

BEWZ  
1993



# TENNESSEE WILDLIFE RESOURCES AGENCY

ELLINGTON AGRICULTURAL CENTER  
P. O. BOX 40747  
NASHVILLE, TENNESSEE 37204

## MEMORANDUM

TO: John Jenkinson - TVA, Chattanooga  
 Dick Biggins - USFWS, Asheville  
 Richard Neves - USFWS, Virginia Tech

FROM: David McKinney

DATE: July 19, 1993

SUBJECT: Inventory of metazoans, including parasites, found  
 in selected freshwater mussels

Please find attached a proposal from the Tennessee Aquarium as requested by TWRA. Your evaluation and recommendations on this proposal would be greatly appreciated. Further, the likelihood of obtaining a multi-year commitment of TWRA state dollars would be enhanced if anyone were interested in cost sharing.

Thank you for taking the time to look this over; please contact me at 615/781-6643 if I may further assist.

ADM/bjs  
Enclosure

*wley 5415*

*Background - us certification for this study  
Zebra  
Qualifications of Sasee Matthews*

Tennessee  
Aquarium



A. David McKinney, Chief  
Environmental Services Division  
Tennessee Wildlife Resources Agency  
P.O. Box 40747  
Nashville, TN 37204

June 14, 1993

Dear Dave,

Enclosed is a draft of the mussel parasite survey proposal. Please note that "Figure 1" is not included in the proposal. I will need your help in supplying me with an appropriate figure with the sampling sites that you noted interest in as per our last meeting.

I have coined this project a survey of the "metazoan symbionts" of mussels instead of just "parasites" because I'm sure that we will also collect many organisms which are not truly parasitic but rather associated as commensals with mussels. Needless to say, all associates need to be collected and identified, so the study might as well formally document them all. Furthermore, although this study specifically deals with the metazoan associates of mussels, we will attempt to identify as many of the protozoan symbionts as well, and if this work proceeds smoothly we will report these findings in similar fashion to the metazoan data.

Several items on the project budget might need explanation. I have allocated money for a compound microscope (see year 1 equipment). We have a fine research compound scope and dissection scope at the Aquarium, however, as this study will involve quite a bit of scope time I need to make provision for use of a compound scope of lesser quality for concurrent routine aquarium duties associated with our fish health program. In year 2 I have allocated money for a lap-top computer. This computer will make the bibliography research much more efficient and less time consuming as references can be indexed into a disk file while at the library rather than spending time handwriting information that eventually needs to be placed on disk. I have waited until year 2 to get the lap-top because I anticipate that during year 1 we will be flat out with the parasites themselves.



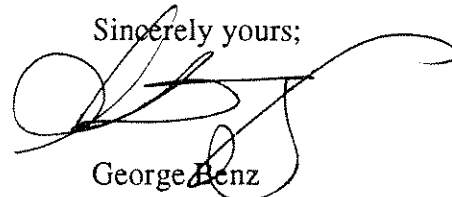
Other than that, the bulk of the project budget is labor. I have only placed items on the supply list that will significantly impact the Aquarium's normally stocked lab supplies.

Concerning the histology fees, the budget makes no provision for money to be allocated to the Aquarium for tissue sectioning, staining and mounting. As we spoke prior, it might be best for TWRA to be billed directly for these fees by the laboratory doing the sectioning. This issue needs to be resolved, along with identifying the laboratory which will do the sectioning. As we spoke last, I believe there is a State lab which routinely does this type of work. I feel the tissue sectioning is an important part of this project. For one, some parasites are small and may burrow into relatively solid tissues where they are best seen in stained sections. Secondly, the examination of tissue sections will lead to other discoveries concerning various disease states. Lastly, the sectioning of tissues as proposed by this study would ultimately generate enough histological information to compile a histological atlas of these two mussel species by the end of the 5 year project. To my knowledge no such comprehensive atlas exists. Such an atlas would be of great interest and utility to biologists interested in both wild and cultured mussels.

The Tennessee Aquarium is excited about working on this project with TWRA. As you are aware, Dr. Susie Matthews will serve as a co-principal investigator on this project along with myself. Realize that the enclosed proposal is a draft. We are interested in working with you to develop this project to the mutual benefit of TWRA and the Tennessee Aquarium.

I look forward to your feedback concerning this proposal, and if I can be of any assistance please feel free to contact me.

Sincerely yours;

A handwritten signature in black ink, appearing to read 'George Benz', with a large, sweeping flourish extending to the right.

George Benz  
Curator of Fishes

Enc.: Draft Mussel Parasite Study

cc: Dr. Matthews  
J. Andrews

# DRAFT

## RESEARCH PROPOSAL

**METAZOAN SYMBIONT SURVEY OF EBONY SHELL (*Fusconaia ebena* [Lea, 1831])  
and MONKEY FACE (*Quadrula metanevra* [Rafinesque, 1820]) MUSSELS FROM  
KENTUCKY LAKE**

Principal Investigator: George W. Benz

Address: The Tennessee Aquarium, One Broad Street, P.O. Box 11048, Chattanooga, Tennessee  
37401-2048

phone: (615) 265-0695      fax(615) 267-3561

Date: June 11, 1993

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### Proposal Abstract

A 5-year project is proposed to survey the metazoan symbiont fauna (both parasitic and non-parasitic) associated with two commercially important freshwater mussels, the ebony shell (*Fusconaia ebena* [Lea, 1831]) and the monkey face (*Quadrula metanevra* [Rafinesque, 1820]) in Kentucky Lake. Mussels will be sampled each year of the survey during three seasonal periods which are transitionally important to aquatic ecosystems: spring, late summer, and mid winter. All metazoan symbionts will be collected, and annual and seasonal estimates of prevalence and density will be calculated for each species. All parasite taxa needing description or redescription will be appropriately detailed for journal publication. Voucher specimens of mussel symbionts will be deposited in the U.S. Helminthological Museum (Beltsville, MD), the U.S. National Museum (Washington, DC), the Manter Museum (University of Nebraska, Lincoln, NE), and in an as yet undesignated collection in Tennessee. A dichotomous identification key to the metazoan symbionts associated with ebony shell and monkey face mussels will be produced. An annotated bibliography of published literature detailing the metazoan parasite fauna of unionid mussels will be compiled.

## 1.0 BACKGROUND

The southeastern United States is the center of biodiversity for freshwater mussels of the family Unionidae. Currently, this rich fauna is experiencing an unprecedented decline due to various environmental factors associated with human activity. Because several unionid species are of considerable commercial importance, and because preliminary studies show that some unionids are excellent indicator species regarding aquatic environmental health, studies are desirable that gather natural history data of potential use in mussel restoration, management, and aquaculture programs.

Parasites can play a dynamic role in the natural history of free-living communities. While some parasite species routinely act as agents of disease, most parasites in natural settings coexist with their hosts by establishing population levels which do not overburden the host. The transmission stage of a parasite's life cycle often represents the limiting phase that ultimately regulates population levels. Environmental factors which alter the probability that the transmission stage will successfully locate and establish itself with a host can cause fluctuations in the parasite load and may alter the overall pathogenicity of the total parasite burden. However, even when parasite populations are stable the pathogenicity associated with them can be variable. This variability is caused by alterations in the overall energy budget of the host and may reflect natural host cycles associated with ontogeny and seasonality or with unnatural or catastrophic environmental events. The pressures placed upon hosts by their parasites undoubtedly are most consequential when the host's energy budget is unbalanced.

Although natural host populations can sometimes be detrimentally impacted by parasites, it is in semi-closed and closed captive environments where parasites often are best known to cause undesirable levels of morbidity and mortality. The physical designs and high stocking densities

of most closed culture systems increase the probability of successful transmission for parasite species (especially those with direct life cycles), and can ultimately overwhelm the capacity for host species to support themselves under the pressures of parasitism.

Systematic studies of the natural parasite burdens of unionid mussels are lacking. Current knowledge of the parasite fauna of freshwater mussels is in the form of geographically scattered parasite records associated with minimal taxonomic and temporal scope. Published records have typically documented the presence of parasites without data concerning the prevalence and density of infections or estimates of the total parasite burden. Because comprehensive background data do not exist for healthy populations, the impact of parasitism on mussel populations is unknown, and the relationship of parasitic infection to "die-off" episodes of mussel populations is impossible to assess.

## 2.0 PROPOSAL STATEMENT

With this in mind a 5-year survey of the metazoan symbionts (both parasites and non-parasites) of two commercially important unionids, the ebony shell (*Fusconaia ebena* [Lea, 1831]) and the monkey face (*Quadrula metanevra* [Rafinesque, 1820]), is proposed to gather baseline data concerning natural parasite burdens which may be of importance in managing both natural and cultured mussel populations.

This proposal has four major objectives:

1. To annually and seasonally document the prevalence and density of metazoan symbionts (both parasitic and non-parasitic) associated with ebony shell and monkey face mussels from Kentucky Lake.

1 location



2. To document the prevalence and density of metazoan parasite species infecting freshwater mussels from populations during "die-off" episodes, and to compare these data to similar data gathered from populations not experiencing "die-offs".

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3. To produce a dichotomous key to the symbiont fauna associated with ebony shell and monkey face mussels.
4. To compile an annotated bibliography of published literature pertaining to the metazoan parasite fauna of freshwater mussels of the family Unionidae.

### **3.0 PROPOSED MATERIALS AND METHODS**

Mussels will be collected by Tennessee Wildlife Resources Agency (TWRA) biologists from two sampling locations in Kentucky Lake (Figure 1). Kentucky Lake has been chosen as the routine study site because the two study species exist in good numbers in the lake, because periodic episodes of mussel population "die-off" have historically occurred in the lake, and because other studies of mussel populations have focused on this lake.

Field collections will take place during the following seasonal periods known to be important to aquatic communities: spring (after water temperature begins to rise), during late summer (when water temperature has reached its highest point), and during mid-winter (when water temperatures have reached lowest values).

Mussel samples from Kentucky Lake (and other bodies of water) will additionally be submitted for examination whenever periods of mussel population "die-off" are noted by biologists. Samples representing periods of "die-off" will not be restricted to the study species (i.e., *Fusconaia ebena* and *Quadrula metanevra*), and the results gathered from the examination of species other than *F. ebena* and *Q. metanevra* may require additional samples to be collected after the "die-off" period has ceased to be best understood.

Each routine seasonal sample of mussels will minimally consist of 25 adult individuals of each study species. Samples associated with "die-offs", likewise, will minimally be composed of 25 adult individuals, however, additional samples may be requested to most accurately detail parasite loads. Individual mussels will be placed in plastic bags and stored on ice. Field biologists will identify each individual mussel to species in a manner transmissible to laboratory personnel. Appropriate field data will be recorded for each sample site. Mussels (stored on ice) will be transported to the Tennessee Aquarium within two days of collection.

At the Tennessee Aquarium the individually bagged mussels will be stored in a refrigerator until they are dissected. Mussel dissections will take place within four days of their arrival at the Aquarium.

*of what size?*  
The following weights (g) will be recorded for each examined mussel during the dissection process: total weight (i.e., shell and soft tissues), soft weight (i.e., total weight of all soft tissues including the retractor and adductor muscles), and shell weight (i.e., weight of shell without retractor and adductor muscles). Muscle shells will be individually marked for later aging by TWRA biologists.

Dissections will generally conform to the following protocol: Water contained in the collection bag will be examined under a dissection scope for living organisms. The outside of

the shell will then be examined under low power for living organisms. Next the shell will be pried open and the mantle water will be drained into a petri dish and examined for living organisms. Next the soft tissues will be examined externally under low power for living organisms. Tissue samples of the following organs will be fixed in bouin's fixative for histological sectioning: mantle, foot, gill, digestive gland/stomach, kidney, gonad. Tissue sections (10µm) will be cut, stained (haematoxylin and eosin or Mallory's trichrome), and permanently mounted using standard histological techniques (Humason, 1972). Prepared tissue sections will be microscopically examined for parasites. Tissues not fixed for histological examination will be dissected and examined under low power for parasites.

All metazoan symbionts (both parasites and non-parasites) will be collected, fixed, preserved, and prepared for specific identification using standard techniques (Pritchard and Kruse, 1982). New parasite species and parasite species in need of redescription will be described in suitable fashion for journal publication. Voucher specimens will be deposited in the U.S. Helminthological Museum (Beltsville, MD), the U.S. National Museum (Washington, DC), the Manter Museum (University of Nebraska, Lincoln, NE), and in an as yet undesignated collection in Tennessee. Annual and seasonal data concerning symbiont prevalence (see Equation 1 below) and density (see Equation 2 below) will be statistically analyzed for differences.

Prevalence is defined as follows:

*No infected*

$$P_{yx} = \frac{N_x}{N_y} \times 100 \quad (\text{Eq. 1})$$

where:

$P_{yx}$  = the prevalence of symbiont species x in host species y,

$N_x$  = the number of hosts found associated with symbiont species x,

and

$N_y$  = the total number of host species y examined for symbiont species x.

Density is defined as follows:

$$D_{yx} = \frac{\sum_1^{N_y} N_x}{N_y} \quad \text{\# / host} \quad (\text{Eq. 2})$$

where:

$D_{yx}$  = the mean density of symbiont species x associated with host species y,

$\sum_1^{N_y} N_x$  = the sum of the total number of symbiont individuals of species x collected from each individual species y host examined,

and

$N_y$  = the total number of host individuals of species y examined.

Any supplemental data accumulated during periods of mussel "die-offs" will be compared to similar data representing non-"die-off" periods of the same mussel species collected from the same waters to ascertain relationship between parasitism and "die-off" phenomena.

A dichotomous key to the metazoan symbionts of ebony shell and monkey face mussels will be prepared. This key will detail all metazoan species known to associate with these mussel species throughout their entire ranges. An annotated bibliography of the published literature concerning metazoan parasites of freshwater unionid mussels (Unionidae) will be prepared from a literature search. The bibliography will contain three indexes: one arranged by mussel species, one arranged by parasite species, and one arranged by geographic location.

#### **4.0 ANNUAL AND FINAL PROJECT REPORTS**

Written Annual Progress Reports will be completed for each of the project's non-terminal years within 30 days of the study calendar year end. The Written Final Report will be completed within 90 days of the calendar year end of the final year of the project. Annual and Final Reports will address all study sections detailed in this proposal.

## 5.0 PROJECT BUDGET

### TOTAL PROJECT BUDGET

Total Year 1 Budget \$24,515  
 Total Year 2 Budget \$23,131  
 Total Year 3 Budget \$20,381  
 Total Year 4 Budget \$20,381  
 Total Year 5 Budget \$20,381

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Total Project Budget \$108,789

### ANNUAL BUDGETS

**Year 1:** Total budget for year \$24,515, broken out as follows:

#### Supplies:

dissection tools (assort.)	\$ 75.00
parafilm (4"x125')	\$ 20.00
glass sample vials (4dr or 3dr, 1cs)	\$ 200.00
Nalgene labels (600ct x 2)	\$ 34.00
micros. slides (1x3; 10 pack)	\$ 209.00
coverslips (22x22; 4oz x 2)	\$ 52.00
fixatives, preservatives, chemicals, reagents, and stains	\$1000.00
photomicrography supplies	\$ 50.00
mailing supplies	\$ 50.00
	<hr/>
	\$1690.00

#### Equipment:

compound microscope (phase)	\$3500.00
microscope slide cabinet	\$ 155.00
books & literature	\$ 200.00
	<hr/>
	\$3855.00

Fees:

xerox costs	\$ 100.00
literature search fees	\$ 100.00
photography fees	\$ 50.00
histology charges	*****
	<hr/>
	\$ 250.00*

\* histology charges to be covered by TWRA.

Labor:

1 biologist @ 3 days per week, for year

$$\frac{52}{3} = 150 \text{ days}$$

$$\frac{50 \text{ weeks/period} \times 3 \text{ periods}}{150 \text{ weeks}}$$

\$18720.00

*\$125/day*

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\$18720.00

**Year 2:** Total budget for year \$23,131.00, broken out as follows:

Supplies:

dissection tools (assort.)	\$ 50.00
parafilm (4"x125')	\$ 20.00
glass sample vials (4dr or 3dr, 1cs)	\$ 200.00
Nalgene labels (600ct x 2)	\$ 34.00
micros. slides (1x3; 5 pack)	\$ 105.00
coverslips (22x22; 4oz x 2)	\$ 52.00
fixatives, preservatives, chemicals, reagents, and stains	\$1000.00
photomicrography supplies	\$ 50.00
mailing supplies	\$ 50.00
	<hr/>
	\$1561.00

Equipment:

lap-top computer	\$2500.00
books & literature	\$ 100.00
	<hr/>
	\$2600.00

Fees:

xerox costs	
literature search fees	\$ 100.00
photography fees	\$ 100.00
histology charges	\$ 50.00
	*****

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\$ 250.00\*

\* histology charges to be covered by TWRA.

Labor:

1 biologist @ 3 days per week, for year	\$18720.00
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	\$18720.00

Years 3, 4, and 5: Total budget for each year \$20,381.00, broken out as follows:

Supplies:

dissection tools (assort.)	\$ 50.00
parafilm (4"x125')	\$ 20.00
glass sample vials (4dr or 3dr, 1cs)	\$ 200.00
Nalgene labels (600ct x 2)	\$ 34.00
micros. slides (1x3; 5 pack)	\$ 105.00
coverslips (22x22; 4oz x 2)	\$ 52.00
fixatives, preservatives, chemicals, reagents, and stains	\$ 750.00
photomicrography supplies	\$ 50.00
mailing supplies	\$ 50.00

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\$1311.00

Equipment:

books & literature	\$ 100.00
	<hr/>
	\$ 100.00



Fees:

xerox costs	
literature search fees	\$ 100.00
photography fees	\$ 100.00
histology charges	\$ 50.00
	*****
	<hr/>
* histology charges to be covered by TWRA.	\$ 250.00*

Labor:

1 biologist @ 3 days per week, for each year	\$18720.00
	<hr/>
	\$18720.00

**6.0 REFERENCES**

- Humason, G. L. 1972. Animal Tissue Techniques, 3rd edition. W. H. Freeman and Co., San Francisco.
- Pritchard, M. H., and G. O. W. Kruse. 1982. The Collection and Preservation of Animal Parasites. University of Nebraska Press, Lincoln and London.



UNITED STATES  
DEPARTMENT OF THE INTERIOR  
FISH AND WILDLIFE SERVICE

Virginia Cooperative Fish and Wildlife Research Unit  
106 Cheatham Hall, Virginia Tech  
Blacksburg, Virginia 24061

cc: Beggs

July 26, 1993

Dave McKinney  
TWRA  
Ellington Agricultural Center  
P. O. Box 40747  
Nashville, TN 37204

Dear Dave:

I received your request for comments on the proposal by George Benz to inventory metazoans in 2 species of mussels. While I certainly understand and appreciate the need for studies of mussel symbionts, I have reservations on the priority, relevance and utility of the proposed work to a wildlife resource agency. The following comments are provided for your consideration.

1. I see no clear justification for this study or obvious management application. The Background section reviews general parasite ecology and clearly states that parasites usually induce morbidity and mortality "in semi-closed and closed captive environments." Then why expect a parasite problem in Kentucky Lake? The incidence of mortality from parasites in natural populations is rare, and there is no evidence of mussel die-offs being linked to parasite loads. The National Shellfish Lab checked for parasites during the die-offs in the early 1980's. Unless TWRA is planning mussel culture, I fail to see the utility of project results. The study is an extreme long shot to provide answers to a management problem (die-offs) not likely related to parasites.
2. Two species from 1 lake; how conclusive will this be for defining "natural parasite burdens" in mussel populations?
3. Of what use or value are the 3 weight measurements to be taken (p. 7)? The author gives no rationale. It seems to me that length is as valuable as weight.
4. How will the results (% infested, number of symbionts per host) be analyzed? Will the analyses compare size/weight classes within species, differences between species, seasons, etc.? There is no discussion given to analysis of data.

Dave McKinney  
Page 2  
July 26, 1993

5. On an annual basis, 50 mussels/season x 3 seasons equals 150 mussels. The budgeted biologist (\$18,720) will be working about 150 days/year. That means that 1 mussel per day will be processed for \$125 (labor only). Are the results worth this cost?

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6. Is 5 years necessary to describe the symbionts of 2 mussel species? This would seem to be a suitable graduate student project (2-3 years).
7. What are the qualifications of Dr. Susie Matthews? I assume she is the biologist in the budget.
8. How will the P.I.'s handle zebra mussels attached to unionids and their associated symbionts? Will these mussels be stressed from the biofoulers and therefore be more susceptible to parasite infestations? Is the question of symbiont loads moot relative to the projected demise of unionids from zebra mussels; i.e., will there be enough ebony shells and monkeyfaces to sample for 5 years?
9. The Proposed Statement (p. 5) says it all in my opinion: "a 5-year survey is proposed to gather baseline data concerning natural parasite burdens which may be of importance in managing both natural and cultured mussel populations." It is a survey of organisms which, in all probability, will not be directly useful to TWRA and to the management of mussel populations. I foresee results consisting of a list of symbionts with perhaps a new species of hydracarinid or other metazoan described. I don't foresee an explanation or remedy to periodic mortality events.
10. It seems to me that a more appropriate sponsor of this type of research is NSF or another basic research agency.

I apologize for seeming to be so negative, but with the impending die-offs of big river mussels from zebra encrustations, a proposal to study symbionts for 5 years and nearly \$109,000 just doesn't get me excited.

Sincerely,



Dick Neves  
Unit Leader

DN/cwl