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Research Grant Proposal Submitted to

Mussel Mitigation Trust

DEVELOPMENT AND EVALUATION OF NON-DESTRUCTIVE METHODS FOR ASSESSING THE NUTRITIVE CONDITION OF FRESHWATER UNIONID MUSSELS

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SUMMARY

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. Thus, the proposed study will assess the potential use of traditional morphological and biochemical condition indices, RNA-DNA ratios, and protein biomarkers, for detecting sublethal nutritive stress in two common Ohio River mussels, *Megalonaias nervosa* and *Quadrula pustulosa*.

The work proposed herein will constitute the first comprehensive analysis of the effect of starvation on the utilization of energy stores by unionid mussels and will provide vital physiological data that will compliment life history and population information currently being accumulated as part of ongoing surveys of mussel populations of the Ohio River drainage. The project will provide scientists and resource managers engaged in mussel conservation efforts with reliable, non-destructive methods for evaluating the vulnerability of natural populations to perturbations and for monitoring the health of species adversely affected by habitat changes, contaminants, and other environmental stressors. Thus, the results of the study will be of particular interest to researchers involved in (1) monitoring the condition of mussels reintroduced to extirpated areas or translocated to new habitats as part of mitigation projects, (2) evaluating and improving relocation procedures and facilities for maintaining mussels in captivity, (3) identifying species amenable to relocation and capable of surviving transplant conditions, and (4) identifying the susceptibility and tolerance of unionid mussels, especially riverine species, to fouling by zebra mussels.

Specific objectives of the study include:

- To determine the impact of starvation and nutritive stress on the energy reserves and biochemical composition of unionid bivalves.
- To compare the relative value of common morphological and biochemical condition indices for assessing the nutritive status of freshwater mussels
- To investigate the possible use of stress proteins and RNA-DNA ratios for the early detection of sublethal starvation in adult mussels

Although the project will focus on two mussel species native to the Ohio River drainage, the results of the study will contribute to a fuller understanding of the basic biology of all freshwater bivalves and will serve as the basis for future comparative research on the impact of

adverse environmental conditions and anthropogenic activities on mussel populations inhabiting lentic and lotic habitats throughout North America.

BACKGROUND

Impact of Nutritive Stress on Freshwater Mussels

Over the past century, the diverse freshwater unionid mussel populations of North America have declined dramatically with more than 45% of the estimated 297 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 changes in water quality, commercial harvesting for the cultured pearl industry, poor land use practices, and loss of suitable habitat from sedimentation, channel dredging, river impoundment, and other anthropogenic activities (Neves 1987, Bogan 1993, Williams *et al.* 1993). More recently, the introduction and spread of the Eurasian zebra mussel *Dreissena polymorpha* has resulted in the extirpation of several mussel species from portions of the Great Lakes (Haag *et al.* 1993, Gillis and Mackie 1994, Nalepa 1994, Schloesser and Nalepa 1994) and is currently considered the most immediate and critical threat to unionid fauna inhabiting large riverine systems, especially the Ohio River and its tributaries (see *Assessing the Impact of Zebra Mussels on the Physiological Condition of Unionid Mussels* below; Bogan 1993, Chaffee 1995, Neves 1995). Current efforts to prevent the decimation of unionid populations include transplanting healthy mussels to extirpated areas to reestablish populations within their historical range and relocating current populations threatened by anthropogenic activities or fouling by zebra mussels (for examples see Dunn 1993, Layzer and Gordon 1993, Dunn and Layzer 1995, Havlik 1995, Hamilton *et al.* 1995, Jenkinson 1995, Kitchel *et al.* 1995, Naimo *et al.* 1995). Unfortunately, evaluating the success of such programs is problematic since long-term, labor intensive mark-recapture studies are often needed to adequately monitor the survivorship, growth, and reproductive activity (*i.e.*, evidence of larval brooding and juvenile recruitment) of transplanted or displaced mussels. Thus, growing concern for endangered and threatened native unionid species emphasizes the need to develop sensitive, economical and non-invasive diagnostic tools for monitoring the physiological condition of natural populations and evaluating the health of mussels relocated to new habitats or maintained under artificial conditions.

Over the past several years, bivalve molluscs have achieved prominence as sentinel organisms and bioindicators of aquatic pollution as evidenced, for example, by the establishment of global "mussel watch" programs (Goldberg 1975, Goldberg *et al.* 1978). Although exposure of molluscs to environmental agents is frequently determined through measurements of morphological and biochemical condition (for review see Mann 1978), the thick opaque shells of adult bivalves often conceal overt evidence of stress, making it difficult to detect the impact of contaminants, environmental perturbations and other stressors on the animal's physiological condition. One common response of bivalve molluscs to environmental perturbation includes the cessation in feeding activity and the subsequent utilization of energy stores to meet metabolic demands. Although there is an extensive literature on the effects of starvation and diet on the physiological condition of marine bivalves, relatively little information is available on the effect of nutritive stress on freshwater mussels.

During prolonged periods of starvation or partial food deprivation, most molluscs experience a reduction in tissue biomass or "degrowth" as energy reserves are mobilized and catabolized (Russell-Hunter 1985), but the relative contribution of stored nutrients and the sequence of utilization varies among species and developmental stages. Although glycogen is generally considered to be the primary reserve of adult bivalves (Walne 1970, Gabbott and Stephenson 1974, Gabbott 1975, Barber and Blake 1981, Lane 1986), recent reports indicate starved oysters derive most of their energy (44%) from protein and only 33% and 23% from carbohydrate and lipid, respectively (Whyte *et al.* 1990). Similar distributions of nutrient stores were reported by Beninger and Lucus (1984) for two species of *Tapes*. Lipids have also been shown to be utilized by adult *Macoma balthica* when carbohydrate stores are depleted (Beukema and De Bruin, 1979). In larval bivalves, lipids are considered the major source of energy (Fraser 1980, Whyte *et al.* 1987, 1989, Albentosa *et al.* 1993), but dietary carbohydrates appear to play an important role in the use of nutrients for synthesis as opposed to energy production (Whyte *et al.* 1989).

In most molluscs, nutritive stress is generally thought to impact survivorship by limiting the mussel's capacity to withstand or tolerate additional disturbances, including diseases, parasitic infections, chemical agents, and changes in environmental conditions (Bayne *et al.* 1975, 1978). Nevertheless, loss of energy reserves and tissue biomass can also have a significant impact on reproductive capacity. Bayne (1972) and Bayne *et al.* (1975) reported that larvae of nutritionally stressed *Mytilus edulis* grow slower than larvae from unstressed mussels. In several marine bivalves, larval viability, growth and survival have been linked to initial lipid content (Helm *et al.* 1973, Gallager and Mann 1986, Gallager *et al.* 1986). Thus, periods of nutritive stress may disrupt the normal energy storage cycle of female unionid mussels and prevent optimal growth and development of glochidial larvae by prohibiting the transfer of sufficient nutrient reserves to eggs during vitellogenesis.

The goal of the proposed study is to determine the impact of starvation on the physiological condition and biochemical composition of freshwater mussels. Traditional morphometric and biochemical measures of bivalve condition will be compared to more modern microanalytical techniques, including RNA-DNA ratios and protein biomarkers, to determine (1) which procedures provide the most reliable estimates of the nutritive condition of freshwater unionid mussels and (2) which techniques hold the greatest promise as economical, nondestructive (*i.e.*, minimally invasive) methods for quantifying the health and physiological status of field and captive populations of bivalves.

Techniques for Measuring Nutritive Stress and Physiological Condition

Morphological and Biochemical Indices

Over the past several years, a variety of condition indices (C.I.'s) have been employed for rapidly assessing the impact of hydrographic conditions, breeding cycles, contaminants, seston availability, parasites, and other environmental stress factors on the nutritive condition and physiology of marine and estuarine bivalves (for reviews see Mann 1978, Lawrence and Scott 1982, Lucas and Beninger 1985, Davenport and Chen 1987, Crosby and Gale 1990, Rainer and Mann 1992). Gross morphological indices, commonly referred to as "meat-to-shell ratios", usually consist of a measure of the animal's soft-tissue weight (numerator) adjusted by a stable

measure of size (denominator), usually shell length, weight or cavity volume. Because numerous derivations of the index have been employed by different authors (for review see Crosby and Gale 1990), comparisons among published studies are difficult and attempts to develop conversion and intercalibration factors have been unsuccessful (Rainer and Mann 1992). Condition indices have also been criticized for being nonspecific indicators of stress and "static" measures of physiological state since they only provide information about the animal's condition at a given point in time (Lucas and Beninger 1985). Nevertheless, C.I.'s have been applied extensively to both laboratory and field populations and have been found to be reliable estimators of the physiological status and biochemical composition of a broad range of bivalve molluscs (Walne 1970, Gabbott and Bayne 1973, Gabbott and Stephenson 1974, Whyte and Englar 1982, Rainer and Mann 1992). Despite their widespread use in bivalve aquaculture, the utility of C.I.'s for detecting nutritive stress and quantifying the condition or health of field and laboratory populations of unionid mussels has never been adequately addressed.

Although static condition indices based on shell and tissue characteristics provide reliable estimates of the physiological status and health of bivalves, tissue composition profiles and biochemical indices, including ones based on glycogen, lipid and organic content, are gaining acceptance as methods for detecting sublethal nutritive stress in a variety of aquatic organisms (for reviews see Walne 1970, Mann 1978, Whyte and Englar 1982). In addition to serving as important diagnostic screening tools of physiological condition, biochemical indices can also be used to monitor the allocation of resources for growth and reproduction and the mobilization of stored energy reserves when food is limited. When coupled with appropriate conversion factors, biochemical analyses also provide indirect estimates of the energy content of specific tissues for bioenergetic studies (Beukema and DeBruin 1979). Although associations between gross morphological indices (meat-to-shell ratios) and the composition of specific tissues has been documented for several bivalves (Walne 1970, Gabbott and Stephenson 1974, Whyte and Englar 1982), published studies demonstrating a causative or physiologic link between starvation or food deprivation and changes in the tissue content and composition of unionid mussels are lacking. Thus, the proposed project will document the mobilization and depletion of important energy reserves in food limited mussels and evaluate the application of several popular biochemical indices, including glycogen concentration, lipid content, and organic content, for condition assessment.

Despite of their popularity as measures of condition in marine bivalves, morphological and biochemical indices have several shortcomings which potentially limit their application to efforts to protect and conserve unionid mussels, especially species currently considered to be threatened or endangered. Most indices are destructive and require sacrificing large numbers of mussels to obtain accurate, population-level estimates of condition. Moreover, the weight and biochemical composition of many bivalves undergo seasonal changes unrelated to environmental stress but attributable to shifts in food quality and quantity and the accumulation and storage of nutrient reserves (Walne 1970, Gabbott and Bayne 1973, Gabbott and Stephenson 1974, Gabbott 1975, 1983, Mann 1978, Blay 1990, Austin *et al.* 1993, Brown and Hartwick 1988a,b). For example, Pekkarinen (1993) reported significantly higher "fatness" levels (proportion of soft-tissue relative to shell volume) in several unionid mussel species during spring and early summer, presumably in response to increased food abundance and higher water temperatures. Similar temporal variability in the adjusted weights of the unionid mussel *Fusconaia ebena* led

Layzer and Madison (1995) to concluded that condition indices based upon dry-tissue weight are unreliable as estimators of physiological condition. Seasonal fluctuations in condition indices have also been shown to coincide with reproduction and are frequently utilized as measures of fecundity and reproductive effort (Bayne *et al.* 1983, Bayne and Worrall 1980, Rodhouse *et al.* 1984, Thompson 1984). Thus, differences in shell shape and thickness, brood chamber design, and the timing and length of glochidial incubation may make inter- and intra-specific comparisons among condition indices difficult (Tankersley and Dimock 1992, Pekkarinen 1993). Changes in weight or body composition may also be the result of less obvious factors. For example, using time series analysis, Austin *et al.* (1993) recently concluded that cyclic seasonal changes in the condition of the oyster *Crassostrea virginica* were partially the result of fluctuations in salinity. Finally, biochemical changes attributable to reproduction or nutritive stress may vary among tissue types (DeZwaan and Zandee 1972). Adachi (1979) reported protein levels in the adductor muscle of *Tapes philippinarum* decreased during gonad maturation while levels in the gills and midgut gland remained relatively constant. Consequently, to avoid errors caused by differential storage and mobilization of energy stores among tissues, the proposed study will utilize only mantle tissue to calculate and evaluate biochemical indicators of stress since (1) adequate samples for biochemcial analysis can be obtained without sacrificing the mussel (Berg *et al.* 1995) and (2) current evidence suggests that the mantle serves as a primary site for energy storage and growth (Bayne 1973).

RNA-DNA Ratios and Stress Proteins

In recent years, considerable attention has been given to biochemical methods of assessing physiological condition and detecting ecotoxicological stress using nucleic acid concentrations and protein expression profiles (for reviews see Mayer *et al.* 1992, Sanders *et al.* 1992). Indices and biomarkers based upon protein syntheses and gene expression are considered cellular-level biomarkers and have the advantage of being among the first detectable and quantifiable responses to environmental change.

Because a primary function of ribonucleic acid (RNA) is the synthesis of protein, it is one of a number of non-specific biomarkers which can be used to provide an estimate of the impact of toxicants and other environmental stressors on growth rate (Mayer *et al.* 1992). Cellular RNA concentrations have been shown to be positively correlated with the rate of recent protein synthesis (Brachet 1960, Sutcliffe 1965) and have been used to predict growth rates in a variety of aquatic organisms including larval and adult crustaceans (Sutcliffe 1970, Sulkin *et al.* 1975) and fish (Haines 1973, Buckley 1979, 1980, 1982, 1984, Clemmesen 1987). In most studies, RNA levels are standardized by deoxyribonucleic acid (DNA) concentrations which are more stable and serve as an estimate of cell number or biomass (Buckley 1984). Although the application of RNA-DNA ratios as indices of bivalve growth rates has been limited, Wright and Hetzel (1985) reported significantly lower RNA-DNA ratios in the mantle tissue of the oyster *Crassostrea virginica* after 8 weeks of starvation stress. Oyster RNA concentrations were also found to be strongly correlated with conventional condition indices based upon wet tissue weight and shell cavity volume. Thus, for the proposed study, we plan to modify the techniques outlined by Wright and Hetzel (1985) to investigate the application of RNA-DNA ratios as indicators of depressed growth and physiological condition in nutritionally stress unionids.

When cells are exposed to physical and chemical agents, gene expression is often altered, resulting in the rapid synthesis and accumulation of a suite of proteins thought to function in cell protection and restoration (for reviews see Sanders 1990, Sanders *et al.* 1992, Hightower 1993). As a result, these "stress proteins" are often considered instantaneous biomarkers of environmental change and can be used to confirm an organism's exposure to a variety of pollutants and adverse conditions (Sanders 1993). Moreover, protein concentrations have been shown to be closely associated with the level of stress (Sanders *et al.* 1992), enabling the degree of protein expression to serve as a quantitative index of environmental contamination (Hightower 1993).

The most well studied inducible stress proteins are the heat shock proteins (HSP's). Although they were originally found to be expressed in response to thermal stress, synthesis of HSP's can be induced by a wide range of chemical, physical or biological agents, making them "general biomarkers" of stress and cell damage (Sanders 1990). Other environmentally induced proteins are more stressor-specific and are only elicited in response to certain chemical contaminants or physiological conditions. Although their function is less understood, most stressor-specific proteins appear to participate in biochemical processes activated during periods of stress or be involved in the metabolism, compartmentalization and sequestration of toxic chemicals, heavy metals and other contaminants (Sanders 1990).

During periods of nutritive stress, the utilization of energy stores and the subsequent decline in physiological condition may induce the production of several heat shock proteins (HSP's) or other general stress proteins known to be synthesized in cells deprived of glucose (*i.e.*, glucose-regulated proteins or GRP's). Moreover, unique nutritive stress-specific proteins may also be synthesized by mussels in response to depleted energy reserves. Novel proteins, which reflect the nutritive state of a wide range of unionid mussels, could serve as important diagnostic screening tools for monitoring the health of natural and captive mussel populations. For example, assays based on antibodies raised against the proteins could be used to construct field "test kits" to quantify the physiological condition of mussels *in situ*. Thus, for the present study, we propose to use two-dimensional electrophoretic gels and stress protein profile analysis to identify unique "nutritive stress proteins" in the mantle tissue of starved mussels which could serve as biomarkers and indices of physiological condition. Future studies will involve determining if relevant stress proteins are present and conserved among unionid species and documenting their expression in field populations.

Application to Conservation Efforts and the Mission of the Trust

Impact of Zebra Mussels on the Physiological Condition of Unionid Mussels

Since its initial colonization of Lake St. Clair and western Lake Erie in the mid-1980's (Hebert *et al.* 1989, Leach 1991), the Eurasian zebra mussel *Dreissena polymorpha* has become a dominant component the benthic fauna throughout the Great Lakes and has quickly spread to several neighboring river systems including the St. Lawrence, Hudson, Mississippi, and Ohio Rivers (Griffiths *et al.* 1991). Throughout its range, *D. polymorpha* has had a profound impact on the structure and composition of plankton communities and adversely affected the density and diversity of native bivalve populations (Bunt *et al.* 1993, Haag *et al.* 1993, Tucker *et al.* 1993, Gillis and Mackie 1994, Nalepa 1994, Schloesser and Nalepa 1994, Strayer *et al.* 1995, Caraco *et*

al. 1995). Unlike unionid mussels, *D. polymorph* is a fouling epibiont that attaches to hard substrata via byssal threads. In many soft-bottom habitats, unionid mussels serve as the primary settlement site for zebra mussel veligers, which quickly attach and form dense aggregations on the exposed posterior edges of the shells. In some areas, zebra mussels densities exceeding 10,000 per mussel have been recorded (Hebert *et al.* 1991, Schloesser and Nalepa 1994).

Although fouling by zebra mussels is thought to have a negative impact on a variety of physiological and behavioral processes in unionid bivalves, including locomotion, burrowing, and shell closure and adduction, most researchers attribute the reported declined in unionid populations to nutritive stress caused by their inability to compete with zebra mussels for suspended food. Dense clusters of zebra mussels near the posterior shell margin are thought to obstruct feeding and respiratory currents flowing through the exhalant and inhalant siphons (Mackie 1991). Encrusting mussels may also effectively act as pre-filters by removing particles entrained in water currents entering the mussel's mantle cavity. Moreover, recent reports suggest that the filtering capacity of zebra mussels may indirectly influence the feeding ecology and physiological condition of native mussels by reducing the biomass of phytoplankton and other nutritionally important seston particles. Bunt *et al.* (1993) estimated populations of *Dreissena* in western Lake Erie filter a substantial portion of the water column daily, subsequently altering the quantity and size-frequency distribution of suspended particles. Using a bioenergetics model, Madenjian (1995) calculated that zebra mussel populations in the western basin of Lake Erie removed approximately 26% of the total primary production from the region. The impact of zebra mussel feeding on seston concentrations has not been restricted to the Great Lakes. Increased light levels (ca. 15%) have also been reported for areas of the Hudson River containing zebra mussels and recent surveys of phytoplankton and zooplankton communities indicate phytoplankton biomass has declined significantly (ca. 85%) since the invasion of *D. polymorpha* (Caraco *et al.* 1995). Changes in turbidity levels and light transmittence may also disrupt phytoplankton composition and permit the growth of unpalatable or toxic algae and benthic macrophytes that could ultimately effect the health of native mussels and other phytoplankton and bacterioplankton grazers.

Several authors have predicted that North American unionid mussel populations will decline rapidly as zebra mussels continue to expand their range and dominate riverine habitats containing dense, diverse bivalve fauna (Bogan 1993, Strayer and Smith 1993, Williams *et al.* 1993, Neves 1993, 1994, 1995). Recent reports by the U.S. Fish and Wildlife Service estimate that more that 20 species of mussels may be at risk of extinction over the next 10 yrs. as a result of the invasion of *D. polymorpha* (Shannon *et al.* 1993). Unfortunately, the sensitivity of native unionid mussels to fouling by zebra mussels appears to vary among species and subfamilies (Haag *et al.* 1993, Nalepa 1994), making it difficult to predict which populations are in greatest need of protection.

Development of sensitive stress indicators for quantifying the physiological condition of native mussels and identifying when fouling densities have reached levels which warrant collection and relocation of infected mussels has recently been identified as a principal research objective of the U.S. Fish and Wildlife Service's proposed "Zebra Mussel Strategic Action Plan" designed to protect vulnerable unionid populations in the Ohio River (Neves 1995, Chaffee 1995). Unfortunately, as detailed above, identifying dependable estimators of nutritive stress has been problematic. Although Nalepa (1994) recently found dry-tissue weight to be an unreliable

indicator of zebra mussel induced physiological stress, other authors have had limited success using biochemical indices, including lipid (Hebert *et al.* 1991) and glycogen content (Haag *et al.* 1993). Nevertheless, it is unclear whether any of the documented changes in biochemical composition are directly attributable to nutritive stress. Thus, studies identifying the susceptibility and tolerance of unionid mussels, especially riverine species, to fouling by zebra mussels are urgently needed.

We anticipate that many of the condition indices and biochemical assays outlined in the current study will serve as sensitive, reliable techniques for evaluating the health of *D. polymorpha* infested mussels and should provide valuable insights into the relationship between zebra mussel fouling densities, physiological condition, and infestation tolerance. Moreover, the development of non-destructive assessment protocols which permit sequential sampling of individual mussels will assist researchers in monitoring the status and health of unionid populations and provide estimates of the amount of time different species might be expected to survive periods of fouling.

Monitoring the Success of Mussel Relocation Projects

The continued decline of native unionid mussel populations coupled with the rapid spread of the of the zebra mussel *Dreissena polymorpha* into several riverine habitats has forced research scientist 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). Artificial propagation studies employing tissue culture techniques and captive rearing (aquaculture) facilities are also being developed to raise juvenile "seed" mussels to help restore decimated populations (Neves 1994, Tankersley, personal observation).

Among the critical challenges faced by many mitigation and captive holding projects is the location of suitable, stable 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. Programs to monitor the success of mitigation and relocation projects typically involve resampling areas to document survivorship and growth and to identify signs of reproduction and recruitment (*i.e.*, presence of gravid females and young juveniles) (for examples see Dunn 1993, Dunn and Layzer 1995, Dunn and Sietman 1995, Hamilton 1995, Waller *et al.* 1995). Unfortunately, the physiological condition of transplanted or captive mussels is rarely determine, primarily because most assays require sacrificing the mussel to obtain sufficient tissue for biochemical analyses or determination of tissue weight (*see discussion above*). Thus, studies examining the effect of nutritive condition on the ability of mussels to survive transplantation or relocation and the effect of prolonged holding under reduced nutritional conditions on the long-term health and survival of mussels are clearly needed. The results of the proposed project will provide valuable techniques for selecting habitats suitable for relocation and mitigation programs, evaluating and improving facilities for

maintaining mussels in captivity, and identifying mussel species amenable to relocation and capable of surviving transplant conditions.

METHODS

Collection and Maintenance of Mussels

Evaluation of nutritional stress indicators in unionid mussels will be conducted using two common Ohio River species, *Megalonaias nervosa* and *Quadrula pustulosa*. Both species were selected for the study because they (1) have a broad distribution that includes much of the Ohio River and its tributaries (2) are susceptible to fouling by zebra mussels, and (3) are known to experience differential mortality when relocated to new habitats as part of mitigation projects (Dunn 1993). Adults of both species will be collected from the Lower Muskingum River in late May 1996 (Watters and Dunn 1995). Following collection, 10 individuals of each species will be randomly selected as "pre-acclimation controls" and their shell lengths (maximum anterior posterior dimension), widths (maximum left-right dimension), heights (maximum dorso-ventral dimension), and whole wet-weights, determined. The soft tissue will then be removed from the shell, the mantle tissue carefully separated from the remaining viscera, and the wet weight of both tissues recorded. Several additional shell features, including shell weight, cavity volume, and cavity capacity will be measured following the procedures of Mann (1978) and Lawrence and Scott (1982). Mantle tissue will be homogenized, separated into five equal aliquots (one each for analysis of organic content, glycogen content, lipid composition, RNA-DNA ratios, and stress protein analysis), freeze-dried, weighed and stored at -80 °C until the appropriate morphological and biochemical analyses can be performed (see descriptions below). The remaining visera will also be homoginized, freeze-dried and weighed for use in calculating gross morphological condition indices (see description below). The remaining mussels will be marked with small plastic tags and transported in a refrigerated holding tank to the laboratory where they will be maintained in recirculating aquaria at collection temperatures and fed mixed algal cultures of *Chlorella vulgaris*, *Ankistrodesmus falcatus*, and *Chlamydomonas reinhardt* (final concentration 2×10^4 cells ml^{-1}) daily via an automatic dosing pump.

Following an initial two week laboratory acclimation period, a second group of ten mussels will be selected as "post-acclimation/pre-treatment controls" and their shells and soft-tissues processed, measured and stored as described above. Half of the remaining members of each species will be randomly assigned to the "starved" treatment and placed in separate aquaria containing river water that is continuously recirculated through a series of filters (100 µm, 25 µm, 4 µm) to remove suspended particles. The remaining mussels will comprise the "fed" group and will be maintained in separate aquaria on the same diet and feeding regime they experienced during the acclimation period. On days 15 and 45, 10 mussels will be randomly selected from each treatment and their shells, viscera, and mantle tissues processed as described previously for the two control treatments.

Calculation of Nutritive Stress Indices

Gross (Morphological) Condition Indices

Measures of dry soft-tissue weight (viscera + mantle tissue), shell weight, shell cavity volume (ml), and internal shell cavity capacity (g) will be used to calculate the following condition indices described by Crosby and Gale (1990):

$$CI_{vol} = \frac{\text{dry soft tissue wt (g) x 1000}}{\text{internal shell cavity volume (ml)}} \quad (1)$$

$$CI_{shell} = \frac{\text{dry soft tissue wt (g) x 1000}}{\text{shell weight (g)}} \quad (2)$$

$$CI_{grav} = \frac{\text{dry soft tissue wt (g) x 1000}}{\text{internal shell cavity capacity (g)}} \quad (3)$$

Biochemical Indices

Since a primary goal of the study is to develop an nondistructive assay for evaluating the physiological state of unionid mussels that can be performed using a small sample of mantle tissue (*i.e.*, "mantle biopsy", Berg *et al.* 1995), all biochemical indices, RNA-DNA ratios, and stress protein characterizations will be conducted using homogenized mantle tissue. Thus, biochemical components will be expressed as a percentage the dry weight of the sample rather than the total animal.

Lipid class composition of the mantle of fed and starved mussels will be determined using the Iatroscan TLC-FID (thin-layer composition-flame ionization detection) techniques described by Fraser *et al.* (1985) and Ackman *et al.* (1990). Lipids will be extracted from homogenized mantle tissue using a mixture of dichloromethane-methanol (2:1 vol./vol.). Lipid content will be determined using an Iatroscan Mark V TLC-FID (IATRON, Inc., Tokyo, Japan) equipped with Chromarod SII rods. Polar lipid classes will be separated in chloroform: methanol:water (70:35:3.5, by vol.) and neutral lipid classes in 1,2 -dichloroethane: chloroform: acetic acid (92:8:0.1, by vol.).

Glycogen levels will be quantified using the phenol-sulfuric acid method of Dubois *et al.* (1956). Briefly, the procedure will involve placing partially purified glycogen from 100 mg of the freeze dried mantle tissue homogenate in a solution of 5% phenol and concentrated sulfuric acid. After 30 min., the optical density of the resulting solution will be read at 430 nm on a UV/VIS spectrophotometer. A standard curve will be constructed using oyster glycogen (Sigma Chemical Company).

Organic content [% of sample dry weight; (dry weight – ash weight)/dry weight] of samples will be determined by ashing 500 mg samples of the tissue homogenate in a muffle furnace (Thermolyne Model F47925) at 400 °C for 12 h.

RNA-DNA Ratios

Quantitative analysis of nucleic acids will be conducted using the modified Schmitt-Thanhauser procedure described by Munro and Fleck (1966) and adapted for use with bivalve mantle tissue by Wright and Hetzel (1985). RNA-DNA determinations will be made by treating homogenized mantle tissue with 0.6N and 0.2N perchloric acid (PCA) to remove acid-soluble free nucleotides and sugars. The remaining pellet will be incubated in 0.3N KOH to obtain the acid-soluble RNA fraction. The RNA-free fraction will then be incubated in 0.6N PCA at 85 °C to obtain the DNA fraction. Absorbencies for both fractions will be measured at 240 nm using a UV/VIS spectrophotometer. Protein content will be assayed using the procedures outlined by Lowry *et al.* (1951) using bovine serum albumin as the standard.

Stress Proteins

Proteins will be extracted from homogenized mantle tissue using cold phosphate-buffered saline (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF, a protease inhibitor). Extracted proteins will be separated on 2D polyacrylamide gels and detected using the silver staining methods described by Ausubel *et al.* (1989). Moreover, the profiles will be scanned for common stress proteins, including HSP_{70}, HSP_{90}, and GRP_{78}, using the immunoassay techniques of Bradley and Ward (1989). Between and within treatment comparisons of mussel protein profiles will be conducted using computerized imaging techniques. Silver stained gels will be digitized using a Silverscanner II flatbed scanner (LaCie Corp.) and novel proteins identified using Prism Image Analysis Software (Analytical Vision Inc.)

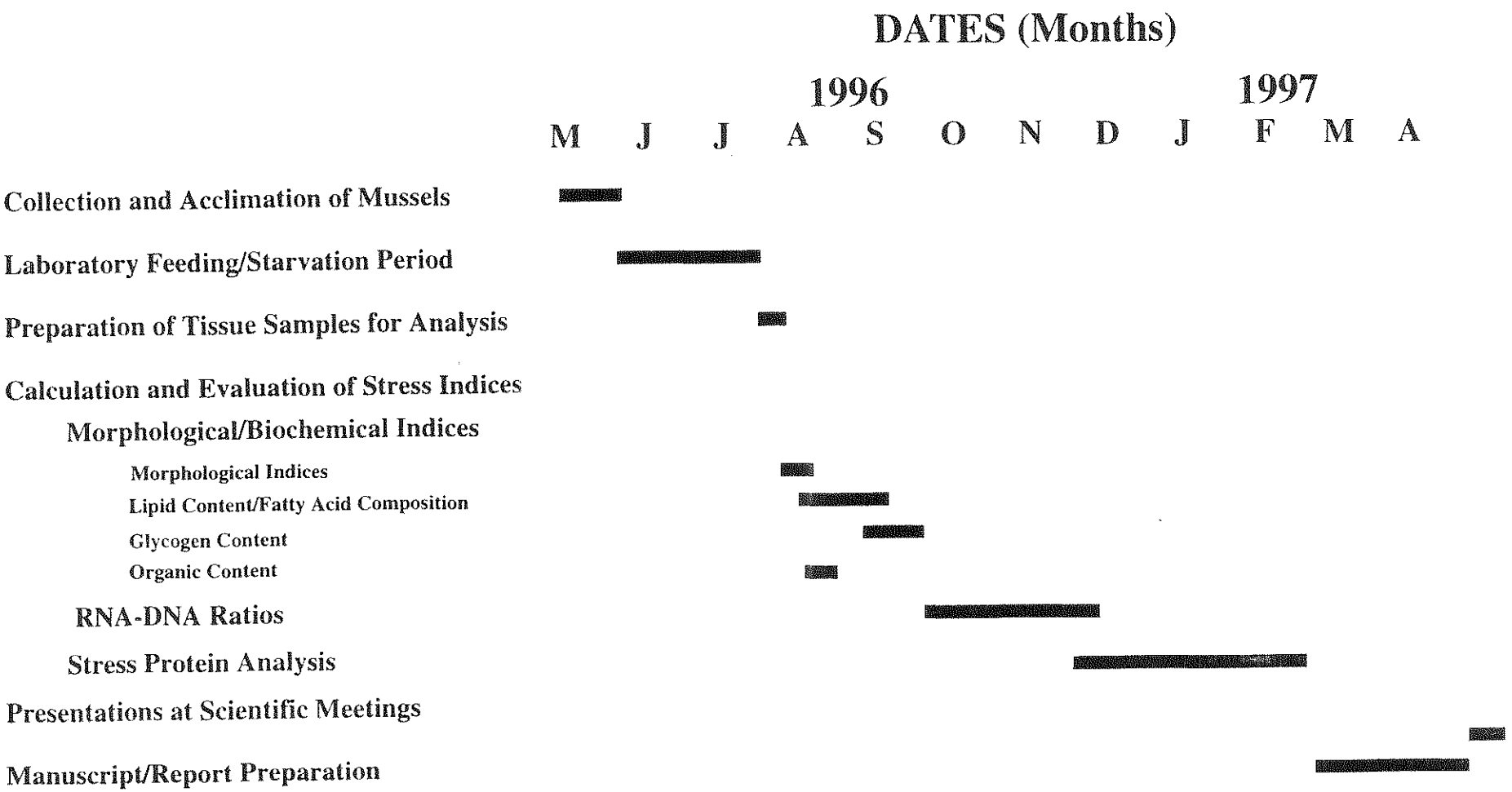
Statistical Analysis

Since it is likely that most of the measured variables will violate one or more of the assumptions of parametric statistical test, comparisons between treatment groups for each of the dependent variables (*i.e.*, morphological and biochemical indices) will be conducted using non-parametric statistics, including Kruskal-Wallis analysis of variance (Zar 1996). If multivariate analyses are needed, rank transformations following the procedures of Conover and Iman (1981) will be employed. Where applicable, Spearman Rank correlations (Zar 1996) will be computed to test for relationships among morphological and biochemical indices of physiological condition.

RESEARCH SCHEDULE

We estimate the project will take approximately 12 months to complete. The schedule of specific research tasks are outline in Figure 1. Collection and maintenance of mussels will take place during the summer 1996. All morphometric and biochemical analyses, including the development of protocols for determining RNA-DNA ratios and analyzing stress protein profiles, will be conducted on freeze dried samples between September 1996 and April 1997. It is anticipated that the results of the project will be presented at the 1997 meeting of the North American Benthological Society and at the Conservation and Management of Freshwater Mussels Meeting scheduled for October 1997.

Figure 1: Time Table of Research Tasks



BUDGET

Budget Item/Description	Amount	Subtotal
A. Total Labor Costs		**$15,180**
Richard Tankersley (PI)	$12,020	
Graduate Research Assistant	$3,160	
B. Fringe Benefits		**$1,195**
C. Non-Expendable Supplies		**$7,850**
Aquarium Chiller Unit	$2,700	
Refrigerated Transport Container	$850	
Electrophoresis Equipment	$1,750	
Glassware	$450	
Benchtop Muffle Furnace	$1,650	
Field/Collecting Equipment	$450	
D. Expendable Supplies		**$2,925**
Aquarium Supplies	$600	
Algal Culturing Supplies	$700	
Mussel Tags	$375	
Supplies/Reagents for Biochemical Analyses	$750	
Gel Electrophoresis Supplies	$500	
E. Equipment Rental		**$1,150**
Boat Rental	$250	
Iatroscan Rental Fee (Lipid Analysis)	$900	
F. Travel		**$1,000**
G. Total Direct Cost		**$29,300**
H. Indirect Cost (47% of TDC-Equipment)		**$10,082**
I. Total (Direct + Indirect Cost)		**$39,382**

BUDGET JUSTIFICATION

A. Total Labor Costs

Principal Investigator: Salary has been requested for summer 1996 at a rate of 2.5/9.5 of the following academic year salary level.

Graduate Assistant: A stipend for summer 1996 is requested. Levels for graduate students are stipulated by the Graduate School of the University of Maryland Baltimore County.

Both researchers will continue to work on the project during the academic year at no additional cost to the grant.

Salary Derivation and Increment

Personnel	Level of Effort		
	Calendar Year	Academic Year	Summer
Principal Investigator	---	20%	100%
Graduate Assistant	---	40%	100%

B. Fringe Benefits

	Principal Investigator	Graduate Assistant
Social Security (7.65%)	Yes	Yes
Medical Insurance (Single)	No	Yes
Unemployment Compensation (0.2%)	Yes	Yes

C. Non-Expendable Equipment

To minimize the stress involved in transporting mussels from the Lower Muskingum River to our aquarium facilities at UMBC, we are requesting funds to purchase a self-contained, refrigerated holding tank (Live Holding Systems, Aquatic Marine Systems, Inc.) capable of maintaining mussels at collection temperatures for 10-12 hrs. Similar transport containers are currently used extensively by aquaculturist to successfully relocate fish and other aquatic organisms to new habitats and holding facilities. Similarly, a refrigerated chiller unit will be used to maintain experimental mussels at ambient collection temperatures in the lab, thus avoiding the confounding effects of thermal stress. Funds are also requested to purchase field supplies and equipment, including mesh bags, buckets, and diving gear, to be used during the two day collecting trip.

Electrophoresis equipment, including minigel electrophoresis units, power supplies, staining trays, and an illuminator will be purchased to process 2D electrophoretic gels for stress protein profile analysis. We have also requested funds to purchase additional laboratory glassware for extracting proteins and nucleic acids from mantle tissue and preparing samples for glycogen and lipid analysis. A small benchtop muffle furnace (Thermolyne Model F47925) will also be needed to ash tissue samples for estimates of organic content.

D. Expendable Supplies:

Mussel tags (Hallprint Ltd., Australia) will be used to label individual mussels following collection. Funds for aquarium and algal culturing supplies will be used to purchase plastic tubing, valves, air pumps, filter cartridges, air stones, and fertilizer/media for maintaining mussels and rearing algae during the two month acclimation and starvation period.

Funds are also requested for chemicals, stains and reagents to extract and quantify the glycogen, lipid, nucleic acid content of mantle tissue samples, prepare samples for gel electrophoresis, and identify known stress proteins using immunoassays.

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 during the two-day collecting trip (2 days @ $125/day).

Lipid content and class composition of mantle tissue samples will be measured using an Iatroscan Mark V TLC-FID (IATRON, Inc., Tokyo, Japan). The method involves separating lipid classes by chromatography on quartz rods coated with silicic acid. The rods are then passed through a flame ionization detector and the amount of lipid in each class quantified. Because of its speed and resolution, TLC-FID is far more cost-effective than other more conventional methods of lipid analysis. Thus, the requested funds will be used to cover modest user fees ($50/hr.), tissue preparation charges, and data processing fees.

F. Travel

Funds are requested to cover travel expenses to collect and transport mussels from the Lower Muskingum River to our laboratory facilities at UMBC. Expenses will include round-trip mileage (1500 miles at $0.28/mile) and room and board for research personnel for 5 days (@ $115/day).

PERSONNEL

Principal Investigator: Richard A. Tankersley

My research interests are in the area of invertebrate physiology and ecology. Many of the projects being 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 1995). We are currently using several of the techniques developed in conjunction with these projects, including flow cytometry and video endoscopy, to document qualitative and quantitative retention and utilization of different algal species by a variety of suspension feeders, including the slipper snail *Crepidula fornicata* and the solitary tunicate *Styela plicata*. We are also studying the development of feeding organs in juvenile unionid mussels. We are interested in documenting the organogenesis of suspension feeding structures, characterizing the food of young bivalves, and developing artificial diets capable of sustaining optimal growth of juvenile and adult mussels maintained in captivity. In recent months, we have constructed a facility for culturing juveniles of two unionid mussels, *Utterbackia imbecilis* and *Anodonta californiensis*, and have completed studies correlating developmental and morphological changes in pallial organs with shifts in feeding mode and particle selectivity.

Research Assistant: Jennifer Shepard

Jennifer Shepard is a first year doctoral student in University of Maryland's systemwide graduate program in Marine-Estuarine-Environmental Sciences (MEES). Her research interests focus on the effect of changes in environmental conditions on the physiology of aquatic organisms, specifically identification and application of protein biomarkers for detecting

contaminant exposure and anthropogenic stress. She has had extensive training in protein biochemistry, microanalytical methods, and the analysis 2D protein profiles using computerized imaging techniques and neural nets.

Additional Personnel/Collaborators

Dr. David Wright, Chesapeake Biological Laboratory, Center for Environmental and Estuarine Studies, University of Maryland System, Solomons, Maryland

Dr. Wright is a toxicologist interested in the impact of environmental contaminants on marine invertebrates and fish. In recent years, he has used RNA-DNA ratio as indicators of nutritive stress in the oyster *Crassostrea virginica* (Wright and Hetzel 1985). As a result, he has agreed to assist us in adapting the protocols developed for oysters to measure RNA concentrations in freshwater unionid mussels.

Dr. Brian Bradley, Professor, Department of Biological Sciences, University of Maryland Baltimore County

Dr. Bradley's research focuses on the genetics and physiology of adaptation of aquatic organisms to marginal and impacted environments. His laboratory is actively involved in the development of protein expression assays for ecotoxicity testing. Dr. Bradley has successfully used the stress protein HSP_{70} to quantify the impact of the protozoan *Perkinsus marinus* on the physiological condition of the oyster *Crassostrea virginica* (Streb and Bradley 1996). He is currently J. Shepard's Ph.D. advisor will participate in those aspects of the study involving the analysis of 2D protein profiles and the identification of starvation-specific stress proteins.

CURRICULUM VITAE
RICHARD A. TANKERSLEY

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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.)

M.S. 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

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

1993-1995 Assistant Professor, Department of Biology, Gonzaga University, Spokane, WA

1992-1993 Post-Doctoral Research Associate, Duke University Marine Laboratory, Beaufort, NC

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, βββ 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

- National Science Foundation Instrumentation and Laboratory Improvement Grant; *Title:* Interactive multimedia resource center for science instruction, $149,965, 1994
- Murdock College Science Research Program; Summer research assistant funding; Gonzaga University, $14,024, 1994

- Gonzaga Research Council Grant Program; *Title:* The effect of silt on the feeding physiology of juvenile freshwater mussels, $700, 1994.
- Conchologists of America Research Grant, *Title:* 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, *Title:* 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 *Title:* 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, *Title:* 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, *Title:* 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., J.J. Hart, and M.G. Wieber. 1996. Developmental shifts in the feeding biodynamics of juvenile Utterbacki imbecilis (Mollusca: Bivalvia). in prep.

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. in review.*

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*: Implications for vertical migration. *in review.*

Forward, R.B., Jr., J. Swanson, R.A. Tankersley and J. M. Welch. 1996. Endogenous swimming rhythms of blue crab megalopae: effects of offshore and estuarine cues. *Mar. Biol. in press.*

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 AND PUBLISHED ABSTRACTS

Developmental shifts in the feeding biodynamics of juvenile *Utterbacki imbecilis* (Mollusca: Bivalvia). Tankersley, R.A., J.J. Hart, and M.G. Wieber. 1995. The Conservation and Management of Freshwater Mussels Workshop, St. Louis, October 1995.

Multipurpose gills: The effect of larval brooding on the feeding physiology of freshwater mussels. Part of the Recent Advances in Invertebrate Feeding Biodynamics Symposium to be held at the Annual Meeting of the American Society of Zoologists. St. Louis, MO, January, 1995.

The effect of silt on the feeding biodynamics of juvenile *Utterbackia imbecilis* (Mollusca: Bivalvia). (with J. Hart) Regional Conference of Undergraduate Research, Murdock College Science Research Program, Gonzaga University. November, 1994

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.

Flood tide transport of crab megalopae: III. Behavioral responses to pressure and salinity. 1st. Annual Larval Ecology Meetings. Port Jefferson, NY. August 1993.

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.

Physiological consequences of larval brooding in the unionid bivalve *Pyganodon cataracta*. Duke University Marine Lab Seminar Series. October, 1992.

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.

Larval brooding by the freshwater unionid mussel *Anodonta cataracta*: its effect on ventilation, respiration and filtration. Biology Department Seminar. Wake Forest University. April 1992.

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.

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.

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.

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

Utilization of multiple orientation cues by the marsh periwinkle *Littorina irrorata*. North Carolina Academy of Science Annual Meeting, Greensboro, NC, March 1991.

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

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.

Not-so-random walks: a computer simulation of chemo-orientation behavior. Annual Meeting of the Association of Southeastern Biologists, Baltimore, MD, April 1990

Simulation simple chemo-orientation behaviors using computers. North Carolina Academy of Science Annual Meeting, Highpoint, NC. March 1990.

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.

The effect of visual cues on the trail-following behavior of *Littorina irrorata*. North Carolina Academy of Science Annual Meeting, Highpoint, NC, March 1988.

Snails, trails, mucus and slime: the trail following behavior of the marsh periwinkle *Littorina irrorata*. Biology Department Seminar, Wake Forest University, September 1987.

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.

The effect of trail-following on the locomotion of the marsh periwinkle *Littorina irrorata*. Benthic Ecology Meetings, Raleigh, North Carolina, March 1987,

Trail-following in *Littorina*. Natural History Seminar. Florida State University. February 1987.

Habitat selection, predation and emigration in juvenile spiny lobsters. W.F. Herrnkind, Mark J. Butler IV and R. Tankersley, Benthic Ecology Meetings, Boston, MA, March 1986.

Factors influencing habitat selection of young juvenile spiny lobsters, *Panulirus argus*. Annual Meeting of the American Society of Zoologists, Baltimore, MD, December 1985.

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.

TEACHING EXPERIENCE

Lecture and Laboratory Classes

Computerized Image Analysis (1 semester)
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

Graduate Committee, Department of Biological Science, University of Maryland Baltimore County
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

American Society of Zoologists
Sigma Xi Scientific Research Society
American Microscopical Society
National Shellfisheries Association
North American Benthological Society

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Education

1995-Present **Ph.D. Student, University of Maryland Baltimore County,** Marine-Estuarine-Environmental Science Program, Research Advisor: Dr. Brian Bradley

Course Work

- Biological Chemistry
- Developmental Biology
- Physiology of Marine & Estuarine Organisms (in progress)
- Introduction to Lab/Field Research
- Aquatic Toxicology (in progress)

Cumulative Grade Point Average: 3.5

1994-1995 **Biology Department University of California- Los Angeles**

Course Work

- Environmental Physiology (Seminar)
- Integrative Biology (Seminar)
- Marine Population Biol. (Seminar)
- Invertebrate Zoology

Cumulative Grade Point Average: 4.0

1989-1993 **B.S. Biology Department, University of Southern California**

Course Work (Major)

- Introduction to Biology
- Molecular Biology
- Biochemistry
- Physiology
- Evolution and Population Genetics
- Marine Vertebrate Biology
- Physics (2 Sem.)
- Marine Invertebrate Biology
- Biological Oceanography
- Directed Research Marine Biology
- Ecology
- General Chemistry (2 Sem.)
- Organic Chemistry (2 Sem.)
- Calculus (2 Sem.)

Spring 1992 **Marine Biology Research Semester** (Catalina Island), Hancock Institute of Marine and Coastal Studies, University of Southern California

Description: Design, development, implementation and presentation of ten day

projects in three areas of marine studies including, Biological Oceanography, Physiology of Aquatic Vertebrates, and Invertebrate Zoology.

Cumulative Grade Point Average: 3.1

Research/Teaching Experience

Aug 1995- Present **Teaching Assistant**, University of Maryland Baltimore County (Introductory Biology Laboratory)

Sept 1994- July 1995 **Teaching Assistant**, University of California - Los Angeles (Introductory Biology Laboratory, Vertebrate Morphology Laboratory, Introductory Biology)

Aug 1993-July 1994 **Administrative Assistant**, Tony Silver Films Associates, Inc., Los Angeles.

Sept 1992-May 1993 **Research Assistant**, Section of Ichthyology, Los Angeles Museum of Natural History (Dr. Martin Miesler)

Job Description: Creating database for the Macintosh of all marine organism found in San Diego. Locating, organizing and converting data. Inputting data into Excel Database and creating maps of the researched area in Hypercard.

June 1992-Aug 1992 **Laboratory Technician**, Department of Molecular Biology, Princeton University (Dr. Michael Cole).

Job Description: Organizing laboratory stocks and use of equipment. Ordering and repairing stock and equipment, autoclaving instruments and glassware. Assisting all staff with individual projects using molecular biology techniques including tissue culture maintenance, DNA purification and amplification using PCR and gel electrophoresis of DNA for sequencing and identification of DNA inserts in bacterial clones.

Honors, Awards and Extracurricular Activities

- Catalina Science Semester- One of eleven chosen to study at the Hancock Institute for Coastal and Marine Studies for one semester
- Dean's List (minimum G.P.A. 3.5) Fall 1990, Spring 1992
- Dean's Scholarship Recipient - 4 years (Academic Scholarship)
- Dean's Scholar Association
- Biological Student Association
- SAFE- Student Action For the Environment

REFERENCES

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Barber, B.J. and N.B. Blake 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irridians concentricus* (Say). J. Exp. Mar. Biol. Ecol. 52: 121-134.

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Bayne, B.L. 1972. Some effects of stress in the adult on the larval development of *Mytilus edulis*. Nature 237: 459.

Bayne, B.L. 1973. Physiological changes in *Mytilis edulis* L. induced by temperature and nutritive stress. J. Mar. Biol. Assoc. U.K. 53: 39-58.

Bayne, B.L. and C.M. Worrall. 1980. Growth and production of mussels *Mytilus edulis* from two populations. Mar. Ecol. Prog. Ser. 3: 317-328.

Bayne, B.L., P.A. Gabbott, and J. Widdows. 1975. Some effects of stress in the adult on the eggs and larvae of *Mytilus edulis* L. J. Mar. Biol. Assoc. U.K. 55: 675-689.

Bayne, B.L., P.N. Salkeld, and C.M. Worrall. 1983. Reproductive effort and value in different populations of the marine mussel, *Mytilus edulis*. L. Oecologia 59: 18-26.

Beninger, P.G. and A. Lucas 1984. Seasonal variation in condition, reproductive activity and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jefferys) and *Tapes phillippinarum* (Adams and Reeve). J. Exp. Mar. Biol. Ecol. 79: 19-37.

Berg, D.J., W.R. Haag, S.I. Guttman, and J.B. Sickel. 1995. Mantle Biopsy: A technique for nondestructive tissue-sampling of freshwater mussels. J. N. Am. Benthol. Soc. 14: 577-581.

Beukeuma, J.J. and W. DeBruin. 1979. Calorific values of the soft parts of the tellinid bivalve *Macoma balthica* (L.) as determined by two methods. J. Exp. Mar. Biol. Ecol. 37: 19-30.

Blay, J. Jr., 1990. Fluctuations in some hydrological factors and the condition index of *Aspatharia sinuata* (Bivalvia, Unionacea) in a small Nigerian reservour. Arch. Hydrobiol. 117: 357-363.

Bogan, A.E. 1993. Freshwater bivalve extinctions (Mollusca: Unionidae): A search for causes. Am. Zool. 33:599-609

Brachet, J. 1960. *The Biological Role of Ribonucleic Acids*. Elsevier, New York.

Bradley, B.P. and J.B. Ward. 1989. Detection of a major stress protein using a peptide antibody. Mar. Env. Res. 28: 471-475.

Brown, J.R. and E.B. Hartwick 1988a. Influences of temperature, salinity, and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*. I. Absolute and allometric growth. Aquaculture 70: 231-251.

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Buckley, L.J. 1980. Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and the effect of starvation. Fish. Bull. U.S. 77: 703-708.

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