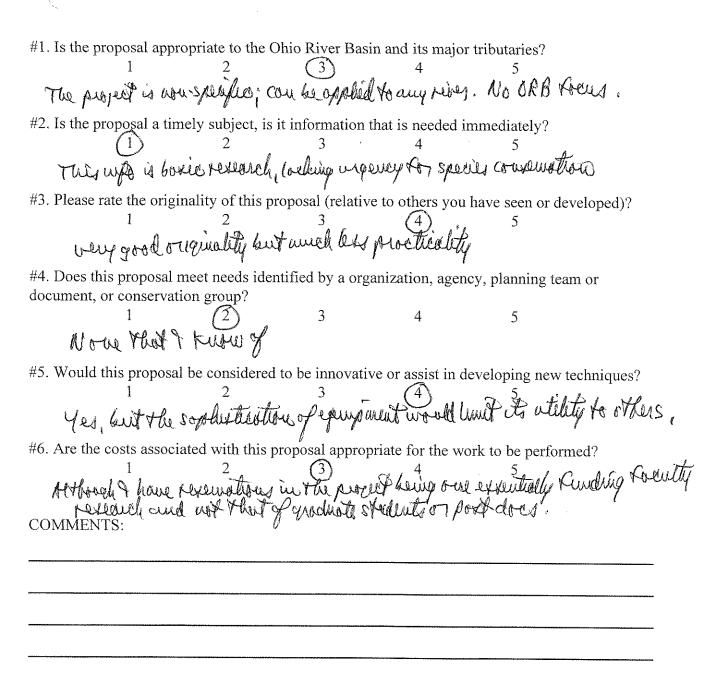
Mussel Mitigation Trust

enclosure

MUSSEL MITIGATION TRUST PEER REVIEW FORM

<u>Instructions:</u> Please review the enclosed proposal and respond to the questions listed below. Each question is self explanatory and all you have to do is circle the appropriate number. The numbers range from 1 to 5 with 1 indicating the proposal is not appropriate to the question at all to 5 indicating the proposal fully embraces the question. If you have any additional comments, please put them on the bottom of the form. When finished, please return the form and proposal to: Robert Schnelle, Mussel Mitigation Trust, c/o Cinergy Corp., 139 East Fourth Street, Room 522-A, Cincinnati, OH 45202.

We appreciate your help.



Comments

This is an excellent basic research proposal to assess the functional feeding structures and mechanisms in unionids. However, there is little applied research (except for perhaps objective 3) that can be used by management or regulatory agencies to benefit the native mussel fauna of the Ohio River Basin. Objective 1 (micro-anatomy of gills, pallial organs and gill cirro), objective 2 (video recordings of particle trajectories and capture), and objective 4 (CD-ROM of endoscopic recordings) have no relevance to the immediate conservation needs of the Ohio River fauna. The proposal is much suited for submittal to an agency that funds basic research such as NSF.

Research Grant Proposal Submitted to

Mussel Mitigation Trust

EFFECTS OF PARTICLE CONCENTRATION AND QUALITY ON THE FEEDING PHYSIOLOGY OF UNIONID MUSSELS

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Project Period: March 1, 1999-February 29, 2000

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Effects of Particle Concentration and Quality on the Feeding Physiology of Unionid Mussels

Richard A. Tankersley, Assistant Professor Department of Biological Sciences, University of Maryland Baltimore County

I. SUMMARY

Studies on a wide range of suspension feeders and planktonic grazers indicate that feeding dynamics are often influenced by differences in particle concentration, composition, and quality, which often vary both spatially and temporally. The proposed study will examine the impact of seston load and composition on the suspension feeding dynamics of three common and ecologically important freshwater mussels from the Ohio River Basin: *Amblema plicata*, *Megalonaias nervosa*, and *Quadrula pustulosa*. The specific objectives of the project include:

- 1. To use light and scanning electron microscopy to describe and compare the microanatomy of suspension feeding structures.
- 2. To use recently developed techniques in video endoscopy to investigate the transport and processing of particles by the pallial organs and to determine the mechanisms and pathways involved in particle selection and rejection as pseudofeces.
- 3. To determine and compare the feeding strategies used by mussels when exposed to quantitatively and qualitatively different assemblages of suspended particles.

Increased knowledge of these aspects of the feeding ecology of unionid mussels will provide researchers and conservation biologists with a greater understanding of (1) the environmental cues that mediate feeding, (2) the impact of temporal changes in seston concentration and composition on the survivorship and physiological condition of mussel populations, and (3) the feeding strategies utilized by mussels for processing particles and acquiring sufficient energy for growth and reproduction during periods of poor food quality. Furthermore, information on the effect of increased siltation and turbidity on mussel feeding and nutrition will help resource agencies evaluate the potential impact of various anthropogenic factors on mussel populations.

II. PROJECT DESCRIPTION AND BACKGROUND

Over the past century, populations of freshwater mussels belonging to the families Unionidae and Margaritiferidae have declined dramatically with nearly half of the estimated 300 species and subspecies currently considered extinct or listed as federally endangered, threatened, or of special concern (Neves 1993, Williams *et al.* 1993). Principal causes of the observed decline include commercial harvesting for the cultured pearl industry, poor land use practices, river impoundment, loss of suitable habitat, changes in water quality and chemistry, and the introduction of non-native species (Neves 1987, Bogan 1993, Williams *et al.* 1993). One common feature of the many factors influencing the survivorship of mussels in riverine habitats is an increase in the concentration of suspended silt and sediment, especially particulate inorganic matter (PIM). Natural and anthropogenic activities that promote the resuspension of

sediment and often cause substantial increases in turbidity and silt load include dredging, channelization, bank erosion, agricultural runoff, and destruction of riparian zones. Unionid mussels, like most bivalves, feed on suspended particles, such as microscopic plant and dead organic material (i.e., seston), and are known to be negatively affected by high turbidity and poor food availability and quality (for review see Bayne 1993 and Navarro and Iglesias 1993). Factors that determine diet quality for suspension feeding bivalves include (1) the size distribution of particles, (2) the relative proportion of inert and metabolizable nutritious particles, and (3) the chemical contribution and digestibility of the nutritious portion (Bayne et al. 1987). Therefore, an increase in the proportion of PIM in seston may "dilute" the nutritional content of the suspended particles available for ingestion by mussels, consequently limiting their growth and reducing their physiological condition. Although numerous studies have examined the effect of food concentration and composition on the feeding strategies of marine and estuarine bivalves (for review see Bayne 1993 and Navarro and Iglesias 1993), the responses of unionid mussels to changing seston conditions have received far less attention. Thus, a detailed examination of the physiological and behavioral processes underlying feeding and ingestion in unionid mussels is needed to determine if declines in mussel abundance and diversity are linked to their ability to compensate for fluctuations in suspended particulate matter (SPM) and to acquire sufficient energy for growth and reproduction during periods of high turbidity.

The compensatory mechanisms and feeding behaviors utilized by bivalve mollusks for maximizing energy uptake under conditions of variable food quality and quantity have been well documented, especially in marine and estuarine species. The amount of food available for ingestion is determined by the concentration of particles in suspension, the efficiency of particle capture, and the volume of water processed (i.e., "pumped") (for review see Jørgensen 1990). Particle capture is mediated by several factors including particle size, composition, and concentration (Sprung and Rose 1988, Reeders and Bij de Vaate 1992), as well as less obvious factors, including electrical charge, shape, and chemical properties, resulting in certain types of particles being retained at higher rates than others (for review see Jørgensen 1990). Many suspension-feeding bivalves regulate the volume of ingested particles under variable seston concentrations by either adjusting pumping and clearance rates or by rejecting filtered material as pseudofeces. Several species are also able to enhance the quality of their diet by selecting or sorting particles based upon size or quality prior to ingestion (e.g., Loosanoff 1949, Kiørboe and Møhlenberg 1981, Newell and Jordan 1983, Shumway et al. 1985, Newell et al. 1989, Prins et al. 1991, MacDonald and Ward 1994, Bougrier et al. 1997, Defossez and Hawkins 1997, Ward et al. 1998). Although additional post-ingestive selection (i.e., preferential digestion) may occur within the stomach (Bricelj et al. 1984, Shumway et al. 1985, Lopez and Levinton 1987, Bayne 1993, Wang and Fisher 1996), the gills and labial palps are thought to be the primary sites of preingestive selection (for review see Ward et al. 1998). Thus, when presented with a diverse assemblage of suspended particles, some species use their gills and palps to retain or sort particles on the basis of their chemical properties so that more nutritious particles are transported to the mouth for ingestion, while less-nutritious, undesirable particles are bound in mucus and expelled from the pallial cavity as pseudofeces. Although past studies have documented the responses of marine and estuarine bivalves to variable food supplies and have examined the roles of ctenidia and palps in particle sorting and selection, no studies have conclusively determined that unionid mussels are capable of selective ingestion or attempted to identify the pallial organs involved in regulating the volume and quality of ingested material.

Our previous studies of suspension feeding in unionid mussels indicated that particles retained by the ctenidia (gills) are transported ventrally toward the free margin of the gills where they are incorporated in a complex mucous-bound cord traveling anteriorly in the heavily ciliated ventral food groove of the medial gills (Tankersley and Dimock 1993b, Tankersley 1996). At the gill-palp interface, mucus-bound particles are transferred as continuous strands to the opposing surfaces of the palps but are broken down into smaller clumps as they travel anteriorly toward the mouth, aided by cilia beating on the inner surfaces of the palp lamellae. However, preliminary results suggest that the exact patterns of particle transport by pallial organs and the mechanisms involved in regulating particle ingestion vary with the quantity and composition of suspended material (R. Tankersley, unpublished data). Although differences in gill type and architecture have been linked to fundamental differences in the mechanisms involved in particle sorting and ingestion (Ward et al. 1993), it is unlikely that the observed shifts in particle processing by unionid mussels in response to changes in seston quality or quantity are the result of differences in gill structure since all members of the Unionidae possess the same gill type (homorhabdic eulamellibranch). We hypothesize that shifts in feeding strategies are more closely linked to the mussel's preferred habitat (e.g., lentic vs. lotic) and may constitute adaptations to exploit prevailing particle concentrations found in their natural environment. Thus, the impact of changes in suspended particles on the nutrition, health, and survivorship of unionid mussels may depend upon their feeding strategy and ability to cope with fluctuations in seston composition and concentration.

Although the basic pathways used by unionid mussels for processing potential food particles are well understood (see Tankersley 1996 for review), the thick opaque shells of adult animals preclude direct observations of the fluid dynamics and structures involved in feeding. Most studies examining the feeding physiology of bivalves, including members of the Unionidae, have been conducted on life history stages possessing transparent shells (Dral 1967, Jørgensen and Ockelmann 1991, Reid *et al.* 1992, Tankersley 1999a) or have been inferred from dissected structures or surgically altered and narcotized animals (*e.g.*, McGinitie 1941, Bernard 1974, Foster-Smith 1975, 1978, Jørgensen 1975, Reid *et al.* 1992). These invasive techniques may dramatically affect the activity of the gill ciliature, alter the patterns of water transport through the mantle cavity/gill, or stimulate excess production to mucus, causing the gill and other pallial structures to function abnormally (for review see Beninger *et al.* 1997). In the present study, we will avoid many of these artifacts by using recently developed video endoscopic techniques to observe and quantify, *in vivo*, the effects of seston load and quality on the feeding biodynamics of unionid mussels (Ward *et al.* 1991, Tankersley and Dimock 1993b).

Endoscopy has several advantages over more traditional and invasive methods of observing and quantifying the mechanisms associated with particle selection and ingestion and has recently been used to examine the intricacies of suspension feeding in a variety of marine and freshwater bivalves (Ward et al. 1991, Tankersley and Dimock 1993b, Beninger et al. 1992, Ward et al. 1993, Ward et al. 1994, Tankersley 1996, Ward 1996, Beninger et al. 1997, Beninger and St-Jean 1997). Endoscopy permits direct, high magnification (150x) observation of particle capture and transport that is impossible to obtain using conventional methods without interfering with normal feeding and significantly altering the flow dynamics and trajectories of particles within the mantle cavity. Combining endoscopy with video recording and image analysis enables observation of real time events and permits post-observational quantitative analysis of physiological processes. As a result, endoscopy has shed new light on the role of the ctenidia (gills) and other pallial organs in particle selection and sorting and has prompted a re-evaluation

of many of the established paradigms related to the biomechanics of particle capture, retention, and transport (see Tankersley 1996 and Ward 1996 for review).

In addition to endoscopy, we will use video-enhanced light microscopy (VLM) and scanning electron microscopy (SEM) as complementary tools to examine and compare the micro-anatomy of suspension feeding structures and pallial organs, including gill filaments, water tubes, cilia and cirri, ventral food grooves, and labial palps. This study will be the first comprehensive investigation of the structures and pathways involved in particle selection and ingestion by unionid mussels and will test hypotheses concerning the feeding strategies used by mussels when exposed to quantitatively and qualitatively different assemblages of suspended particles. Therefore, the results of the project will provide valuable information concerning the impact of temporal changes in food quality and concentration on feeding rates, the regulation of ingestion volume, the production of pseudofeces, and the ability of mussels to discriminate among potential food particles.

III. PROJECT GOALS AND OBJECTIVES

The goals and objectives of the project include:

- 1. To use traditional microscopic methods (both video-enhanced light microscopy and scanning electron microscopy) to describe and compare the suspension feeding structures of three common Ohio River mussels, the threeridge *Amblema plicata*, the washboard *Megalonaias nervosa* and the pimpleback *Quadrula pustulosa*. We will focus on the micro-anatomy of the gills and other pallial organs (e.g., palps), the presence and distribution of gill cilia and cirri, the arrangement of gill filaments and water tubes, and the structure and morphology of dorsal and ventral feeding tracts.
- 2. To use recently developed techniques in video endoscopy (Ward et al. 1991, Tankersley and Dimock 1993b) to make direct, minimally invasive observations of the structures and mechanisms of feeding in intact unionid mussels. Video recordings of particle trajectories, capture, and transport, as well as the processes involved in the regulation of ingestion volume and pseudofeces production will be used (1) to quantify and analyze suspension feeding processes and events, (2) to determine the principal patterns of water flow and particle transport, and (3) to investigate the mechanisms and pathways involved in particle acceptance and rejection.
- 3. To determine and compare the feeding strategies used by mussels when exposed to quantitatively and qualitatively different assemblages of suspended particles. Using flow cytometry, we will test the hypothesis that unionid mussels can selectively ingest highquality particles and regulate the composition of ingested material under different seston loads.
- 4. To develop and distribute an instructional/educational CD-ROM containing hyper-linked text, high-magnification photographs, digitized video clips of endoscopic recordings, and computerized animations, that illustrate and describe the processes and structures involved in bivalve suspension-feeding.

IV. METHODS

A. Collection and Maintenance of Mussels

Evaluation of the effects of seston quality and quantity on the feeding physiology of unionid mussels will be conducted using three common Ohio River species, *Amblema plicata*, *Megalonaias nervosa*, and *Quadrula pustulosa*. These species were selected for the study because (1) they have a broad distribution that includes much of the Ohio River and its tributaries, (2) they appear to utilize different strategies for coping with changes in seston load and quality, and (3) preliminary studies indicate they are amenable to endoscopic examination. Adults of both species will be collected by hand (using SCUBA if necessary) from the Lower Muskingum River and other selected sites in the Ohio River Basin during the summer and fall 1999. Following collection, mussels will be transported in a refrigerated holding tank to the laboratory where they will be maintained at collection temperatures in a recirculating aquaculture system (Tankersley and Butz 1999) and fed a multi-algal diet of live or spray-dried *Chlorella vulgaris*, *Schizochytrum* sp., and *Neochloris oleoabundans* (final concentration 2 x 10⁵ cells ml⁻¹).

B. Histological Examination of Suspension Feeding Structures

Histological examination of the gills, palps, and other pallial organs of *Amblema plicata*, *Megalonaias nervosa* and *Quadrula pustulosa* using video-enhanced light (VLM) and scanning electron microscopy (SEM) will follow the methods described by Tankersley and Dimock (1992). Both techniques will be used to describe and compare the distribution and spatial arrangement of the cilia and cirri on the gills, the structure of the ventral food groove, and the morphology of the inner surface of the palp lamellae, including the arrangement and distribution of the palp ridges and associated cirri.

<u>Video-Enhanced Light Microscopy</u>

Tissues for light microscopy will be excised and fixed in Bouin's fixative for 24 h and stored in 70% ethanol. For histological examination, tissues will be dehydrated in a graded ethanol series and xylene prior to being embedded in paraffin by vacuum infiltration. Serial frontal sections (7-8 μ m thick) will be cut on a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin. Sections will be examined using a Zeiss Photomicroscope and quantitative measurements of pertinent structures will be made from digitized images using a Scion LG-3 frame grabber (Scion Corp., Frederick, MD) and image analysis software (NIH-Image version 1.61).

Scanning Electron Microscopy

Specimens for SEM will be fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) at 4°C for 2 h, post-fixed in 2% cacodylate buffered (pH 7.4) osmium tetroxide for 1 h, and then rinsed with buffer. Specimens will then be dehydrated through a graded ethanol series, dried using liquid CO₂ (Ladd Critical Point Drier), mounted on aluminum SEM stubs, and sputter-coated with gold-palladium (Polaron Sputter Coater). Features of the gills and other pallial tissue will be examined and photographed with a JEOL-JSM-35CF scanning electron

microscope operating at 15 kV. A total of 5 specimens of each species will be used for microscopic (both LM and SEM) observations.

C. Examination of Particle Transport and Processing Using Endoscopy

Endoscopic examination of the gills and feeding structures of *Amblema plicata*, *Megalonaias nervosa*, and *Quadrula pustulosa* will follow the basic procedures described by Tankersley and Dimock (1993b) and Tankersley (1996). Observations will be made using a rigid endoscope (1.7 mm diam. x 19 cm long; Fibertron Corp., Carrolton, TX) attached to a xenon (250 W) light

source (Fig. 1). The tip of the scope provides a 60° field of view and a maximum magnification of about 150X. (Fig. 2). Maximum resolution is estimated to be about 4 µm. The viewing direction of the endoscope can be adjusted from 0° (direct view) to 90° (side-view) by attaching a mirror sleeve to the tip. Video recordings of pallial structures will be made by attaching the endoscope's ocular to a color CCD camera. Images will be recorded for later analysis using a S-VHS recorder (JVC Model HRS 6900).

For endoscopy, mussels will be placed in a refrigerated holding tank (30 cm x 15 cm x 15 cm) containing filtered (<0.45 µm) river

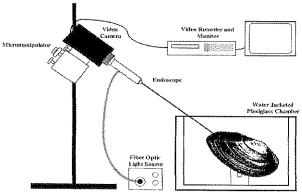


Figure 1: Diagrammatic representation of the endoscope and video equipment used to monitor the suspension feeding dynamics of intact unionid mussels. Feeding structures are observed and recorded using a videocamera and recorder attached to the scope's ocular. (Adapted from Tankersley and Dimock 1993).

water. Mussels will be held stationary in a vertical guide by a nylon bolt cemented to one value (Fig. 1). A rubber plug will be inserted between the valves near the incurrent margin of the shell to prevent the mussel from closing on the endoscope's tip. Mussels will be allowed to acclimate to the chamber for several hours before the endoscope is inserted into the infra- or suprabranchial cavities. Recording will commence after they appear to resume normal pumping activity (*i.e.*, valves open and siphons extended).

To observe and quantify the processes involved in particle capture, retention and ingestion, the optical insertion tube (OIT) of the endoscope will be placed within the infrabranchial chamber so that it rests within the inter-demibranchal space between the gill lamellae

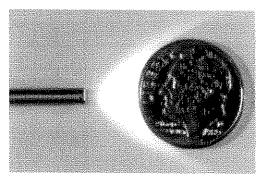


Figure 2: Image of the tip of the endoscope's optical insertion tube (OIT) showing the relative size and angle of illumination.

(mediolateral cavity; Fig. 3, position B) or near the gill-palp junction (Fig. 3, position C). Observations of the water tubes and flow through the gills will be accomplished by inserting the OIT in the suprabranchial cavity via the excurrent siphon and positioning it above either the lateral or medial gill (Fig. 3, position A). Because the excurrent siphons are relatively large (>12-18 mm²) compared to the endoscope's OIT (3 mm²) (Fig. 2), the presence of the tip of the scope within the cavity has no apparent effect on the pumping activity of the mussel or the flow of water traveling through the ctenidia and exiting the siphon. Endoscopic video

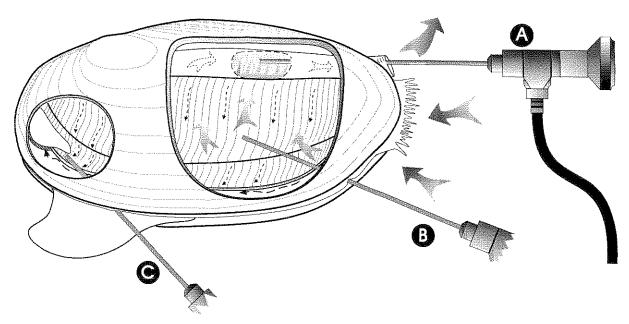


Figure 3: Diagrammatic representation of the placement and orientation of the optical insertion tube (OIT) of the endoscope for recording the suspension feeding activities of unionid mussels. Observation of the water tubes and inner surface of the ctenidia is accomplished by inserting the scope through the exhalent siphon and into the suprabranchial chamber (Position A). Particle capture, transport and ingestion are analyzed by placing the OIT near the surface or edge of the gills (Position B) or near the gill-palp junction (Position C). Large arrows indicate the general flow of water currents entering the mantle cavity. Small, dashed arrows indicate the predicted direction of particle transport. (Adapted from Tankersley 1996)

micrographs will be made from video recordings by digitizing single video frames using a Scion LG-3 frame grabber (Scion Corp., Frederick, MD) and image analysis software (PrismView, Analytical Vision, Raleigh, NC) running on a Macintosh PowerPC 9500 computer. Images will be calibrated by comparing the distance between filaments to similar structures on dissected ctenidia.

To examine the effect of seston concentration and "diet quality" (defined as mass of organic material in suspension per unit volume of particulate matter) on particle transport, processing, and pre-ingestive selection, endoscopic observations will be conducted while mussels are exposed to one of four experimental seston concentrations (1, 5, 10, 15 mg dry weight L⁻¹) and one of three levels of diet quality (25, 50, or 75% particulate organic matter). Thus, twelve (4 x 3) treatment (diet) combinations will be tested. Treatment levels for both seston quantity and quality include the range of values typically experienced by mussels under natural conditions (R. Tankersley, unpublished data). Diets will be prepared by varying the concentrations and relative proportions of organic (freshwater alga *Neochloris oleoabundans*; 8-10 µm dia.) and inorganic (silica; 6-12 µm dia.) particles added to the suspension in the refrigerated holding tank. *Neochloris oleoabundans* was chosen for the "high-quality" portion of the diet since recent studies indicate it is readily ingested and assimilated by unionid mussels (Patterson *et al.* 1999) and is an effective diet for the long-term maintenance of adult mussels in captivity (Tankersley and Butz 1999).

During each trial, the OIT of the endoscope will be placed within the mantle cavity and the particle solution (*i.e.*, diet combination) will be slowly added to the suspension using a variable speed parastaltic pump. The tip of the scope will be positioned so that it is possible to observe and record the accumulation and processing of mucous-bound particles in ciliated food grooves, the transfer of particles from the food grooves to the labial palps, and the processes involved in

particle rejection as pseudofeces. Since pseudofeces are only expected to be produced at higher particle concentrations (R. Tankersley, personal observation), particular attention will be paid to the influence of seston quality (organic content) on the initiation and formation of pseudofeces. Observations will be conducted on all three species and will be repeated at least four times at each treatment (diet) combination (3 species x 12 diets x 4 replicates/diet).

D. Effect of Seston Quality and Concentration on Particle Selectivity and Feeding

To test the hypothesis that unionid mussels are able to selectively ingest high-quality particles and regulate the composition of ingested material under different seston loads, two different methods will be used: (1) a traditional **filtration assay** in which the depletion of particles in suspension is monitored over time (Shumway *et al.* 1985), and (2) an *in vivo* **sampling assay** in which endoscopy is used to sample captured particles at various locations on the ctenidia and labial palps while the animal is actively feeding (Ward *et al.* 1998). Although both methods can be used to document selective ingestion of particulates based on particle size or quality, the latter assay also provides information on the relative role of various pallial organs in particle selection.

Filtration Assay

In the filtration assay, mussels will be placed in 18 cm x 20 cm x 15 cm plastic containers filled with mixed suspensions of the freshwater alga Neochloris oleoabundans and silica. Feeding activity and particle selection will be determined by monitoring and comparing the relative depletion (removal) of particles from suspension over time. As in the previous experiment, mussel clearance rates will be measured under four total particle concentrations (1, 5, 10, 15 mg dry weight 1⁻¹) and three levels of diet quality (25, 50, or 75% POM). During each trial, the water within the chamber will be maintained at collection temperatures by a thermostatically controlled water bath and gentle aeration will be used to maintain normoxic conditions, ensure mixing, and prevent particles from settling. Each trial will last < 1 h and clearance rates will be determined by periodically (≈ 15 min) removing a small sample of the suspension in the chamber and analyzing the concentration and composition of particles using flow cytometry (see below). Control chambers containing the same diet suspensions and empty mussel shells will be used to adjust for particle settlement and algal cell division. At the end of the trial, pseudofeces will be collected and analyzed separately using flow cytometry (see below). Mussels will only be tested once and each feeding trial will be repeated five times at each particle/diet combination.

In Vivo Sampling Assay

The *in vivo* sampling assay will follow the methods recently described by Ward *et al.* (1998). Mussels will be prepared for endoscopic examination (see *Examination of Particle Transport and Processing Using Endoscopy*) and exposed to one of the 12 inorganic/organic particle diet combinations listed above for the traditional filtration rate assay. After the mussel begins normal feeding, a micropipet mounted to the tip of the endoscope and connected to a peristaltic pump will be used to sample mucous-bound post-capture/pre-ingested particulate matter at four locations: (1) the surface of the gill, (2) the ventral food grove of the ctenidia, (3) the gill-palp junction, and (4) the inner surface of the labial palps. Preliminary observations indicate that sampling does not interfere with normal feeding and we have recently used a similar technique to

successfully sample particles from the infra- and suprabranchial cavities of unionid mussels to document changes in filtration efficiency of marsupial and non-marsupial gills during brooding periods (Tankersley 1996). As with the filtration assay, any pseudofeces produced during the trial will be collected and all five samples will be analyzed separately using flow cytometery (see below). Differences in the relative composition of the two groups of particles in suspension and in the samples collected at each location will be used to identify the site(s) of particle selection and to assess the role of the gills, ventral food groove, and palps in controlling the quality of ingested particles and in the rejection of non-nutritive particles as pseudofeces.

Analysis Using Flow Cytometry

Quantitative analysis of the feeding dynamics of suspension feeding organisms has been greatly assisted by the development of sophisticated particle sorting and enumeration techniques, such as flow cytometry, which permit rapid, simultaneous analysis of several properties of natural particles including size and chemical composition (Cucci *et al.* 1985, Cucci *et al.* 1989, Yentsch *et al.* 1983). Flow cytometry has several advantages over more traditional methods of determining particle abundance and quantifying selective feeding including (1) the ability to discriminate among various subcomponents of natural seston, including algae and other organic and inorganic particles, that occupy the same size range but have different optical properties, (2) the ability to analyze extremely small samples, and (3) increased sensitivity and resolution permitting detection of small changes in cell concentration over time (Cucci *et al.* 1985, Shumway *et al.* 1985).

For the proposed study, flow cytometry will be used to quantify the relative abundances of organic and inorganic particles in all samples (water, gills, ventral food grove, palps and pseudofeces). The procedures outlined by Yentsch *et al.* (1983) and Cucci *et al.* (1985) will be used to sort and enumerate particles based on their optical properties using a Coulter EPIC XL flow cytometer fitted with a 15 mW argon ion laser emitting an excitation wavelength of 488 nm. This instrument utilizes the fluorescence derived from each particle to simultaneously measure six parameters (relative size, granularity, and four fluorescence colors). We will collect data on three parameters, forward light scatter (an indicator of size), orange fluorescence from phycoerythrin (centered at 585 nm), and red fluorescence from chlorophyll (>650 nm). Nonfluorescent particles will be considered to be inorganic. Thus, the flow cytometric signatures of each particle will be used to simultaneously determine the concentration of organic phytoplankton cells (*i.e.*, Neochloris oleoabundans cells) and inorganic (silica) particles in each sample based upon their size and relative chlorophyll and phycoerythrin content.

Flow cytometry data obtained for filtration assay samples will be used to calculate clearance (filtration) rates (CR), defined as the volume of water filtered free of particles per unit time. Clearance rates will be calculated separately for each particle type (organic vs. inorganic) using the formula of Coughlan (1969):

$$CR = \frac{v}{t} \ln \left(\frac{C_o}{C_t} \right)$$

where CR is the clearance rate (L hr⁻¹), v is the volume of the experimental chamber, t is the duration of the experiment, and C_o and C_t are the initial and final concentrations of particles. All rates will be standardized by the wet weight of the mussel. The effect of particle concentration,

diet quality (organic content), and particle type on clearance rate will be determined using a two-factor repeated measures analysis of variance (ANOVAR).

For the *in vivo* sampling assay, data obtained from flow cytometry will be used to compare the relative proportion of *N. oleoabundans* cells and silica particles in the post-capture/preingestion samples (gills, ventral food groove, palps, pseudofeces). Selectivity for one particle type or the other will be determined using the following electivity index (*E*) described by Jacobs (1974) and Bayne *et al.* (1977):

$$E = \frac{n-p}{(n+p) - (2np)}$$

where n is the proportion of N. oleoabundans cells in the samples and p is the proportion of cells in suspension (pre-capture diet). A positive E indicates an increase or "preference for" N. oleoabundans cells in the sample compared to water and a negative E indicates a decrease or "selection against" N. oleoabundans cells and "preference for" inorganic particles. The effect of particle concentration and diet quality on the calculated electivity indices for each group of samples will be determined using a two-factor Kruskal-Wallis test. Differences in the E's calculated for samples obtained from different locations along the transport route (gill \rightarrow food groove \rightarrow palps \rightarrow pseudofeces) will be used to identify the site of particle selection. Moreover, to test the hypothesis that mussels have the ability to sort captured seston so that nutritious food particles are preferentially ingested and others are expelled as pseudofeces, the E for the pseudofeces samples will be compared to 0 (i.e., no preference) using a Wilcoxen Rank-Sum test (Zar 1996, Ward et al. 1998). If mussels preferentially ingest high-quality particles, the E's for the pseudofeces samples are expected to be significantly less than 0 (i.e., selection against N. oleoabundans), while the E's for one or more of the other sample groups should be significantly greater than 0.

V. SIGNIFICANCE AND RELEVANCE TO THE MISSION OF THE TRUST

A. Impact of Seston Concentration and Quality on Mussel Physiology, Growth, and Survivorship

Information regarding changes in feeding behavior under variable food conditions is critical to the analysis of bivalve energetics (Bayne *et al.* 1988). Thus, a comprehensive understanding of the mechanisms involved in particle capture, sorting, and selection in unionid mussels is needed (1) to determine the environmental cues that mediate feeding, (2) to predict the potential impact of temporal variability in food quantity and seston load on the survivorship and physiological condition of mussel populations, especially threatened and endangered species, and (3) to investigate possible links between the susceptibility of mussels to changes in silt load and turbidity and their ability to compensate for changes in seston condition and meet energetic demands during periods of poor diet quality. Data on the ability of mussels to sort and selectively ingest particles based upon size or chemical composition is needed to assess the contribution of different components of natural seston, phytoplankton, and detritus to mussel bioenergetics and growth. Furthermore, information on the effects of increased siltation and turbidity on mussel feeding and nutrition will enable resource agencies to evaluate the potential

impact of various anthropogenic factors, including dredging, channelization, erosion, agricultural runoff, and destruction of riparian zones, on threatened mussel populations.

B. Effect of Relocation and Captive Maintenance on Feeding Dynamics

The continued decline of native unionid mussel populations coupled with the rapid spread of the zebra mussel Dreissena polymorpha into several riverine habitats has forced research scientists and federal and state agencies to develop effective, innovative methods for preventing the extirpation and extinction of native mussels (Shannon et al. 1993). Consequently, most recovery plans for state and federally listed species include recommendations for restocking areas or reintroducing mussels into their natural range (Neves 1993, 1995, Shannon et al. 1993). Several projects are currently underway to evaluate the use of protected areas, including impoundments, ponds, raceways, and fish hatcheries, as temporary holding facilities for mussels vulnerable to anthropogenic stress or zebra mussel infestation (Neves 1994, 1995, Gatenby et al. 1999). Among the critical challenges faced by many mitigation and captive refugia projects is the location of suitable habitats that meet the nutritional and physiological requirements of transplanted or relocated mussels. In new habitats and artificial containment facilities, the quality or quantity of food available may be insufficient to meet energy demands, reducing the likelihood of survival and hampering reproduction and growth. Thus, studies examining the factors regulating the quality of ingested material and the effects of seston quantity and phytoplankton composition on the filtration dynamics of unionid mussels are clearly needed. The results of the proposed project will help researchers and conservation biologists refine protocols for identifying suitable relocation sites and evaluate and improve facilities for maintaining mussels in captivity. For example, information on the effects of seston quality and quantity on ingestion rates and feeding strategies can be used to help establish feeding protocols and diets for captive animals that maximize ingestion and prevent particle rejection as pseudofeces.

C. Impact of Bivalve Suspension Feeding on Community Structure and Dynamics

In many lentic and lotic systems, bivalve mollusks are dominant members of benthic infaunal communities and their suspension-feeding activities have been shown to have pronounced effects on community dynamics and ecosystem processes including energy flow, biodeposition, and nutrient cycling (for reviews see Winter 1978 and Jørgensen 1990). In the Ohio River, mussels filter and process seston containing a large proportion of inorganic mineral particles and silt. Thus, if unionid mussels, like many other bivalves, are able to selectively remove phytoplankton and other nutrient-rich particles from suspension, their feeding activities may have a significant impact on the population dynamics and composition of the plankton and ultimately effect the growth rates of other suspension feeders. The proposed research will be the first comprehensive analysis of the feeding biology of unionid mussels and will provide important information concerning the impact of their feeding activities and changes in turbidity and seston on the trophic dynamics of lentic and lotic communities.

D. Outreach and Dissemination of Information on the Biology and Ecology of Unionid Mussels

As outlined in the objectives, the results of the project will be summarized in an educational CD-ROM containing information on the ecology, anatomy, and physiology of unionid mussels

with hyperlinks to detailed drawings and high magnification micrographs (light and SEM) of pallial structures, digitized video clips of suspension feeding processes, and animations of particle capture and transport. Although the CD-ROM will focus on the feeding physiology of mussels from the Ohio River, many of the topics and concepts covered on the disk will be common to filter-feeding bivalves, making it a valuable educational tool.

VI. PUBLICATION AND DISSEMINATION OF RESULTS

We anticipate that the project will result in at least two publications in leading scientific journals (*e.g.*, Biological Bulletin, Invertebrate Biology, Journal of the North American Benthological Society). Manuscripts will be submitted for publication during the spring 2000. We also plan to present the results of the project at the year-2000 meetings of the Freshwater Mollusk Conservation Society and the Society for Comparative and Integrative Biology.

The results of the project will also be summarized in an educational CD-ROM. The CD will be advertised at scientific meetings and on the internet (via the UNIO list-server) and will be distributed to interested researchers, resource managers, and educators for a nominal fee (*i.e.*, to cover packaging and postage).

VII. RESEARCH SCHEDULE

The specific tasks to be completed are listed below in chronological order. The proposed startup date for the project is March 1, 1999 and we anticipate it will take approximately one year to complete:

March-April 1999

Construct chambers for feeding and endoscopic studies

Collect specimens for histological and microscopic analysis of gills and pallial organs

June-August 1999

Complete microscopic analysis of gills and pallial organs

Collect new mussels for endoscopic examination

Conduct endoscopy observations of particle transport and processing

September-December 1999

Analyze endoscopic video recordings of particle transport and processing

Collect new mussels for particle selectivity and feeding studies

Conduct feeding studies (both traditional feeding and in vivo sampling assays)

Acquire images and digital video clips for CD-ROM

December-March 2000

Analyze data from the feeding studies

Prepare manuscripts and final report detailing the results of the study

Present results at the annual meetings of the Freshwater Mollusk Conservation Society and the Society for Comparative and Integrative Biology

Complete and distribute educational CD-ROM

VIII. BUDGET

| Budget Item/Description | | Amount | Subtotal |
|--------------------------------|--|----------|----------|
| A. | Total Labor Costs | | \$16,830 |
| | Richard Tankersley, Principal Investigator | \$12,930 | , |
| | Trevor Marshall, Undergraduate Research Assistan | \$3,900 | |
| В. | Fringe Benefits | | \$1,265 |
| C. | Equipment (Non-capitalized) | | \$2,450 |
| | Digital Imaging Hardware and Software | \$1,600 | . , |
| | Endoscopy and Video Equipment | \$850 | |
| D. | Expendable Supplies | | \$6,950 |
| | Aquarium Supplies | \$900 | . , |
| | Algal Culturing Supplies | \$800 | |
| | Latex Beads | \$900 | |
| | Video Supplies (Cables, Connectors, Tapes) | \$600 | |
| | Photography Supplies | \$600 | |
| | Histology Supplies, Chemicals and Reagents | \$1,000 | |
| | Electron Microscopy Supplies/Expendables | \$800 | |
| | Flow Cytometer Supplies | \$600 | |
| | Misc. Lab/Field Supplies | \$750 | |
| E. | Equipment Rental | | \$3,300 |
| | Electron Microscope User Fees | \$1,200 | |
| | Flow Cytometer User Fees | \$1,300 | |
| | Boat Rental | \$800 | |
| F. | Travel & Lodging | | \$2,470 |
| G. | Publication/Dissemination Costs | | \$800 |
| G. | Total Direct Cost | | \$34,065 |
| H. | Indirect Cost (10% of TDC-Equipment) | | \$3,407 |
| I. | Total (Direct + Indirect Cost) | | \$37,472 |

IX. BUDGET JUSTIFICATION

A. Labor Costs

<u>Principal Investigator (Richard Tankersley)</u>: Salary has been requested for summer 1999 at a rate of 2.5/9.5 of the following academic year salary level. The PI will continue to work on the project during the academic year (20% of time) at no additional cost to the grant.

<u>Undergraduate Assistant (Trevor Marshall):</u> Funds are requested to hire an undergraduate student (Trevor Marshall) to assist with the project during the summer (full-time, 40 hrs/week x 12 wks @ \$6.50/hr) and fall (part-time, 10 hrs/week x 12 wks @ \$6.50/hr) 1999. Mr. Marshall will help with the collection and maintenance of mussels, preparation of samples for light and scanning electron microscopy, and the analysis of feeding strategies using endoscopy.

B. Fringe Benefits

| | Principal | Undergraduate | |
|----------------------------------|--------------|---------------|--|
| | Investigator | Assistant | |
| Social Security (7.65%) | Yes | Yes | |
| Unemployment Compensation (0.2%) | Yes | Yes | |

C. Equipment (Non-Capitalized)

Most of the more expensive capital equipment needed to complete the study, including the light microscope, scanning electron microscope, and endoscopy setup, are currently available in our laboratory at UMBC. However, funds are requested to update our digital image analysis hardware and software for capturing, enhancing, and quantifying light micrographs and video images of suspension feeding structures and to replace or repair any video, microscopy or endoscopy equipment that may become damaged during the study.

D. Expendable Supplies

Funds are requested to purchase materials and supplies to maintain mussels in captivity. Supplies including aquarium filters, chemicals and mineral supplements, and algae culture media. Additional expendable supplies and consumables include histology chemicals (*e.g.*, stains, fixatives, buffers and reagents) and microscopy and endoscopy supplies (*e.g.*, tissue mounts, slides, fluorescently-labeled latex particles, video tapes, and photographic film).

E. Equipment Rentals

Since collection of mussel samples in the Lower Muskingum River will require SCUBA, funds are requested to cover the rental of a surface support boat to assist divers collecting. Support is also requested to cover user fees for the scanning electron microscope (40 hr. @ \$30/hr) and the flow cytometer (20 hrs @ \$65/hr). Charges include fees for technical support and supervision, tissue preparation, and data processing.

F. Travel and Lodging

Funds are requested to cover travel expenses to collect and transport mussels from the Lower Muskingum River and other selected sites in the Ohio River Basin to our laboratory facilities at UMBC. Expenses include round-trip mileage (3 trips x 1200 miles/trip @ \$0.30/mile) and lodging for 6 days (@ \$90/day). Support is also requested for project personnel to attend and present the results of the study at national and regional meetings. Estimated costs (\$850) includes travel, lodging, and meeting registration.

G. Publication Costs/Documentation/Dissemination

Funds are requested to cover charges associated with publishing the results of the project. Anticipated charges include publication fees/page charges, manuscript preparation fees, reprint costs, and postage. Based upon the success of previous projects, we expect 4-5 presentation abstracts and 2-3 peer reviewed papers to be published in conjunction with the project.

X. PERSONNEL

A. Principal Investigator: Richard A. Tankersley

My research interests are in the area of invertebrate physiology and ecology. Many of the projects conducted by members of my lab focus on the physiological and behavioral mechanisms responsible for food capture by suspension feeding invertebrates, especially bivalves. In recent years, we have completed studies on the effect of larval brooding by freshwater unionid mussels on their feeding and respiratory physiology (Tankersley & Dimock, 1992, 1993a,b,c, Tankersley 1996). We are also studying the development and organogenesis of suspension feeding structures in juvenile unionid mussels and have recently completed studies correlating developmental and morphological changes in pallial organs with shifts in feeding mode and particle selectivity (Tankersley 1999a). We have also been actively involved in efforts to propagate and rear threatened and endangered mussels and have developed facilities for culturing and maintaining juvenile and adult mussels in captivity (Tankersley 1999b, Tankersley and Butz 1999). Financial support for many of these projects has been provided by the Mussel Mitigation Trust (see *Results of Prior Support* below).

B. Undergraduate Assistant: Trevor Marshall

Field and lab support for the project will be provided by Mr. Trevor Marshall. He will assist with the collection and maintenance of mussels and will be responsible for fixing and sectioning ctenidia and other pallial organs for light and scanning electron microscopy. Mr. Marshall is a very talented, bright, and capable student who has been working in my laboratory for the last 14 months. He is currently a 2nd semester Junior biology major and has earned a 4.0 GPA while at UMBC. Over the past year, he helped design, construct and evaluate a new recirculating aquaculture facility for the rearing newly transformed unionid mussels in captivity. The system has been extremely successful and has permitted us to raise large numbers of *in vitro* transform juveniles for experiments examining the impact of diet composition on growth and early development. The results of his project will be presented as a poster at this year's meeting of the Freshwater Mollusk Conservation Society (see "Results of Prior Support" below).

XI. RESULTS OF PRIOR SUPPORT

Our previous Mussel Mitigation Trust supported project entitled "Development and evaluation of non-destructive methods for assessing the nutritive condition of freshwater unionid mussels" was completed last May. Four presentations describing the results of the study were presented at the *Conservation, Captive Care and Propagation of Freshwater Mussels Symposium* held last March in Columbus, OH. Three manuscripts detailing the results of the studies have been submitted for publication in the Symposium Proceedings. Detailed summaries of the results of these studies are listed below. One additional paper is currently being prepared for submission to the Journal of North American Benthological Society later this spring. Two additional papers entitled "A recirculating aquaculture facility for the captive propagation of juvenile unionid mussels" and "State of the Unio: Methods for assessing the physiological condition of captive mussels" will be presented at this year's meeting of the Freshwater Mollusk Conservation Society.

A. Use of Condition Indices, Protein Biomarkers, and RNA:DNA Ratios for Detecting Nutritional Stress in Freshwater Unionid Mussels

With the growing concern for the protection of North American unionid mussels and the increased acceptance of captive rearing and relocation programs as methods for preventing the extirpation of threatened and endangered species, there is an immediate need to develop sensitive, non-destructive methods for assessing the physiological condition and health of existing populations. Although numerous methods have been developed for evaluating the nutritive condition of bivalve molluscs, especially commercially important marine species, their application to unionid mussel conservation efforts has been limited. Using Elliptio complanata as a model, we determined the impact of starvation on the physiological condition of adult mussels and investigated the sensitivity of several common morphological and biochemical condition indices for detecting nutritional stress in captive mussels. To assess the effects of starvation on physiological condition, we compared the nucleic acid, glycogen, lipid, protein, carbohydrate and organic content of the mantle tissue of mussels maintained on one of three feeding regimes: (1) starved, (2) fed (i.e., fed mixed algal cultures daily), and (3) partially fed (i.e. fed mixed algal cultures on alternate days). The biochemical composition of the mantle was also compared to more traditional condition indices based upon soft-tissue weight, shell weight, and shell cavity volume. In general, biochemical indices were better predictors of physiological condition and feeding regime than gross morphological indices based upon tissue mass and shell size. Although glycogen, lipid, protein and carbohydrate levels declined rapidly in starved mussels, RNA:DNA ratios in the mantle tissue were the most sensitive indicators of nutritive stress. Throughout the experiment, protein and lipid levels and RNA:DNA ratios of starved mussels were significantly lower than in fed and partially fed mussels, but glycogen levels among the three treatments were highly variable and did not display a consistent pattern. These results suggest that glycogen may not be reliable indicator of nutritional stress in unionid mussels.

B. Fluorescence Techniques for Evaluating the Lipid Content of Larval and Juvenile Mussels

Early growth and viability of juvenile unionid mussels is thought to depend upon the accumulation of neutral-lipid energy reserves. To test this hypothesis, the lipid-specific fluorophore nile red (NR) was used to document the concentration and distribution of lipid stores in glochidia and newly transformed juvenile mussels. When viewed under epifluorescence illumination (450-490 nm excitation), lipid droplets stained with NR fluoresce a bright yellow/orange, making them easy to detect and visualize through the thin, semi-transparent shells of larval and juvenile mussels. Digital image analysis was used to quantify the relative concentration of neutral lipid reserves and to determine the optimal concentration and timecourse for staining. To examine the spatial distribution of lipid deposits, confocal microscopy was used to optically section and three-dimensionally reconstruct intact larvae and juveniles. In mature larvae, lipid droplets (2-8 µm diameter) were dispersed throughout the mantle, with the highest concentrations occurring in the lateral pit cells. Following transformation, lipid levels increased rapidly in developing juveniles and were concentrated in the foot, gills and ventral margin of the mantle. In subsequent experiments, NR was used to 1) document the accumulation of lipid deposits in glochidia during metamorphosis, 2) compare the lipid content of juveniles transformed using traditional and "lipid fortified" culture media, and 3) determine the effect of

starvation on the lipid reserves of juveniles. Results of these studies indicate NR is a sensitive, inexpensive probe for assessing the lipid content of individual mussels and may serve as a valuable, non-destructive tool for evaluating the physiological condition of glochidia and the nutritional status of juveniles.

C. Design, Construction and Evaluation of a Laboratory-Scale Recirculating Aquaculture System for the Captive Care of Freshwater Mussels

We constructed a closed, recirculating freshwater aquaculture system suitable for the longterm maintenance and quarantine of adult unionid mussels in captivity. The system was designed 1) to accommodate mussels with different habitat requirements, 2) to enable strict control of environmental conditions and prevent the accumulation of debris and metabolic wastes, 3) to include several tanks that could serve as independent experimental units (replicates) in laboratory studies, and 4) to incorporate integrated filtration and feeding systems to help minimize the time required for daily maintenance and care. The system consists of two duplicate units, each with eighteen 38 L rectangular glass tanks. Conditions within each tank, including substrate, temperature, water level and flow rate, can be adjusted independently. Water draining from the tanks empties into a 170 L insulated holding tank and is filtered by a 120 L min⁻¹ modular system equipped with mechanical and chemical filters and an ultraviolet sterilizer. Nitrogenous wastes are removed using a trickle-style biological filter, and temperature within each unit is controlled (± 0.5 °C) using a flow-through chiller unit. Water quality parameters, including pH, temperature, and oxidation-reduction potential (ORP), are monitored continuously and logged to a remote computer. An automatic feeder is used to dose the system with concentrated suspensions of live and spray-dried algae at programmed intervals. The system is capable of holding up to 1000 mussels and has been used to maintain populations of Elliptio complanata for >5 months with 100% survival. A smaller-scale intermittent-flow system employing many of the same design features and components is currently being evaluated for culturing juvenile mussels.

D. Effects of Commercial Algal Preparations on Growth and Survival of Juvenile Unionid Mussels

Although commercially available algal diets, including spray-dried powders and concentrated microalgal pastes, have been used extensively by marine aquaculturalists to rear larval and juvenile shellfish, their application for culturing unionid mussels in captivity has been limited. We examined the growth and survivorship of newly transformed juvenile *Utterbackia imbecillis* fed spray-dried *Schizochytrium* and *Chlorella*, or a multialgae paste composed of *Thalassiosira pseudonana*, *Skeletonema* sp., *Chaetoceros calcitrans* and *Isochrysis galbana*. Ten days following transformation, juvenile mussels were assigned to one of five diets: *Schizochytrium* alone, *Chlorella* alone, multialgal paste alone, a 1:1:1 mixture of all three diets, and a starved (no algae) control. Although initial growth rates of mussels were similar among treatments, after 3-wks mussels fed *Schizochytrium* alone had significantly higher growth rates (shell length, 54.4 µm wk⁻¹) and survival (77.8%) than all other treatments. Longer-term experiments were conducted to compare growth rates of juvenile *Pyganodon cataracta* (2-wks post-transformation) reared on spray-dried *Schizochytrium* alone and in combination with fresh cultures of *Chlorella vulgaris*. Juvenile mussels were assigned to 3 diets, *Schizochytrum*, live *Chlorella*, and a 1:1 mix of both diets, plus silt. Only juveniles fed the combination of *Chlorella* and *Schizochytrum*

survived to 10-wks (17%). At the end of the experiment (22-wks), survivorship of juvenile *Pyganodon cataracta* on the combination diet was nearly 16%. Mussels reached a mean shell length of 5.3 mm, representing about a 12 fold increase, and had a mean growth rate of about 271 μ m wk⁻¹.

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- Shannon, L., R.G. Biggins, and R. Hylton. 1993. Freshwater mussels in peril: Perspective of the U.S. Fish and Wildlife Service. Conservation and Management of Freshwater Mussels: Proceedings of a UMRCC Symposium, pp 66-68
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- Tankersley, R.A. 1996. Multipurpose gills: effect of larvae brooding on the feeding physiology of freshwater unionid mussels. Invert. Biol. 115:243-255.
- Tankersley, R.A. 1999a. Developmental shifts in the feeding biodynamics of juvenile *Utterbacki imbecilis* (Mollusca: Bivalvia). *Invert. Biol.* (*in prep*).
- Tankersley, R.A. 1999b. Fluorescence techniques for evaluating the lipid content of larval and juvenile mussels. In: R.A. Tankersley, T. Watters, B. Armatage, and D. Warmolts (eds). *Conservation, Captive Care and Propagation of Freshwater Mussels.* Ohio University Press., Columbus. (in review)
- Tankersley, R.A. and R.V. Dimock, Jr. 1992. Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel *Anodonta cataracta*. Biol. Bull. 182:145-154
- Tankersley, R.A. and R.V. Dimock, Jr. 1993a. The effect of larval brooding on the filtration rate and particle-retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae). Can. J. Zool. 71:1934-1944
- Tankersley, R.A. and R.V. Dimock, Jr. 1993b. Endoscopic visualization of the functional morphology of the ctendia of the unioid mussel *Pyganodon cataracta*. Can. J. Zool. 71:811-819

- Tankersley, R.A. and R.V. Dimock, Jr. 1993c. The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta*. *Am. Midl. Nat.* 130: 146-163.
- Tankersley, R.A. and S. Butz. 1999. Design, construction and evaluation of a laboratory-scale recirculating aquaculture system for the captive care of freshwater mussels In: R.A. Tankersley, T. Watters, B. Armatage, and D. Warmolts (eds). *Conservation, Captive Care and Propagation of Freshwater Mussels*. Ohio University Press., Columbus. (in review)
- Wang, W.-X. and N.S. Fisher. 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Ocean. 41: 197-207.
- Ward, J.E. 1996. Biodynamics of suspension feeding of adult bivalve molluscs: particle capture, processing and fate. Invert. Biol. 115:218-231.
- Ward, J.E., P.G. Beninger, B.A. MacDonald and R.J. Thompson. 1991. Direct observations of feeding structures and mechanisms in bivalve molluscs using endoscopic examination and video image analysis. Mar. Biol. 111:287-291.
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- Yentsch, C. M., P. K. Horan, K. Muirhead, Q. Dortch, E. Haugen, L. Legendre, L. S. Murphy, M. J. Perry, D. A. Phinney, S. A. Pomponi, R. W. Spinrad, M. Wood, C. S. Yentsch, and B. J. Zahuranec. 1983. Flow cytometry and cell sorting: a technique for analysis and sorting of aquatic particles. Limnol. Oceanogr. 28: 1275-1280.
- Zar, J. 1996. Biostatistical Analysis. Prentice-Hall, Inc. New Jersey.

XIII. CURRICULUM VITAE

RICHARD A. TANKERSLEY

Department of Biological Sciences University of Maryland, Baltimore County Baltimore, MD 21228

(410) 455-3381 (Voice) (410) 455-3875 (Fax) tankersl@umbc.edu (Email)

Date of Birth: June 6, 1962 Marital Status: Married

Children: 2

EDUCATION

Ph.D. Wake Forest University, Department of Biology, Ph.D. 1987-1992 (Advisor: Dr. Ronald V. Dimock, Jr.)

MS. Florida State University, Department of Biological Science, M.S. 1984-1987 (Advisor: Dr. William F. Herrnkind)

B.A. Wake Forest University, 1980-1984, Cum Laude with Honors in Biology

PROFESSIONAL EXPERIENCE

Assistant Professor, Department of Biological Sciences, University of Maryland Baltimore County (UMBC), 1995-present

Research Associate, Smithsonian Environmental Research Center, Edgewood, MD 1998-present

Assistant Professor, Department of Biology, Gonzaga University, Spokane, WA 1993-1995 Post-Doctoral Research Associate, Duke University Marine Laboratory, Beaufort, NC, 1992-1993

AWARDS AND HONORS

- Robert R. Bryden Graduate Research Award, North Carolina Academy of Science, 1991
- Elton C. Cocke Outstanding Graduate Student Award, Department of Biology, Wake Forest University, 1989-90.
- McClung Research Award, βββ Biological Honor Society, 1986
- Frank G. Brooks Award for Excellence in Student Research, $\beta\beta\beta$ Biological Honor Society, 1984
- Sigma Xi Student Research Award, 1984
- Carolina Biological Supply Company Award for Excellence in Student Research, 1984
- John B. Derieux Research Award, North Carolina Academy of Science, 1984

GRANTS AND FELLOWSHIPS

- Columbus Zoo Small Grants Program, Endoscopic and microscopic examination of the feeding biodynamics of Tridacnid clams. \$ 4,880 1999-2000
- National Science Foundation, Settlement site location and development of an estuarine crustacean: responses to chemical and current cues, \$ 90,175, 1999-2001

- Maryland Sea Grant College Program, Project Development Grant, Roles of estuarine hydrodynamics and larval behavior in determining the spatial distribution of blue crab (*Callinectes sapidus*) larvae in the Chesapeake Bay, \$10,713, 1997
- Mussel Mitigation Trust, Development and evaluation of non-destructive methods for assessing the nutritive condition of freshwater unionid mussels, \$31,455, 1996
- National Science Foundation Instrumentation and Laboratory Improvement Grant; Interactive multimedia resource center for science instruction, \$149,965, 1994
- Murdock College Science Research Program; Summer research funding; Gonzaga University, \$14,024, 1994
- Gonzaga Research Council Grant Program; The effect of silt on the feeding physiology of juvenile freshwater mussels, \$700, 1994.
- Conchologists of America Research Grant, Endoscopic analysis of the ctenidia and suspension feeding dynamics of three Northwest American freshwater bivalves, \$3,000, 1993.
- Grady Britt Teaching Fellowship, Department of Biology, Wake Forest University, 1991-92
- National Science Foundation Doctoral Dissertation Improvement Grant, Larval brooding by the freshwater mussel *Anodonta cataracta*: Its effect on ventilation, filtration and respiration, \$10,946, 1990.
- Sigma Xi Grant-in-Aid of Research; The effect of larval brooding on the filtration efficiency and ciliary activity of the freshwater mussel *Anodonta cataracta*, \$500, 1989.
- Theodore Roosevelt Memorial Grant, American Museum of Natural History; The effect of larval brooding on the physiology and morphology of the marsupial gills of *Anodonta cataracta* (Mollusca: Unionidae), \$850, 1988
- Sigma Xi Grant-in Aid of Research; Effect of conspecific trail following on the locomotion of the marsh periwinkle *Littorina irrorata*, \$350, 1985.
- John Yarborough Memorial Undergraduate Research Award, North Carolina Academy of Science, 1984

PUBLICATIONS

- Tankersley, R.A. and K. Kachurak. 1999. Potential use of condition indices, protein biomarkers, and RNA:DNA ratios for detecting nutritional stress in freshwater unionid mussels. *J. N. Am. Benthol. Soc. (in prep)*.
- Tankersley, R.A. 1999. Developmental shifts in the feeding biodynamics of juvenile *Utterbacki imbecilis* (Mollusca: Bivalvia). *Invert. Biol.* (*in prep*).
- Tankersley, R.A. 1999. Fluorescence techniques for evaluating the lipid content of larval and juvenile mussels. (eds) R.A. Tankersley, T. Watters, B. Armatage, and D. Warmolts *In:* Conservation, Captive Care and Propagation of Freshwater Mussels. Ohio University Press., Columbus. (in review)
- Tankersley, R.A. and S. Butz. 1999. Design, construction and evaluation of a laboratory-scale recirculating aquaculture system for the captive care of freshwater mussels. (eds) R.A. Tankersley, T. Watters, B. Armatage, and D. Warmolts In: *Conservation, Captive Care and Propagation of Freshwater Mussels*. Ohio University Press., Columbus. (in review)
- Tankersley, R.A. and M.G. Wieber. 1999. Physiological responses of postlarval and juvenile crabs (*Callinectes sapidus*) to hypoxia and anoxia. *Mar. Ecol. Prog. Ser. (in review)*

- Tankersley, R.A., R.V. Dimock, Jr., and M. Whitton. 1999. Growth and survival of juvenile *Utterbackia imbecillis* and *Pyganodon cataracta* (Bivalvia: Unionidae) reared on commercial algal diets. (eds) R.A. Tankersley, T. Watters, B. Armatage, and D. Warmolts *Conservation*, *Captive Care and Propagation of Freshwater Mussels*. Ohio University Press., Columbus. (*in review*).
- Forward, R.B., Jr., K.A. Reinsel, D.S. Peters, R.A. Tankersley, J.H. Churchill, L. B. Crowder, W.F. Hettler, S.M. Warlen, and M.D. Greene 1998. Transport of fish larvae through a tidal inlet. *Fisheries Oceanography (in press)*
- Forward, R.B., Jr., M.C. De Vries, R.A. Tankersley, D. Rittschof, B. Hettler, J. Burke, J.M. Welch, and D.E. Hoss. 1998. Behavior and sensory physiology of Atlantic menhaden, *Brevoortia tyrannus*, during horizontal transport. *Fisheries Oceanography (in press)*.
- Tankersley, R.A., M.G. Wieber, M. A. Sigala, and K. Kachurak. 1998. Migratory movements of ovigerous blue crabs, *Callinectes sapidus*: Evidence for selective tidal-stream transport. *Biol. Bull.* 195: 168-173.
- Forward, R.B., Jr., R.A. Tankersley, and K.A. Reinsel. 1998. Selective tidal stream transport of spot (*Leistomus xanthurus*) and pinfish (*Lagodon rhomboides*) larvae: Contribution of circatidal rhythms in vertical migration. *J. Exp. Mar. Biol. Ecol.* 226: 19-32.
- Fitzgerald, T.P., R.B. Forward, Jr. and R.A. Tankersley. 1998. Metamorphosis of the estuarine crab *Rhithropanopeus harrisii* (Gould): effect of water type and adult odor. *Mar. Ecol. Prog. Ser.* 165:217-223
- Forward, R.B., Jr., R.A. Tankersley, D. Blondel, and D. Rittschof. 1997. Metamorphosis of the blue crab *Callinectes sapidus*: effects of humic acids and ammonium. *Mar. Ecol. Prog. Ser.* 57: 277-286.
- Forward, R.B., Jr., J. Swanson, R.A. Tankersley and J.M Welch. 1997. Endogenous swimming rhythms of blue crab megalopae: effects of offshore and estuarine cues. *Mar. Biol.* 127:621-628.
- Tankersley, R.A. 1996. Multipurpose gills: The effect of larval brooding on the feeding physiology of freshwater mussels. Proceedings of the Recent Advances in Invertebrate Feeding Biodynamics Symposium. *Invert. Biol.* 115:243-255.
- Forward, R.B., Jr., R.A. Tankersley, J.S. Burke, and W.F. Hettler, Jr. 1996. Endogenous swimming rhythms of larval Atlantic menhaden, *Brevoortia tyrannus* Latrobe: Implications for vertical migration. *J. Exp. Mar. Biol. Ecol.* 204: 195-207
- Forward, R.B., Jr., R.A. Tankersley, M.C. DeVries, and D. Rittschof. 1995. Sensory physiology and behavior of blue crab (*Callinectes sapidus*) postlarvae during horizontal transport. *Mar. Fresh. Behav. Physiol* 26: 233-248.
- Tankersley, R.A. L.M. McKelvey and R.B. Forward, Jr. 1995. Behavioral responses of crab megalopae to hydrostatic pressure, salinity and light. *Mar. Biol.* 122: 391-400.
- Tankersley, R.A. and R.B. Forward, Jr. 1994. Endogenous activity rhythms in two estuarine crab megalopae: implications for flood tide transport. *Mar. Biol.* 118: 415-424.
- DeVries, M.C., R.A. Tankersley, R.B. Forward, Jr., W.W. Kirby-Smith and R.A. Leuttick. 1994. Abundances of crab megalopae are associated with tidal hydrologic variables. *Mar. Biol.* 118: 403-414.
- Tankersley, R.A. and R.V. Dimock, Jr. 1993. The effect of larval brooding on the filtration rate and particle retention efficiency of *Pyganodon cataracta*. *Can. J. Zool*. 71:1934-1944.

- Tankersley, R.A. and R.V. Dimock, Jr. 1993. The effect of larval brooding on the respiratory physiology of the freshwater unionid mussel *Pyganodon cataracta*. *Am. Midl. Nat.* 130: 146-163.
- Tankersley, R.A. and R.V. Dimock, Jr. 1993. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta*. *Can. J. Zool.* 71:811-819.
- Tankersley, R.A. 1992. Larval brooding by the freshwater unionid mussel *Anodonta cataracta*: its effect on filtration, ventilation and respiration. Ph.D. Dissertation. Wake Forest University, Winston-Salem, NC. 199 p.
- Tankersley, R.A. and R.V. Dimock, Jr. 1992. Morphological analysis and 3D reconstruction of the marsupial gills of the freshwater mussel *Anodonta cataracta*. *Biol Bull*. 182: 145-154.
- Tankersley, R.A. and W. E. Conner. 1990. Not-so-random walks—computer simulation of chemo-orientation behavior. *BioScience* 40: 392-395.
- Tankersley, R.A. 1990. Trail-following in *Littorina irrorata*: the influence of visual stimuli and the possible role of tracking in orientation. *Veliger* 33: 116-123.
- Tankersley, R.A. 1989. The effect of conspecific trail-following on the locomotion of the marsh periwinkle *Littorina irrorata*. *Mar. Behav. Physiol.* 15: 89-100.
- Herrnkind, W.F., M. Butler, and R. Tankersley. 1988. The effects of siltation on recruitment of spiny lobsters (*Panulirus argus*). Fish. Bull. 86: 331-338.
- Tankersley, R.A. 1987. The trail-following behavior of *Littorina irrorata* (Mesogastropoda: Littorinidae): Its effect on locomotion and the influence of visual orientational cues. Master's Thesis. Florida State University, Tallahassee, Florida.
- Tankersley, R.A. 1986. The effect of several environmental variables on the locomotion of the mud snail *Ilyanassa obsoleta*. *BIOS* 56: 224-233

PRESENTATIONS, POSTERS AND PUBLISHED ABSTRACTS

- Bullock, T. R.A. Tankersley, R.B. Forward, Jr. and D. Rittschof. Larval release behaviors in the blue crab *Callinectes sapidus*: Role of chemical Cues. Benthic Ecology Meetings, Scheduled for March 1999.
- Tankersley, R.A., T. Marshall, and S. Butz. 1999. A recirculating aquaculture facility fo rthe captive propagation of juvenile unionid mussels. Freshwater Mollusk Conservation Society. Scheduled for March 1999.
- Tankersley, R.A. State of the Unio: Methods for assessing the physiological condition of captive mussels. Freshwater Mollusk Conservation Society. Scheduled for March 1999.
- Sigala, M.A. and R.A. Tankersley. Behavioral response of blue crab (*Callinectes sapidus*) megalopae to chemical cues. Benthic Ecology Meetings, Melbourne, Fl., April 1998
- Tankersley, R.A., M.G. Wieber, M.A. Sigala, and K. Kachurak. Migratory movements of ovigerous blue crabs, *Callinectes sapidus*: Evidence for selective tidal-stream transport. Benthic Ecology Meetings, Melbourne, Fl., April 1998
- Forward, R.B., R.A. Tankersley, J.M. Welch, and M.G. Wieber. Settlement of blue crab (*Callinectes sapidus*) postlarvae during selective tidal stream transport. Benthic Ecology Meetings, Melbourne, Fl., April 1998

- Dimock, R.V., Jr., R.A. Tankersley, and M. Whitton. Growth and survival of juvenile *Utterbackia imbecillis* and *Pyganodon cataracta* (Bivalvia: Unionidae) reared on commercial algal diets. Conservation, Captive Care and Propagation of Freshwater Mussels Symposium, Columbus OH., March 1998
- Tankersley, R.A., M.G. Wieber, K. Kachurak and S. Butz. Potential use of condition indices, protein biomarkers, and RNA:DNA ratios for detecting nutritional stress in freshwater unionid mussels. Conservation, Captive Care and Propagation of Freshwater Mussels Symposium, Columbus OH., March 1998
- Tankersley, R.A. Fluorescence techniques for evaluating the lipid content of larval and juvenile mussels Conservation, Captive Care and Propagation of Freshwater Mussels Symposium, Columbus OH., March 1998
- Tankersley, R.A. and S. Butz. Design, construction and evaluation of a laboratory-scale recirculating aquaculture system for the captive care of freshwater mussels. Conservation, Captive Care and Propagation of Freshwater Mussels Symposium, Columbus OH., March 1998
- Tankersley, R.A. and M.G. Wieber. Physiological responses of postlarval and juvenile crabs (*Callinectes sapidus*) to hypoxia and anoxia. Benthic Ecology Meetings, Portland, ME, April 1997.
- Tankersley, R.A. and M.G. Wieber. Ontogentic changes in the feeding dynamics of juvenile freshwater unionid mussels. National Shellfisheries Association Annual Meeting, Baltimore, April 1996.
- Tankersley, R.A. and M.G. Wieber. Developmental shifts in the feeding biodynamics of juvenile *Utterbackia imbecilis* (Mollusca: Bivalvia). The Conservation and Management of Freshwater Mussels Workshop, St. Louis, October 1995.
- Tankersley, R.A. Multipurpose gills: The effect of larval brooding on the feeding physiology of freshwater mussels. Recent Advances in Invertebrate Feeding Biodynamics Symposium, Annual Meeting of the American Society of Zoologists. St. Louis, MO, January, 1995.
- Hart, J. and R.A. Tankersley. The effect of silt on the feeding biodynamics of juvenile *Utterbackia imbecilis* (Mollusca: Bivalvia). Regional Conference of Undergraduate Research, Murdock College Science Research Program, Gonzaga University. November, 1994
- Tankersley, R.A. and R.B. Forward, Jr. Endogenous swimming rhythms in two estuarine crab megalopae: implications for flood tide transport. Annual Meeting of the American Society of Zoologists, Los Angeles, CA, December, 1993.
- Tankersley, R.A. and R.B. Forward, Jr. Flood tide transport of crab megalopae: III. Behavioral responses to pressure and salinity. 1st. Annual Larval Ecology Meetings. Port Jefferson, NY. August 1993.
- Tankersley, R.A. and R.V. Dimock, Jr. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta*. Annual Meeting of the American Society of Zoologists. December, 1992.
- Tankersley, R.A. Physiological consequences of larval brooding in the unionid bivalve *Pyganodon cataracta*. Duke University Marine Lab Seminar Series. October, 1992.

- Tankersley, R.A. and R.V. Dimock, Jr. The effect of marsupium formation and larval brooding on ciliary activity and particle transport in the freshwater mussel *Anodonta cataracta*. North American Benthological Society 40th Annual Meeting, University of Louisville, May 1992.
- Tankersley, R.A. Larval brooding by the freshwater unionid mussel *Anodonta cataracta*: its effect on ventilation, respiration and filtration. Biology Department Seminar. Wake Forest University. April 1992.
- Tankersley, R.A. The effects of hypoxia on the respiration and ventilatory behavior of the marine polychaete *Chaetopterus variopedatus*. North Carolina Academy of Science Annual Meeting, Fayetteville, NC, March 1992.
- Tankersley, R.A. and R.V. Dimock, Jr. New techniques for the analysis of ciliary activity in freshwater eulamellibranch bivalves and their possible use in biomonitoring programs. Second Annual Southern Appalachian Man and the Biosphere Conference. Gatlinburg, Tenn., November 1991.
- Tankersley, R.A. Stalks, slopes and trails: the effect of conflicting directional cues on the orientation of the marsh periwinkle *Littorina irrorata*. American Malacological Union Meeting, San Francisco, CA, July 1991.
- Tankersley, R.A. *A priori* identification of brooding unionid mussels using stepwise discriminant analysis. Annual Meeting of the Association of Southeastern Biologists, Appalachian State Univ., NC., April 1991
- Tankersley, R.A. Utilization of multiple orientation cues by the marsh periwinkle *Littorina irrorata*. North Carolina Academy of Science Annual Meeting, Greensboro, NC, March 1991.
- Tankersley, R.A. and R.V. Dimock.. Morphological analysis and 3D reconstruction of the marsupial gills of the freshwater mussel *Anodonta cataracta*. Annual Meeting of the American Society of Zoologists. San Antonio, TX, December 1990
- Tankersley, R.A. and R.V. Dimock, Jr. Quantitative morphological analysis of the marsupial gills of *Anodonta cataracta* using light and scanning electron microscopy. Annual Meeting of the American Malacological Union, Woods Hole, MA. June 1990.
- Tankersley, R.A. and W. Conner. Not-so-random walks: a computer simulation of chemoorientation behavior. Annual Meeting of the Association of Southeastern Biologists, Baltimore, MD, April 1990
- Tankersley, R.A. and W. Conner. Simulation simple chemo-orientation behaviors using computers. North Carolina Academy of Science Annual Meeting, Highpoint, NC. March 1990.
- Tankersley, R.A. The influence of visual orientational cues on the trail-following behavior of the marsh periwinkle *Littorina irrorata*. Annual Meeting of the American Society of Zoologists, San Francisco, CA, December 1988.
- Tankersley, R.A. The effect of visual cues on the trail-following behavior of *Littorina irrorata*. North Carolina Academy of Science Annual Meeting, Highpoint, NC, March 1988.
- Tankersley, R.A. Snails, trails, mucus and slime: the trail following behavior of the marsh periwinkle *Littorina irrorata*. Biology Department Seminar, Wake Forest University, September 1987.

- Tankersley, R.A. The trail-following behavior of the marsh periwinkle *Littorina irrorata*: its effect on locomotion and the influence of visual orientational cues. Thesis Defense, Department of Biological Science, Florida State University, Tallahassee, FL, June 1987.
- Tankersley, R.A. The effect of trail-following on the locomotion of the marsh periwinkle *Littorina irrorata*. Benthic Ecology Meetings, Raleigh, North Carolina, March 1987,
- Tankersley, R.A. Trail-following in *Littorina*. Natural History Seminar. Florida State University. February 1987.
- Herrnkind, W.F., M.J. Butler IV and R.A. Tankersley. Habitat selection, predation and emigration in juvenile spiny lobsters., Benthic Ecology Meetings, Boston, MA, March 1986.
- Tankersley, R.A., W.F. Herrnkind, M. J. Butler, IV. Factors influencing habitat selection of young juvenile spiny lobsters, *Panulirus argus*. Annual Meeting of the American Society of Zoologists, Baltimore, MD, December 1985.
- Tankersley, R.A. The effect of several environmental variables on the locomotion of the mud snail *Ilyanassa obsoleta*, Annual Meeting of the North Carolina Academy of Science, Winston-Salem, NC, March 1984.

INVITED SEMINARS

College of Marine Studies, University of Delaware, April 1998
Department of Zoology, University of Maryland College Park. November 1996
Smithsonian Environmental Research Center, Edgewater, MD. June 1996
Maryland Department of Natural Resources, Annapolis, MD. January 1996
Center of Marine Biotechnology, Baltimore, MD. December 1995
Chesapeake Biological Laboratory, Solomons, MD. November 1995
Old Dominion University, Norfolk, VA. October 1995

TEACHING EXPERIENCE

Lecture and Laboratory Classes

Marine and Estuarine Ecology, UMBC (1 semester)

Ecology and Evolution Research Seminar, UMBC (5 semesters)

Computerized Image Analysis, Gonzaga University (1 semester), UMBC (1 semester)

Ecology and Evolution, UMBC (3 semesters)

Ecology, Gonzaga University (1 semester),

Advanced Topics in Animal Behavior, Gonzaga University (1 semester)

Advanced Topics in Aquatic Ecology, Gonzaga University (1 semester)

Comparative Physiology, Gonzaga University (2 semesters)

Human Ecology, Gonzaga University (2 semesters)

Biostatistics, Wake Forest University (1 semester); Gonzaga University (1 semester)

Animal Behavior, Florida State University (2 semesters)

Graduate Teaching Assistantships (Laboratory Classes)

Graduate Student Coordinator, Comparative Physiology Laboratory, Wake Forest University (1 semester)

Marine Invertebrate Zoology, Duke University Marine Laboratory (1 semester)

Marine Biology, Wake Forest University (2 semesters)

Comparative Physiology, Wake Forest University (4 semesters)

Introductory Biology, Florida State University (2 semesters), Wake Forest University (2 semesters)

Graduate Student Coordinator, Introductory Biology Laboratory, Florida State University (3 semesters)

SPECIAL PROGRAMS AND COMMITTEE PARTICIPATION

Co-Chairman, Subcommittee on Propagation and Reintroduction, Freshwater Mollusk Conservation Society. 1997-present

Co-Organizer and Proceedings Co-Editor, Conservation, Propagation and Captive Care of Freshwater Mussels Symposium, Columbus, OH, March 1998.

Committee Member, Computer Policy Committee, UMBC, 1998-present

Faculty Advisor, Undergraduate Council of Majors, Department of Biological Sciences, UMBC, 1996-present

Aquarium Science Curriculum Coordinator, Department of Biological Sciences, UMBC, 1996-present

Faculty Representative to the Student Life Committee, UMBC, 1996-1998

Committee Member, Graduate Committee, Department of Biological Sciences, UMBC 1995-1997, 1998-present

Co-Chairman and Organizer, Recent Advances in Invertebrate Feeding Biodynamics Symposium, Annual Meeting of the American Society of Zoologists, January 1995

Faculty Advisor, Gonzaga Environmental Organization (GEO), 1994-1995

Graduate Student Coordinator, Wake Forest Summer Undergraduate Research Experience Program (SURE), Summers 1989 & 1990.

Graduate Student Representative to the Faculty, Department of Biology, Wake Forest University. 1989-91

Graduate Student Representative, Graduate Committee, Department of Biology, Wake Forest University, 1988-89

Residence Hall Director, Florida State University and Wake Forest University, 1984-90.

Graduate Student Coordinator, Florida State University Summer Math and Science Camp, Summer 1987.

PROFESSIONAL SOCIETIES

Sigma Xi Scientific Research Society
American Microscopical Society
North American Benthological Society
National Shellfisheries Association
American Malacological Union
Freshwater Mollusk Conservation Society

GRADUATE STUDENTS

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Dear Dick,

In my opinion, the experiments proposed in "Effects of Particle Concentration and Quality "" will provide some useful information for captive holding of adult freshwater mussels, but the authors have over-sold the utility of future results. I am very interested in understanding the particle sorting mechanisms of freshwater mussels, and feel that the information generated by these studies will be fascinating and important. Also, the ability of mussels to alter their feeding behavior to compensate for fluctuations in suspended sediment in the water column is of great interest.

I must preface my critique of this proposal with a description of my interpretative frame of reference. My work and thinking have been pointed toward the generation of practical information for future large-scale holding of mussels at fish hatcheries. In my opinion, fish hatcheries will play a central role in future culturing efforts. From this experience and position, I must interpret the authors' statement that "the results of the proposed project will help researchers and conservation biologists refine protocols for identifying suitable relocation sites and evaluate and improve facilities for maintaining mussels in captivity" as overly ambitious. The authors leap (a gigantic leap) from the results generated under objectives 1, 2, and 3 to "a greater understanding of 1) the environmental cues that mediate feeding, 2) the impact of temporal changes in seston concentration and composition on the survivorship and physiological condition of mussel populations, and 3) the feeding strategies utilized by mussels for processing particles and acquiring sufficient energy for growth and reproduction during periods of poor food quality." All this, from examinations of the feeding structures, and experiments that last less than one hour, using one species of algae, one type of inorganic particulate matter. with mussels glued to a plastic bolt in a plastic container, wedged open, and with a rod stuck into them. If the above points represent the benefits that the authors wish to provide the funding source, as well as those who are actually going to be culturing mussels on a large scale, then they need to reexamine their proposed methods. The results from this study will be so narrow that the level of generalization proposed by the authors will be unjustified. Contrary to the authors' desire to show long-term effects of particle concentration and quality on mussel feeding, they will show short-term effects.