NATIONAL SCIENCE FOUNDATION

PROPOSAL EVALUATION FORM

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PROPOSAL NO.	INSTITUTION	PLEASE RETURN BY
BSR-9019998	PA St U University Park	09/15/90

PRINCIPAL INVESTIGATOR	NSF PROGRAM
Jay R. Stauffer	ECOLOGY PROGRAM

TITLE: Role of Fish Host Specificity in Reproductive Ecology of Mussels

Please evaluate this proposal using the criteria presented on the back of this review form. Continue on additional sheet(s) as necessary.

This proposal provides a sound although somewhat disjunct approach to its primary goal; namely, to develop techniques for the identification of glochidia and host fishes for a selected group of mussel species. The participants are excellent scientists in their respective disciplines and locations. The research team appears to consist of an ichthyologist, fish biologist, and entomologist at Penn State, and a malacologist (Dr. Davis) at the Academy of Natural Sciences in Philadelphia (subcontractor). Personnel at Penn State, according to Biographical Sketches, have had no experience working with mollusks, and yet such knowledge and background seem imperative to those studies to be conducted at Penn State. Without any track record in this discipline, the likelihood of great success with their proposed studies is suspect. The proposal seems to have been prepared such that each team member's discipline is accommodated, and as a result, some studies are somewhat obtuse to the primary goal of the project.

Some of the studies (I-VIII) are more important than others in their scientific merit and contribution to basic science and the conservation of freshwater mussels. Brief comments on each of the studies are presented below.

I. There is no mention of how ages of mussels will be determined. Previous attempts to correlate fish distributions with those of mussels have been unsuccessful because of the disjunct distribution of mussels and multiple hosts for most species. An evaluation of known hosts for congeneric species would seem more appropriate and fruitful than the proposed scheme.

Identity of reviewers will be kept confidential to maximum extent possible.

OVERALL RATING: ☐ EXCELLENT ☒ VERY GOOD ☐ GOOD ☐ FAIR ☐ POOR

REVIEWER'S SIGNATURE	REVIEWER'S NAME (TYPED)

OTHER SUGGESTED REVIEWERS (OPTIONAL)

Dr. Richard J. Neves
Virginia Polytechnic Institute & State University

PROPOSAL FILE

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II. This is an exciting and worthwhile study that offers the potential for a significant contribution to science.

III. Another worthwhile study that can assist in species identification of glochidia. The utility of results to the overall project, however, is not explained adequately. With the techniques and technology available, why not describe the glochidia (taken from gravid females) of all mussel species in French Creek? Use of those data for identifications of glochidia on freshly collected fishes and museum specimens would be enhanced.

IV. This is a thorough but labor intensive study, fraught with numerous difficulties in accomplishing the proposed objective. For example, no indoor laboratory has been able to sustain healthy adult mussels for more than roughly 3 months. If the investigators intend to do so, they will need to test proposed methods. Similarly, culture of putative host species for F1 progeny will be difficult, particularly cyprinids. The routine manner in which these activities are presented reflects some naivete or inexperience on their part.

V. The immunity studies by Reuling and Arey used primitive techniques and cannot be considered definitive. The proposal does not define how immunity will be determined. There is recent evidence that complete immunity does not occur, even after multiple infestations of glochidia. Because results will not be an all or none situation, how do the authors propose to "observe immunity" or quantify its occurrence? This question is fundamental to the study.

VI. How do the authors propose to identify newly metamorphosed juveniles that have excysted from wild-caught fish? There are 22 mussel species, yet the authors propose only 10 of them for study. Surely the mussel beds will contain other than the 10 species under study. Even if study IV is successful, the authors will likely encounter juveniles from unstudied species that cannot be identified with certainty. The protocol to accomplish this objective is lacking here.

VII & VIII. This study, although of considerable interest to behaviorists, is not directly relevant to the primary goal of the project (identification of glochidia and host fishes). How fish become infested, whether by Lampsilis spp. or any other species, is a separate project in itself, requiring more detail and broader scope than this study can provide. Study VII is only the glamorous tip of a much larger iceberg. As such, time and funds devoted to this effort would be better spent on the more goal-oriented studies.

The budget is well within reason for the amount of priority work to be accomplished. In fact, I feel that the co-P.I.'s have underestimated the time commitment (5%) required of them to assist the 2 Ph.D. students in completing the proposed work.

NATIONAL SCIENCE FOUNDATION 90-19998

FOR CONSIDERATION BY NSF ORGANIZATIONAL UNIT (Indicate the most specific unit known, i.e. program, division, etc)	PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE
Ecology/Division of Biotic Systems & Resources	NSF 83-57 15 June 1990

SUBMITTING INSTITUTION CODE (if known)	FOR RENEWAL ☐ CONTINUING AWARD ☐ ACCOMPLISHMENT BASED RENEWAL ☐ REQUEST, LIST PREVIOUS AWARD NO.:	IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? Yes___ No X ; IF YES, LIST ACRONYM(S)
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The Pennsylvania State University - University Park Campus

ADDRESS OF ORGANIZATION (INCLUDE ZIP CODE)
Sponsored Programs & Contracts Office
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IS SUBMITTING ORGANIZATION: ☐ For-Profit Organization; ☐ Small Business; ☐ Minority Business; ☐ Woman-Owned Business

TITLE OF PROPOSED PROJECT
Role of Fish Host Specificity in Reproductive Ecology of Mussels

REQUESTED AMOUNT	PROPOSED DURATION	DESIRED STARTING DATE
184,231	3 years	1 January 1991

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW:

- ☐ Animal Welfare
- ☐ Endangered Species
- ☐ Human Subjects
- ☐ Marine Mammal Protection
- ☐ Pollution Control
- ☐ National Environmental Policy Act
- ☐ Research Involving Recombinant DNA Molecules
- ☐ Historical Sites
- ☐ Interdisciplinary
- ☐ International Cooperative Activity
- ☐ Research Opportunity Award
- ☐ Facilitation Award for Handicapped
- ☐ Proprietary and Privileged Information

PI/PD DEPARTMENT	PI/PD ORGANIZATION	PI/PD PHONE NO. & ELECTRONIC MAIL
School of Forest Resources	Penn State University	814/863-0645

PI/PD NAME/TITLE	SOCIAL SECURITY NO.*	HIGHEST DEGREE & YEAR	SIGNATURE
J. R. Stauffer	180-42-0644	Ph.D.	Jay R Stauffer
ADDITIONAL PI/PD (TYPED) G. M. Davis		Ph.D.	See subcontract
ADDITIONAL PI/PD (TYPED) B. A. McPheron	286-56-6281	Ph.D.	Bruce A. McPheron
ADDITIONAL PI/PD (TYPED) R. F. Carline	149-32-7895	Ph.D.	Robert F Carline
ADDITIONAL PI/PD (TYPED)			

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TO BE COMPLETED BY THE AUTHORIZED ORGANIZATIONAL REPRESENTATIVE. By signing and submitting this proposal, the prospective grantee is providing the certifications set forth in (1) *Grants for Research and Education in Science and Engineering*, NSF 83-57 (rev. 11/87), and (2) Appendix C, 45CFR 620, Subpart F (Requirements for a Drug-Free Workplace).

(If answering *yes* to either, please provide explanation.)	YES	NO
Is the organization delinquent on any Federal Debt?		X
Is the organization presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency?		X

AUTHORIZED ORGANIZATIONAL REP.	SIGNATURE	DATE	TELEPHONE NO.
NAME/TITLE (TYPED) Kenneth J. Yost, Associate Vice President for Research & Technology Transfer	Kenneth J Yost	6-7-90	814/865-1372
OTHER ENDORSEMENT (optional)			
NAME/TITLE (TYPED) A. D. Sullivan	H D Garbold for A D Sullivan	June 5, 1990	814/865-7541

*Submission of social security numbers is voluntary and will not affect the organization's eligibility for an award. However, they are an integral part of the NSF information system and assist in processing the proposal. SSN solicited under NSF Act of 1950, as amended

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PROJECT SUMMARY

French Creek, a tributary to the upper Allegheny River in Pennsylvania, harbors the most speciose aquatic fauna of any stream system in the state. Extensive surveys throughout the past decade yielded 22 species of freshwater mussels (Bivalvia: Unionidae) and 71 species of fishes (Teleostei). Mussels are long-term breeders, and eggs develop through a veliger stage in the gill lamellae of the female mussels. The veliger, referred to as a glochidium, is an obligate parasite of a fish host. There is currently a significant void in knowledge of mussel/fish host relationships, and in many cases the glochidium attached to a host cannot be identified. It is imperative to know the fish hosts of mussels to understand and appreciate the interactions between fishes and mussels in order to manage these resources effectively. This proposal will generate information on specificity of parasitic mussels to particular fish hosts, the behavioral and ecological relationships that exist between these organisms, and mussel reproductive ecology. In so doing, we will provide age/distribution maps of all the unionid species that occur in French Creek, and we will obtain glochidia from as many of the species as possible in sufficient quantity to enable molecular genetic characterization and scanning electron microscope (SEM) analysis. Simultaneously, we will record the timing of glochidial production and any behavioral attributes of the unionid species to facilitate the infection of the fish host. Ten unionid species have been selected for in-depth study, including artificial inoculation, immunity studies, and metamorphosis of glochidia from wild-caught fishes. Additionally, observations in both artificial streams and *in situ* will be made of behavioral interactions between mussels of the genus *Lampsilis* and their respective hosts. This group of mussels was chosen because its members wave a fish-like mantle flap that may lure the hosts into close proximity of the mussels before the glochidia are discharged.

Several of the mussel species that inhabit French Creek are being considered for placement on both state and federal rare and endangered species lists. There is some speculation that it is possible to reintroduce mussel species to their native ranges where populations are threatened or extirpated. In order for such programs to be successful, it is necessary to determine if the potential fish hosts needed for mussel reproduction are present and to understand the interactions between hosts and mussels.

RESULTS OF PRIOR NSF SUPPORT

J. R. Stauffer was a co-principal investigator of an NSF funded proposal entitled "Mate Choice and the Behavior Significance of Nest Form Among Cichlid Fishes of Lake Malawi, Africa" (BNS86-06836; $176,594; 1987-1990). Stauffer spent four to six months per year in Malawi working on the project. During that time he observed approximately 4,000 eggs being individually laid on the breeding arenas, and he took over 50 hours of underwater videos, which documented the behavior of many Lake Malawi fishes. Within the *Lethrinops lituris* species-group, bower forms of five populations were measured and examined by principal component analysis (McKaye et al., ms). Genetic variation of enzyme loci from breeding males and females of these same populations was analyzed using starch-gel electrophoresis. The phylogenies derived from both the similarity of bower form and genetic distances were congruent. No such congruence would be expected if bower forms were governed by local ecological conditions leading to adaptive functional responses in bower building or by nonheritable variance among populations. Results from this study, therefore, provide preliminary evidence that bower form differences in these cichlid populations are correlated with genetic distances. The operating hypothesis is that such differences probably arose from non-functional differences in female choice.

Publications and manuscripts acknowledging NSF awards, 1986 to present.

Stauffer, J. R., Jr. in press. Description of a facultative cleanerfish (Teleostei: Cichlidae) from Lake Malawi, Africa. *Copeia*. 9 pp, 2 tables, 4 figures.

McKaye, K. R., J. H. Howard, J. R. Stauffer, Jr., R. P. Morgan, F. Feresu. manuscript. Cichlid bower form and genetic distance are correlated: Consequence of sexual selection? 12 pp., 1 table, 1 figure.

McKaye, K. R., S. M. Louda, and J. R. Stauffer, Jr. in press. A field test of the importance of bower size to male reproductive success in a cichlid fish lek. *Am. Nat.*

LoVullo, T. J., K. R. McKaye, J. Likongwe, and J. R. Stauffer, Jr. in press. Use of indigenous fishes in tropical aquaculture: Case study from Malawi. *J. Bunda College of Agri.*

Stauffer, J. R., Jr. and J. M. Boltz. 1989. Description of a new species of Cichlidae, from Lake Malawi, Africa. *Proc. Bio. Soc. Wash.* 102(1): 8-13.

Stauffer, J. r. and K. R. McKaye. 1988. A decription of a new genus and three deep water species of fishes (Teleostei: Cichlidae) from Lake Malawi, Africa. Copeia. 1988(2):441-449.

McKaye, K. R. and J. R. Stauffer, Jr. 1988. Seasonality, depth, and habitat distribution of breeding males of *Oreochromis* spp. 'Chambo' in Lake Malawi National Park. *J. Fish Biol.* 33:825-834.

Stauffer, J. R., Jr. 1988. Descriptions of three rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Copeia*. 1988:663-668.

McKaye, K. R., J. R. Stauffer, Jr. and S. M. Louda. 1986. Fish predation as a factor in the distribution of Lake Malawi gastropods. *J. Exp. Biol*. 45:279-289.

McKaye, K. R. and J. R. Stauffer, Jr. 1986. Description of a gold cichlid (Teleostei: Cichlidae) from Lake Malawi, Africa. *Copeia* 1986 (4):870-875.

Stauffer, J. R., Jr. and K. R. McKaye. 1986. Description of a paedophagous deep-water cichlid (Teleostei: Cichlidae) from Lake Malawi, Africa. *Proc. Biol. Soc. Wash.* 9:29-33.

G. M. Davis was principal investigator of an NSF funded proposal entitled "Systematics of Coastal Hydrobioid Snails of Eastern United States" (BSR-8500279; $130,000; 1985-1988). The proposal was to study hydrobioid snails from coastal marshes from Maine through the Delmarva Peninsula. Species composition, systematics, ecology, and distribution were investigated. Techniques employed included habitat analysis, comparative anatomy study, and investigation of molecular genetics using allozymes. Detailed comparative anatomy studies showed no significant differences, excepting size, among three Long Island populations of *Hydrobia* living in significantly different environments (marsh pools; intertidal mud flats of a harbor; sandy riffles at the lowest tide zone of exposed shore on Long Island Sound). On the basis of conchology and habitat, we would have previously relegated these populations to two species: *Hydrobia totteni* and *H. truncata*. The comparative anatomical evidence, combined with results of molecular genetic analysis, led to the conclusion that a single *Hydrobia* species, not two, was found on Long Island. This species is distributed from Cape Cod through New Jersey; it is called *Hydrobia totteni* until topotypical *H. truncata* (the older named species) from the Delmarva Peninsula can be found and studied.

Publications acknowledging NSF awards

Davis, G. M., V. Forbes, and G. Lopez. 1988. Species status of northeastern American *Hydrobia*: Ecology, morphology, and molecular genetics. *Proc. Acad. Nat. Sci. Phila.* 140(2): 191-246.

Davis, G. M. and M. McKee. 1989. A new species of *Heleobops* (Prosobranchia: Hydrobiidae: Littoridininae) from Maryland. *Proc. Acad. Nat. Sci. Phila.* in press.

Davis, G. M., M. McKee, and G. Lopez. 1989. The identity of *Hydrobia truncata* (Gastropoda: Hydrobiidae): Comparative anatomy, molecular genetics, ecology. *Proc. Acad. Nat. Sci. Phila.* in press.

PROJECT DESCRIPTION

INTRODUCTION

There are more than 1,000 species of freshwater mussels (Bivalvia: Unionacea) worldwide, and 230 species of Unionidae in the 48 contiguous United States (Pennak 1989). Thirteen species are thought to be extinct and approximately 30 are listed as endangered (Turgeon et al. 1988). Mussels become reproductively active between one and eight years of age, depending on the species and environmental conditions. Unionids are typically long-term breeders and eggs fertilized during the summer may be carried until the following spring or summer (Pennak 1989). The eggs develop through the veliger stage in the gill lamellae of the female mussels. The veliger, referred to as a glochidium, is an obligate parasite usually of a fish host, although some species may parasitize amphibians. Upon release, glochidia attach to a host. Glochidia that possess hooks attach to fins and other external body parts of fishes, while those without hooks attach to gills, where they usually remain for 10 to 30 days until metamorphosis is complete. They then drop from the fish and burrow into the substrate (Barnes 1980, Pennak 1989). Reports of attachment to fish hosts for as long as 190 days have been recorded (Pennak 1989). The duration of attachment is probably temperature dependent (Pennak 1989). The attachment of the glochidia is triggered by a response to specific molecules present in fish mucus; therefore, some glochidia species can only parasitize specific fish species (Neves et al. 1985). It is hypothesized that many of the freshwater mussels have a single or restricted number of viable fish-hosts of which only a handful of are known (Fuller 1974, Wiles 1975, Stern and Felder 1978, Trdan and Hoehn 1982, Zale and Neves 1982, Neves et al. 1985, Waller et al 1985, Yeager and Neves 1986, Neves and Wildlak 1988, Waller and Holland-Bartels 1988), which is a major stumbling block in determining mussel/fish interactions. Development of techniques for identifying glochidia from museum specimens would allow identification of fish/mussel associations that no longer exist today, because one or both species have been extirpated from a given drainage basin.

From 1985 through 1987, we conducted studies to determine the biodiversity of the ichthyofauna of Pennsylvania. These studies indicated that French Creek (Allegheny River drainage) in northwestern Pennsylvania harbors 71 species of fishes; this constitutes the most diverse assemblage of fishes in any stream system in the Commonwealth. Twenty species of mussels, some of which are being considered for rare and/or endangered status by both state and federal governments, inhabit French Creek. It is imperative to know the fish hosts of mussels to reestablish populations of endangered mussel species and to manage the existing species effectively. To fill the significant void in knowledge of mussel/fish host relationships, we need to develop techniques for identifying glochidia that have parasitized fishes. We propose to develop both morphological and molecular genetic techniques for glochidial identification. In so doing, we will generate information on: 1) specificity of parasitic mussels to particular fish hosts; 2) the behavioral and ecological relationships that exist between these organisms; and 3) mussel reproductive ecology.

SCOPE OF WORK

We will provide age/distribution maps of all the unionid species that occur in French Creek (Section I) and will attempt to obtain glochidia from as many of the species as possible in sufficient quantity to enable molecular genetic characterization and SEM analysis (Sections II, III). In so doing we will record the timing of glochidial production and any behavioral attributes of the unionid species that facilitate infection of the fish host. We will presumably deal with a majority of the unionid species while investigating metamorphosis of glochidia from wild-caught fishes (Section VI).

It is unrealistic to expect to discover and study the glochidial fish species systems for all 22 unionid species of French Creek. We have selected ten unionid species for in-depth study (see Table 1), including artificial inoculation and immunity studies (Sections IV, V, VI). These ten were chosen because, with one exception (*Lampsilis ovata*), they had not been previously studied relative to glochidium/fish interactions, they represent divergent clades and different ecological settings, possess different posterior mantle flap papillae or fish-like flaps, or are potentially endangered or threatened with extinction. The artificial stream studies and *in situ* observations (Sections VII, VIII) will concentrate on *Lampsilis* species because these mussels wave a fish-like mantle flap (Appendix I), and they discharge glochidia singly or in aggregated clusters (conglutinates).

I. Distribution of mussels and fishes

Historical The distributions of the mussels native to French Creek are shown in Appendix II. A portion of the mussel beds in French Creek consists of multiple age classes, while others consist only of older (i.e. > 30 years old) mussels (pers. obs.).

Methods The age distribution of each of the of mussel beds within French Creek will be determined. An overlay of the distributions of each fish species present in the drainage since 1985 will be superimposed on each mussel age-distribution map. Thus, a first order approximation of congruence between mussel distribution and fish distribution will be established. The results of this analysis will suggest which mussel/fish host interactions should be investigated first.

II. Molecular genetics

Historical The ability to amplify selected regions of DNA exponentially in a short time period via the polymerase chain reaction (PCR) (Saiki 1990) presents a novel approach to the classical problem of glochidial identification. PCR is an extremely versatile process for *in vitro* replication of specific DNA segments. In brief, the technique involves selection of two oligonucleotide primers that flank the DNA of interest. In the presence of nucleotides and a heat stable DNA polymerase, repeated cycles of denaturation of DNA strands, annealing of

primers, and extension of these primers yields complementary copies of the original template DNA. This rapid (< 4 hours) accumulation of DNA sequences circumvents the need to clone and screen genomic DNA from the organism of interest, thus greatly facilitating analysis of many samples. Primer design is a key to the success of PCR. Depending on the choice of primers, PCR can be used to amplify DNA of one particular species or of species over various degrees of phylogenetic separation. Specific primers have been used successfully to screen for the presence of parasite DNA's within vector species. Moser et al. (1989) designed a highly specific primer pair that amplified only DNA of *Trypanosoma cruzi* and used it to screen insect vector samples for presence of this protozoan, which is the causative agent of American trypanosomiasis. In a similar approach, ticks infected with *Borrelia burgdorferi*, the spirochete responsible for Lyme disease, can be separated from uninfected ticks by use of spirochete-specific primers (Persing et al. 1990). In contrast to this specific approach, primers to more conserved regions of DNA may be used to investigate genetic diversity among many taxa, from congeners (Sheppard & McPheron, unpublished data on the honey bee genus, *Apis*) to members of different classes or phyla (Kocher et al. 1989).

A further advantage of PCR over previous molecular techniques is that it can be used to amplify DNA that has been preserved by a variety of methods and/or has been badly degraded. Shibata et al. (1988) successfully amplified DNA from human tissue fixed in formalin and preserved for 40 years in paraffin. Pääbo et al. (1988) examined PCR-amplified mtDNA fragments from a 7000-year old human brain discovered in a Florida spring. Most recently, Golenberg et al. (1990) extended the range of PCR orders of magnitude by successfully amplifying a fragment of the chloroplast *rbc*L gene from a Miocene *Magnolia* leaf dated at 17-20 million years of age. PCR has been successfully applied to dried, frozen, and ethanol-preserved tissues (Thomas et al. 1989). This adaptability means that, in addition to freshly collected fishes and mussels, museum specimens, which are generally fixed in formalin followed by alcohol preservation, are likely to be available for analysis (Arnheim et al. 1990). Such an approach has at least two immediate benefits: it will greatly increase the available samples of protected species, and it will permit the analysis of historical patterns of glochidial associations.

Methods Initial approaches to using PCR to establish fish-mussel relationships will involve screening pairs of primers to establish two parameters. First, this survey, across a variety of mussel species, will identify primer pairs amplifying DNA with sufficient heterogeneity to permit species-level discrimination. Second, we will compare sizes of the DNA segments amplified by individual primer pairs from adult mussel samples and fish tissues with no glochidial infestation (e.g., internal organs). Primers conservative enough to amplify both fish and mussel DNA must bracket variable stretches of DNA in order to be useful in unambiguous identification of glochidia species. These primers can be used only if the product resulting from amplification of fish DNA is different in size than the amplification product of mussel DNA. Such

products could be easily separated on agarose gels for subsequent analysis. This is an important point, because glochidial cysts extracted from fishes are likely to be contaminated with host fish tissue. A second method for identification of glochidial DNA sequences is to develop mussel-specific primers. These primers would amplify genes found in mussels but not fish or would be designed to anneal only to mussel sequences even when fish DNA contaminates the template. One approach to the latter is to identify primer regions where, within the same gene, the 3′ end of the primer sequence differs substantially between mussel and fish. A survey of mussel DNA sequences in various data banks is currently in progress.

All PCR examinations of mussel-fish relationships will involve standard reaction protocols (e.g., Kocher et al. 1989, Saiki 1990) in 50 µl reaction volumes, with specific times and temperatures empirically optimized for each primer and template DNA. Amplified products will be analyzed by dideoxy sequencing of single-stranded template, using either unbalanced amplification techniques (e.g., McCabe 1990) or λ-exonuclease digestion (Higuchi and Ochman 1989) to generate the single-stranded DNA. Sequences will be analyzed to determine the most straightforward method of species discrimination. Options, in addition to direct comparison of sequences to identify single base mutations or insertion/deletion events, include development of species specific probes, identification of species specific restriction sites in the amplified product, or diagnostic species specific size differences among amplified products.

Primers already available for screening include mitochondrial DNA primers to the D-loop (Kocher et al. 1989), a highly variable region of DNA in most organisms, a series of primers originally designed for direct sequencing of the nuclear small subunit ribosomal RNA (Hamby and Zimmer 1988) but optimized for PCR by one of us (B.A.M.), and primers for the internal transcribed spacer (ITS) between the nuclear small and large subunit ribosomal RNA gene (McPheron and Sheppard, unpublished). These regions, particularly the mitochondrial D-loop and the nuclear ITS, are sufficiently variable that differences are likely to exist among even congeneric mussels. In addition, these regions are sites of substantial length variation among taxa, implying that separation of fish and glochidial amplification products should be possible. Some other primer sets will be available if those mentioned above fail to provide diagnostic characters; specifically, a region of the nuclear glucose-6-phosphate dehydrogenase gene (H. Robertson, University of Illinois) and the nuclear large subunit ribosomal RNA (B. Hedges and L. Maxson, Penn State University).

III. Morphology of glochidia

Historical Glochidia have been examined under light microscopes and described in terms of shape (semi-elliptical, subtriangular, head shaped, kidney shaped), hinge line shape and size (short, curved; long; long, slightly curved), ventral margin characters (obliquely rounded, etc.), and presence of spines or hooks (spine at tip of each valve; two

spines, one at each of the ventral covers of the shell; no spine) (Surber 1912, 1915; Lefevre and Curtis 1910; Coker et al. 1921). Most recently Hoggarth has completed a Ph.D. dissertation (Ohio State, 1988, unpublished) on SEM comparisons of 75 species of 30 genera of unionids making clear the value of examining sculptural features and fine structure of the hooks. The wealth of characters seen on the glochidial valves prescribes SEM analysis of glochidia from French Creek species, including recently encysted glochidia to attempt correlating unionid species with fish hosts.

Morphology has always played an important role in the study of the systematics and evolution of organisms. Attempts have been made to describe the shape of organisms both qualitatively and quantitatively. Historically, biological shapes have been delineated by single measurements or a small number of measurements (Oxnard 1978) that have been standardized by the use of ratios (Strauss 1980). The use of ratios is now generally considered invalid (Atchley 1978, Mosimann and James 1979, Humphries et al. 1981, Reyment et al. 1984, Bookstein et al. 1985). Traditionally, when these measurements were subjected to principle component analysis, the first principal component was regarded as a size component, while the additional components were considered to be dependent on the shape of the individual. This technique has also been questioned because it has been recognized that there is an effect of size on components other than the first one. Consequently, Humphries et al. (1981) developed a sheared principal component analysis, which restricts the variation due to size to the first component, so that the subsequent components are strictly shape related. Stauffer and Boltz (1989) and Stauffer and Hocutt (in review) successfully utilized this technique to distinguish between two sympatric species of rock-dwelling fishes in Lake Malawi and scale shapes of four North American fishes, respectively.

Methods Glochidia will be digested from the unionid marsupial demibranch, or from fish tissue dissected free from the fish using a weak solution of commercial Clorox (sodium hypochlorite). Cleaned glochidia will be washed with distilled water and dried prior to mounting on double sided tape for coating. Extremely fragile glochidia will be prepared using techniques of Davis and McKee (1989), where specimens are fixed in 2.5% glutaraldehyde, dehydrated to 100% ethanol, then transferred to 100% amyl acetate and dried in a Denton DCP-1 Critical Point dryer using CO_2. Dried specimens will be mounted on double sided tape. A Cambridge S200 SEM will be used.

Inner and outer surfaces will be examined. Data recorded will include glochidial size, shape, sculpture, and attachment devices (hooks, barbs, etc.). Measurements will involve glochidial length, width at 20%, 40%, 60%, 80% along the length axis; and length of hinge line. Measurements will be digitized from SEM photographs of glochidia at a standard magnification.

The above data will be analyzed using sheared principal component analysis. It is hypothesized that this shape analysis will enable us to

distinguish among the glochidia that parasitize a given fish species collected in French Creek. These analyses can be applied not only to freshly collected specimens, but also to glochidia on artificially inoculated fishes (see next section), and on museum specimens. Furthermore, we will be able to determine spatial/temporal differences in attachment patterns between mussel species that utilize the same host fish.

IV. Artificial propagation of freshwater mussels

Historical Lefevre and Curtis (1912) were the first to attempt artificial propagation of freshwater mussels using fish blood plasma; however, their techniques were not successful. Ellis and Ellis (1926) and Ellis et al. (1930) reported the metamorphosis of parasitic mussel glochidia raised on an artificial nutrient medium; however, they used glochidia that had been encysted for some time on fish hosts, and not glochidia taken directly from gravid female mussels. The methods and composition of the media were not reported. It has only been recently that a thorough investigation of *in vitro* methods has been reported (Isom and Hudson 1982, Hudson and Isom 1984, Isom 1987).

Methods We will artificially inoculate putative fish hosts with glochidia removed directly from a female mussel. To sustain glochidial production, adult mussels must be supplied with a diet that will ensure that they remain reproductively active. The diet of mussels consists of detritus and animal plankters (Fuller 1974). For laboratory maintenance of unionids, we will artificially create aquatic plant disintegration chambers to obtain sufficient detritus to suspend in the well aerated aquaria. This diet will be supplemented by daily feeding with powdered trout food and a commercial invertebrate diet obtained from Hawaiian Marine Imports Inc., Houston, Texas. Studies by Zale and Neves (1982) demonstrated that gravid females provided with this diet yield infective glochidia.

Several artificial streams will be constructed to maintain laboratory cultures of gravid female mussels. In addition to providing glochidia for artificial inoculation of fish hosts, the streams will provide a reservoir of gravid females with glochidia that will be needed for the molecular genetic and morphological studies. The artificial stream will consist of a 2.44 m x 1.22 m x 0.5 m plexiglass tank (Figure 1). The tank, constructed from 1.25-cm thick plexiglass, has slightly rounded corners. A center divide along the long axis of the tank creates unidirectional flow and establishes areas with reduced current near the center of the tank. Current is provided by an electric trolling motor with variable speed settings, which is mounted in a corner with 1.25-cm mesh screening surrounding the propeller. Current speed will be measured with a Marsh-McBirney model 201 meter and adjusted for optimum velocity. A mixture of sand (0.5-1.0 mm) and gravel (4-10 mm) approximately 10 cm deep will be used to allow for the burrowing behavior of the mussels. Lewis and Riebel (1984) concluded that the exact nature of the substrate did not affect the burrowing ability of three freshwater mussels tested, thus the same substrate will

be used throughout the tests. This is consistent with the fact that adult mussels are highly mobile (Negus 1966, Kat 1982) and encounter a variety of substrates during their lifetime.

Platforms scattered throughout the tank will provide cover for fish host species. They are made from 15 cm x 15 cm red "quarry stone" tiles separated by 3-mm pieces of plexiglass glued to one of the tiles. The tiles are elevated 10 cm above the bottom of the tank on a 10-cm diameter PVC pipe. The entire tank rests on a table. A cooling unit with a submersible pump, placed beneath the table, controls the water temperature in the artificial stream.

Initially, young-of-the-year fishes will be collected from French Creek. Prior to exposure, each fish will be examined under a dissecting microscope to determine if there is any pre-existing encystment by glochidia, which may confound the results of our studies or elicit an immune response. Each fish will also be examined for external parasites, because infestation by copepods may produce an immune response to glochidia in fishes (Wilson 1916).

Subsequently, putative host fishes will be cultured in the laboratory and F1 generations will be tested as discussed in later sections. This technique will ensure that previous exposure to glochidia has not occurred. Stream fishes will be cultured in aquaria fitted with a glass partition positioned at a 45° angle. A series of airstones will be placed at the base of the glass to create a circular current (Figure 2). This apparatus has been used successfully in our laboratory to culture several riffle species artificially.

Glochidia are released from the mussel either as conglutinates (clumps of glochidia within a casing) or as clouds of free-swimming individuals. Glochidia become encysted on fish hosts' fins, body, or gill filaments. Theoretically, glochidia in either conglutinate or free-swimming form can come in contact with gill filaments when fish ingest them. Another possible way in which gills may become infected with glochidia involves the propulsion of glochidia onto the gill filaments of the fish that venture near or are lured close to a gravid mussel. For some mussels, fish hosts must be within centimeters of a gravid female mussel if she is to transfer the glochidia to the host successfully. Presumably to facilitate this process, *Lampsilis* spp. wave their mantle flap, which is of similar color and shape to the putative host (Kraemer 1970).

To identify possible fish hosts of the mussel species that inhabit French Creek, two methods will be used to perform artificial inoculations of fishes. Glochidia will be collected for both procedures by separating the valves of a gravid female a few millimeters and inserting a sterile Pasteur pipette into the interlamellar space. The glochidia will be extracted with the pipette, placed in a watch glass, and rinsed with deionized water to remove the mucus covering.

The first method of artificial inoculation will allow free-swimming glochidia to come in contact with a host. The glochidia will be transferred to a 40 l aquarium that is fitted with a piece of glass to create a current in the aquarium (Figure 2). Fishes to be infected will be placed into the aquaria with the suspended glochidia for 10 minutes. Waller et al. (1985) exposed fish to glochidia for 30 seconds while Trdan and Hoeh (1982) determined that 1-5 min was sufficient for glochidia attachment. One hour after exposure, fish will be examined under a dissecting microscope for presence of glochidia. The number and location of attached glochidia will be recorded. Infected fish will be held in separate aquaria at 15°C and examined daily to determine whether or not glochidia encystment occurred. Successful metamorphosis will be indicated by the presence of juvenile mussels on the bottom of the aquaria. The water along the bottom of each aquarium will be siphoned daily and passed through a 130-µm nylon mesh net. The number of detached glochidia and transformed juveniles will be counted. Daily siphoning will continue for five days after the last glochidium or juvenile is found. Transformation of the encysted glochidia to the juvenile stage will suggest an appropriate fish host.

The second method of artificial inoculation will involve direct application of the glochidia to the gill filaments of the putative host. Glochidia will be collected as stated previously. The conglutinates from mussel species that produce them will be teased apart. Glochidia will be tested for activity by stimulating them with a fine probe. Active glochidia will respond by rapidly closing their valves (Zale and Neves 1982). Ten individuals of each fish species to be tested will be anaesthetized with MS-222 and rinsed in clean water to remove any of the remaining chemical. Zale and Neves (1982) reported that exposure to MS-222 did not affect glochidial activity. Several hundred glochidia will then be directly pipetted onto the gill filaments. After being revived, each fish species that was exposed to a particular species of glochidium will be placed in its own 40-l aquarium. One hour after exposure, a subsample of fish will be examined under a dissecting microscope for evidence of glochidial encystment. Thereafter, fish will be examined daily under a dissecting microscope for encystment, and the water along the bottom of each aquarium will be siphoned and passed through a 130-µm nylon mesh net. The contents will be examined under a dissecting microscope and the number of juveniles and unattached glochidia counted. Photographs of encysted glochidia and juveniles will be made using an Olympus camera mounted on a dissecting microscope. Aquaria will be siphoned for five days after the last juvenile is found. A fish species will be considered an appropriate host if the glochidia develop to the juvenile stage. Unsuccessful host species will be tested a second time for verification using previously unexposed fish.

We realized that conclusions drawn from artificial inoculations are somewhat suspect, in that behavorial interactions of fish and mussel species are eliminated. Habitat and behavior differences that might keep some fish and mussel species apart in the field are circumvented in the above studies, as is the role of the female mussel in timing the glochidial release. Moreover, glochidia that successfully parasitize

fish *in situ* may be unsuccessful when introduced artificially. Therefore, the number of potential hosts may be over- or underestimated. These studies are necessary, however, to: 1) verify that the glochidia identified by the morphological and molecular genetic studies described earlier can in fact parasitize the fish species from which they were removed, since glochidia are sometimes "attached" to unsuitable hosts for as long as a week before they are sloughed off by the host (Tedla and Fernando 1969); 2) identify mussel/fish pairs to be tested in the artificial streams (see following section); and 3) identify which mussel/fish interactions should be observed *in situ*.

V. Acquired immunity of host fishes to glochidia

Historical It has been demonstrated that host fishes acquire immunity to glochidia upon repeated exposure to them (Reuling 1919, Arey 1923a, 1923b, 1932). The duration and species-specificity of this immunity is not well established, however. Gaining additional insight into these aspects of the fish-mussel relationship is essential if one is to determine what size host population is necessary to sustain a naturally reproducing mussel population.

Reuling (1919) reported that immunity to glochidia of the Lake Pepin mucket (*Lampsilis luteola*) could be induced in largemouth bass (*Micropterus salmoides*) via two to three successive gill infections of approximately 2,000 glochidia each. *L. luteola* glochidia attached normally to immune fish but dropped off after 24-72 hours. Fish that had acquired immunity to *L. luteola* glochidia were immune to glochidia of *Lampsilis ventricosa* and *Lampsilis ligamenta*, as well. The duration of this immunity was believed to be at least one year, and possibly permanent (Reuling 1919).

Arey (1923a) also showed that immunity to *L. luteola* glochidia could be induced in *M. salmoides*, although four or more infections were sometimes necessary, "with a gradual building up of semi-immunity." It was further demonstrated that *M. salmoides* individuals that were exposed to infections three to four times heavier than those to which other individuals were subjected acquired immunity slightly earlier. The number of successive infections, rather than the intensity of exposure, appeared to be the more important determinant of when immunity was acquired, however.

The discrepancy in the number of successive infections required to produce immunity as reported by Reuling (1919) and Arey (1923a) is most likely due to the fact that both investigators tested wild-caught adult fishes; hence, neither investigator knew the previous infection histories of their subjects. To determine the necessary number of infections with certainty, it is imperative that one know the exact number and approximate intensities of infections to which host fishes have been exposed.

Reuling's (1919) observation of *M. salmoides'* immunity to several *Lampsilis* species led him to conclude that immunity was not species-

specific, but general. Wilson (1916) claimed that host fish simultaneously gain immunity to both glochidia and copepods. He reported an inverse relationship between the number of copepods on a fish's gill and its susceptibility to infection by glochidia and vice versa (Wilson 1916). This relationship was contradicted, however, by Cope (1959), who observed that sticklebacks (*Gasterosteus aculeatus*) with the smallest numbers of glochidia harbored the smallest numbers of copepods. Additional tests are needed to determine the specificity of acquired immunity to glochidia. In particular, it is necessary that fishes that have become immune to one species of glochidia be tested for immunity to as wide a range of additional species from as great a variety of genera as is possible, within the constraints of their natural abilities as hosts.

Methods In a series of investigations, we propose to raise fishes from eggs in the laboratory and expose them to natural levels of glochidial infections to determine whether or not immunity to specific glochidial parasites is acquired by particular fish species. Based on our observations of metamorphosis of glochidia from wild-caught fishes, we will calculate the median rate of glochidial infection per particular fish species. Fishes will be repeatedly exposed to this median (±10%) in a series of trials. After the initial exposure, fish will be held in aquaria until glochidial metamorphosis is complete and will then be re-inoculated. These trials will be repeated until immunity is observed or the fish have been repeatedly exposed for a period of not less than 12 months. If immunity is observed, the same fishes will be inoculated with glochidia from another mussel species that is a verified parasite to determine if immunity is species specific. If a particular fish species acts as a host to several mussel species, the mussel species that is most closely related to the mussel that triggered an immune response will be tested before more distantly related species. These data are needed to determine if the reproductive potentials of selected mussel species are limited by other mussels. It is realized that the following questions must be addressed at some point: What infection schedules are required to produce immunity? How long does the immunity persist? Although these questions are important in addressing the population biology of mussel species, they are beyond the scope of this study.

VI. Observations of metamorphosis of glochidia from wild-caught fishes

Methods Fishes captured in the vicinity of known mussel beds will be transported back to our laboratory. Fishes will be examined for glochidia under a dissecting microscope, and each fish species will be held in a separate aquarium (Figure 2). The water along the bottom of each aquarium will be siphoned weekly and passed through a 130-µm nylon mesh net. The transformed juveniles will be identified. These observations will provide information concerning host-parasite specificity. We realize that not all hosts of all mussel species can be detected in this manner, but it will serve to verify the molecular genetic and morphological data, as well as support data generated from artificial inoculations.

VII. Behavioral observations using artificial streams

Historical Kraemer (1970) reported that *Lampsilis siliquoidea* and *Lampsilis fasciola* wave a mantle flap. Kraemer (1970) did not study the other *Lampsilis* species that inhabit French Creek. *Lampsilis* species discharge glochidia singly or in aggregated clusters (conglutinates).

Methods Initial observations will concentrate on the four *Lampsilis* species that inhabit French Creek. Direct observations of spawning behaviors and the ecological relationships between mussels and fish hosts will be conducted using the artificial streams described previously. Each mussel species will be tested separately. Several fish species, selected based on the results of the artificial propagation experiments, will be introduced into the stream. Initially, each fish species will be tested individually. All fishes introduced into the stream will be screened for external parasites or mussel infestation as described previously, or will be F1 generations of fishes spawned in the laboratory. Temperature and photoperiod in the artificial streams will be regulated to simulate ideal conditions for reproduction of the mussel species being tested. We propose to examine whether these mussels release glochidia randomly or in response to the presence of a potential fish host.

Four experimental regimens will be established. Each of the eight artificial streams will contain ten mussels with ripe glochidia (determined by examination -- see section on artificial inoculation) that have been individually marked and randomly assigned. Two of the streams will have no fish, two will have a viable host fish species, two will have a non-host fish species, and two will contain host fish-conditioned water, but no fish. Fish-conditioned water will be supplied through continuous exchange of water between the artificial stream and a nearby holding tank containing the same number of host fish that are placed in the artificial streams. Behavior (eg. waving a mantle flap) will be observed directly and recorded on a Sony 8mm video camera. A series of 130-µm nets will be positioned in the tank to collect any glochidia that are released by the mussel. Nets will be examined every day for a total of twenty-eight days. Six times during the study period, the nets will be examined every six hours for 24 hours to determine when glochidia are released. The mode of release (i.e. singly or conglutinate) will also be recorded. Periodically, the fish will be examined for any glochidia encystment. In the streams with non-host fish, glochidia may attach temporarily and subsequently be sloughed off by the fish.

During daylight periods, six 30-minute observations will be recorded for each artificial stream using a Sony 8mm video recorder. The time of day will be superimposed continuously on each video tape. A pair of the observations for each artificial stream will be made during the first two hours after the streams are illuminated, a second pair during the middle of the photoperiod, and the final pair during the two hours immediately before the streams are darkened. A series of concentric rings will be established around each marked mussel at 20 cm

intervals to a distance of 60 cm. The concentric rings will be divided by a line perpendicular to the short axis of the mussel. After the observation period, the tapes will be critically reviewed. The times at which individual fish enter or leave each ring segment will be recorded. The position and orientation of each fish relative to the mussel just prior to mantle flap exposure will be determined by rewinding the tape. Any changes in fish orientation to the mussel while the mantle flap is exposed will be monitored. These observations are necessary to determine if the waving of the mantle flap is correlated with the presence of a fish and if the fish are lured closer to the mussel when the mantle is exposed. These observations will also enable us to determine if non-host fishes elicit or respond to flap-waving.

VIII. In situ observations

Observations of several French Creek *Lampsilis* mussel beds will be made to determine whether or not the *in situ* observations are congruent with those from artificial streams. Underwater video cameras will be positioned at a particular mussel bed. The use of a remote system will permit us to observe mussel/fish interactions without the influence of a diver. A series of transects will be established around the mussel beds at 20 cm intervals to a distance of 100 cm. Ten mussels of each species represented in the bed will be randomly selected and marked so that they can be individually identified. When a fish enters or leaves a transect, it will be identified to species and time of entry/exit will be recorded. The times at which marked mussels wave their mantle flaps, and the total number and duration of waving episodes will be recorded. Again, we will determine the position and orientation of fishes just prior to mantle flap exposure by rewinding the tape. These observations are necessary to confirm the conclusions drawn from data collected in the artificial streams.

SIGNIFICANCE

The primary goal of this research will be to develop techniques for the identification of glochidia that have parasitized fish hosts. In so doing, we will generate information on the host specificity of mussels, the behavioral and ecological relationships that exist between these organisms, and on mussel reproductive ecology. All facets of the proposed research are necessary to achieve our primary goal.

The mapping of the distribution of each fish species in the French Creek drainage with an overlay of the age distribution of each mussel species will indicate probable host fish/mussel interactions. We assume that the presence of young mussels demonstrates that the appropriate fish hosts are also present; thus, these data will suggest which mussel/fish host interactions should be investigated first.

Verification of the identity of the glochidia will be made by searching for congruence between the morphological and molecular genetic techniques used for mussel identification. If there is not complete congruence between these techniques, data from the artificial

inoculation tests, observations of metamorphosis of glochidia from wild-caught fishes, observations of interactions in the artificial streams, and *in situ* observations will be used to formulate the most parsimonious hypotheses concerning which fish species are suitable hosts for which mussel species. Data relevant to the partitioning of fish hosts and the temporal/spatial differences in attachment patterns among mussel species will be collected. Finally, information will be gathered concerning host immunity and duration of glochidial attachment. The use of molecular genetic and morphological techniques for the identification of glochidia will provide valuable information with which systematists may refine mussel systematics.

Several of the mussel species that inhabit French Creek are being considered for placement on both state and federal rare and endangered species lists. There is some speculation that it is possible to reintroduce mussel species to their native ranges where populations are threatened or extirpated. For such programs to be successful, it is necessary to determine if the potential fish hosts needed for mussel reproduction are also still present in the system. This research represents the most extensive effort to date to identify the ecological conditions that will make mussel preservation possible.

IMPORTANCE OF EDUCATION AND HUMAN RESOURCES

The integration of molecular techniques and ecological questions is a relatively new association. The breadth of this project will permit students to experience firsthand how different scientists and techniques may work in concert to solve problems. The proposed project will support the research expenses for two doctoral graduate students, plus provide money for publication costs and travel to scientific meetings. Penn State currently conducts an annual Graduate Research Exhibition which is open to all graduate students at the university. The exhibits are judged and sponsored by the Graduate School. The students involved with this project will be encouraged to participate in this exhibition. One of the co-PI's graduate students was awarded second prize in the life-sciences division for the 1990 exhibition.

Undergraduates will be involved in the research program through work-study programs, independent studies, and summer employment (B.A.M. is an honors program advisor for the Department of Entomology). The co-principal investigators teach a variety of courses including: ichthyology, ecology of fishes, systematics and evolution of fishes, insect systematics, and molecular methods. The video tapes developed from the *in situ* observations will be used in these lectures. Undergraduate students will receive hands-on experience working with the artificial streams and the laboratory-held mussels and fishes. Exposure of highly motivated high school students to this research project will be possible through the Pennsylvania Governor's School in Agricultural Sciences, a highly competitive summer program held annually on the University Park campus. The PI's will likely seek supplemental NSF funding for undergraduate research opportunities if this proposal is supported.

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Zale, A. V. and R. J. Neves. 1982. Fish hosts of four species of Lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Can. J. Zool.* 60: 2535-2542.

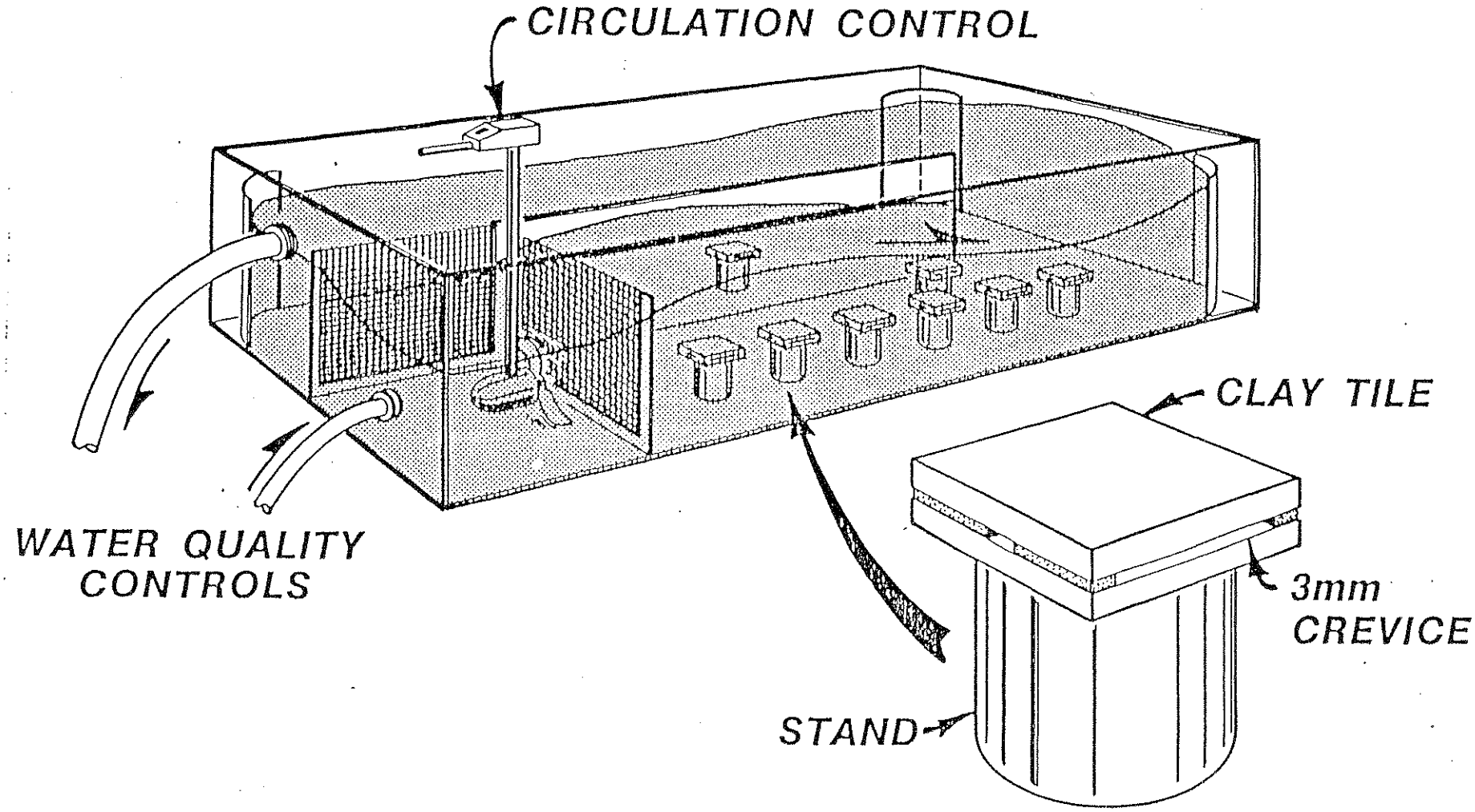

Figure 1. Schematic of artificial stream and fish breeding substrates.

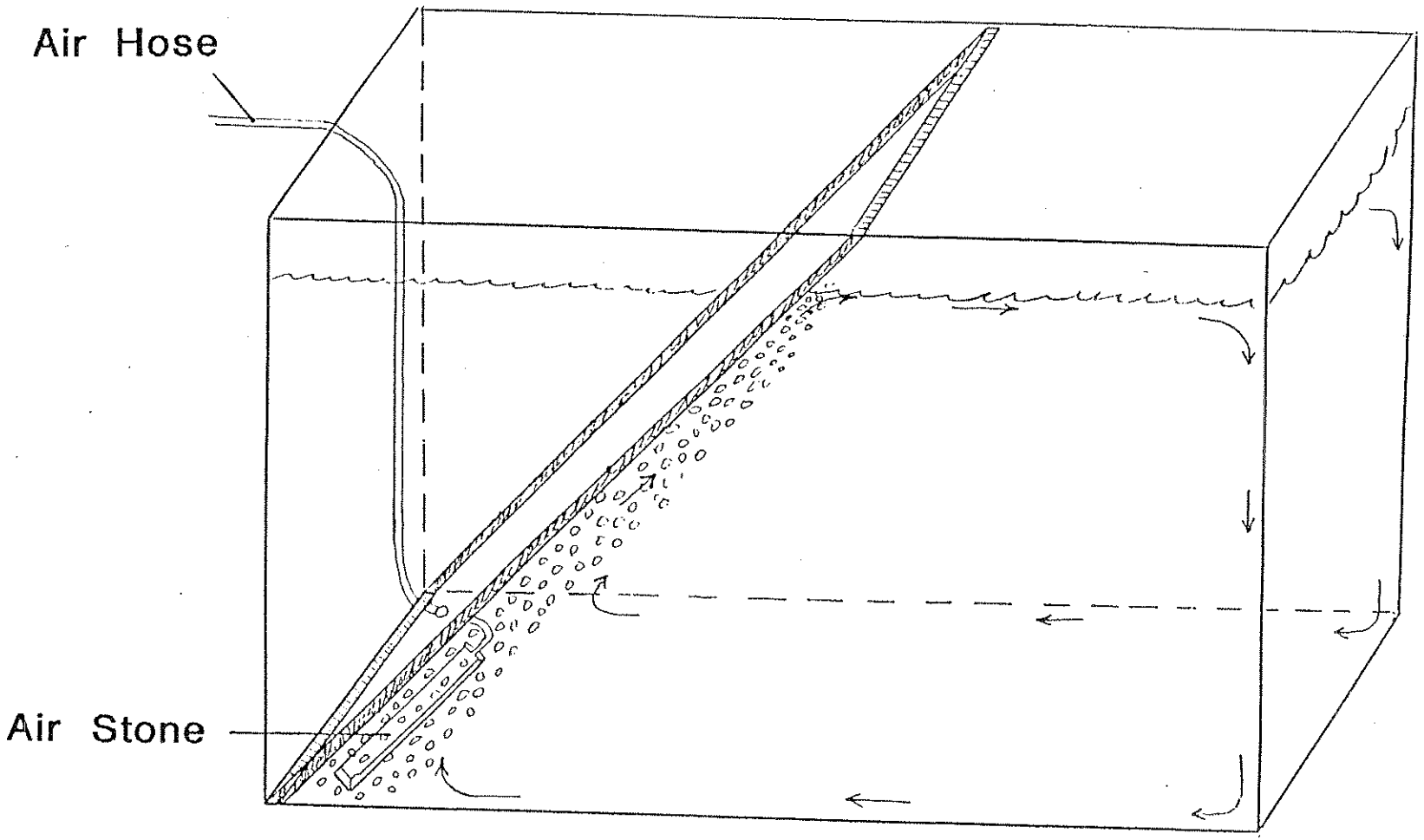

Figure 2. Artificial inoculation aquarium. Glochidia are suspended in the water column by the current created by the air stone.

Table 1. Extant Unionid Mussels of French Creek.

Actinonaias ligamentina	Mucket
Alasmidonta marginata	Elktoe
Amblema plicata	Threeridge
Anodonta grandis	Giant floater
Anodontoides ferussacianus	Cylindrical papershell
Elliptio dilatata	Spike
**Epioblasma torulosa*	Tubercled blossom
**Epioblasma triquetra*	Snuff box
Fusconaia subrotunda	Long-solid
+**Lampsilis cardium*	Plain pocketbook
+**Lampsilis fasciola*	Wavy-rayed lampmussel
+**Lampsilis ovata*	Pocketbook
+**Lampsilis siliquoidea*	Fatmucket
Lasmigona compressa	Creek heelsplitter
Lasmigona costata	Fluted-shell
**Ligumia recta*	Black sandshell
**Pleurobema clava*	Club shell
Pleurobema coccineum	Round pigtoe
Ptychobranchus fasciolaris	Kidney shell
**Quadrula cylindrica*	Rabbitsfoot
Strophitus undulatus	Squawfoot
**Villosa fabalis*	Rayed bean

* To be used in artificial inoculations and immunity studies.
+ To be studied in artificial streams and *in situ*.

Biographical Sketch
of
Jay Richard Stauffer, Jr.

Business Address:

The Pennsylvania State University
School of Forest Resources
8B Ferguson Building
University Park, PA 16802

Education:

Cornell University - B.S., December 1972
Virginia Polytechnic Institute and State University - Ph.D., June, 1975.

Doctoral Dissertation:

The influence of temperature on the distribution, community structure and condition of fish of the New River, Glen Lyn, Virginia.

Positions Held:

Appalachian Environmental Laboratory, Center for Environmental and Estuarine Studies, University of Maryland
Assistant Professor, April 1975 - June 1980
Associate Professor, July 1980 - June 1984

The Pennsylvania State University, School of Forest Resources
Associate Professor Fishery Science, July 1984- June 1988
Professor Fishery Science, July 1988 - present

Awards:

Phi Sigma Award, 1974. For outstanding graduate research in the biological sciences at VPI & SU
Sigma Xi Research Award, 1974. For outstanding graduate students and promoting scholarly achievement.
Certified as Professional Fisheries Biologist by American Fisheries Society.
Member, American Institute of Fishery Biologists.

Students Advised During Last Five Years:

Charles Denoncourt, MSc.
Suzanne Winterbottom, MSc.
Martin Gutowski, MSc.
Sylvia Feldman, MSc.
Jeffrey Boltz, Ph.D.
Richard Raesly, Ph.D.
M. Jon Siemien, Ph.D.
Laura White, Ph.D.
T. J. LoVullo, Ph.D.

Postdoctoral scholars sponsored:

J. M. Boltz

Total Number of Graduate Students Advised Since 1975 - 27

Colleagues:
R. F. Denoncourt
C. H. Hocutt
W. R. Courtenay, Jr.
K. R. McKaye

Thesis Advisor:
K. L. Dickson

Refereed Journal Articles:

Stauffer, J. R., Jr., K. L. Dickson, J. Cairns, Jr., and D. S. Cherry. 1976. The potential and realized influences of temperature on the distribution of fishes on the New River, Glen Lyn, Virginia. Wild. Mon. 50:1-40.

Stauffer, J. R., Jr., C. H. Hocutt, and R. F. Denoncourt. 1979. Status and distribution of the hybrid *Nocomis micropogon* X *Rhinichthys cataractae*, with a discussion of hybridization as a viable mode of speciation. Amer. Midl. Nat. 101(2):355-365.

Stauffer, J. R., Jr. 1981. Temperature behavior of the bluespotted sunfish *Enneacanthus glorisus* (Holbrook) with an evaluation of the interpretation of thermal behavioral data. Water Res. Bull. 17(3):504-507.

Hocutt, C. H., R. F. Denoncourt, and J. R. Stauffer, Jr. 1982. Observations of behavioral responses of fish to environmental stress *in situ*. J. Applied Ecology 19:443-451.

Stauffer, J. R., Jr., C. H. Hocutt, and W. F. Goodfellow. 1985. Effects of sex and maturity on preferred temperatures: a proximate factor for increased survival of young *Poecilia latipinna*. Arch. fur Hydrobio. 103(1):129-132.

Stauffer, J. R., Jr. and K. R. McKaye. 1985. *Cyrtocara macrocleithrum*, a deep-water cichlid (Teleostie: Cichlidae) from Lake Malawi, Africa. Copeia 1985(3):591-596.

Stauffer, J. R., Jr. 1986. Ontogenetic changes in the preferred temperatures of the blackchin tilapia, *Sarotherodon melanotheron*. Archiv. fur Hydrobio. 105(3):397-402.

Stauffer, J. R., Jr. 1986. Effects of salinity on preferred and lethal temperatures of the Mozambique tilapia, *Oreochromis mossambicus* (Peters). Arch. fur Hydrobio. 110(1):163-164.

Raesly, R. L., J. R. Stauffer, Jr. and R. F. Denoncourt. in press. Hybridization between two darters, *Etheostoma zonale* and *Etheostoma olmstedi* (Teleostei: Percidae), following an introduction event. Copeia.

McKaye, K. R., S. M. Louda, and J. R. Stauffer, Jr. in press. An experimental field test of the importance of bower size to male reproductive success in a cichlid fish lek. Amer. Nat.

Biographical Sketch
of
George M. Davis

Personal Information

Birthdate:	21 May 1938; Bridgeport, Connecticut
Martial Status:	Married, two children
Citizenship:	United States of America

Education

Marietta College - B.A., 1960
University of Michigan - M.S., 1962
University of Michigan - Ph.D., 1965

Work History

Curator and Chairman, Department of Malacology,
Academy of Natural Sciences of Philadelphia - 1978 to date

Pilsbry Chair of Malacology - 1989 to date

Honors/Awards

Honorary Docotor of Science, Marietta College, Marietta, Ohio	1989
Fellow, Linnean Society	1979
Fellow, American Association for the Advancement of Science	1976
NIH Traineeship	1964 to 1965
Rackham First-year Fellowship	1960 to 1961

National/International Responsibilities

Current:

Member Public Responsilbilities Committee, A.I.B.S.	1990 to date
Chairman, Building and Finance Committee, ATCC	1990 to date
Immediate Past Chairman, Board of Directors, ATCC	1990 to date
Editor-in-Chief, Malacologia, International Journal of Malacology	1988 to date
Council Member: American Institute of Biological Sciences	1986 to date

Past:

Chairman, Board of Directors, ATCC	1987 to 1990
NIH-NIAID Panel: Special Review Committee; Jan. 4	1990
Chairman, Auditing Committee, Unitas Malacologica	1990
Co-Chairperson: American Society of Zoologists (ASZ) Public Affairs Discussion Group III: Roundtable on the national need for specialists in Systematics (with Carl Gans); Boston; Dec. 30	1989

Students Advised

1989-1990 Ana Marie Dutra, Drexel University. M.S. canditate; Systematics and biogeography of South American Succineidae.

1989-1990 Christian Altabe, University of Pennsylvania. Ph.D. candidate; Systematics and Evolution of **Melanopsis**.

Publications

1986. Anatomy and systematics of Triculini (Prosobranchia: Pomatiopsidae: Triculinae), freshwater snails from Yunnan, China, with descriptions of new species. Proceedings of the Academy of Natural Sciences of Philadelphia 138(2):466-575 (with Y. H. Guo, K. E. Hoagland, P. L. Chen, L. C. Zheng, H. M. Yang, D. J. Chen, and Y. F. Zhou).

1986. In search of Tricula (Gastropoda: Posobranchia): Tricula defined, and a new genus described. Proceedings of the Academy of Natural Sciences of Philadelphia 138(2):426-442 (with N.V.S. Rao and K. E. Hoagland).

1987. The Succineid snail fauna of Chittenango Falls, New York: taxonomic status with comparisons to other related taxa. Proceedings of the Academy of Natural Sciences of Philadelphia 139:465-526 (with K. Elaine Hoagland).

1988. Species status of northeastern American Hydrobia (Gastropoda: Prosobranchia): ecology, morphology and molecular genetics. Proceedings of the Academy of Natural Scisences of Philadelphia 140(2):191-246 (with V. Forbes and G. Lopez).

1988. The Stenothyridae of China. No. 2: Stenothyra humanensis. Proceedings of the Academy of Natural Sciences of Philadelphia 140(2):247-266 (with C. E. Chen, X. G. Xing and C. Wu).

1989. A new species of Heleobops (Prosobranchia: Hydrobilidae) from Maryland. Proceedings of the Academy of Natural Sciences of Philadelphia 141:213-249.

1989. Notes on the anatomy of a small Hubendickia (Gastropoda: Pomatiopsidae: Triculinae) from Yunnan, China. Proceedings of the Academy of Natural Sciences of Philadelphia 141:321-333.

1989. The Identity of Hydrobia truncata (Gastropoda: Hydrobiinae): Comparative Anatomy, Molecular Genetics, Ecology. Proceedings of the Academy of Natural Sciences of Philadelphia 141:333-359. (with . McKee and G. Lopez).

1990. Rectification of the nomenclature of certain species of triculine snails transmitting Paragonimus and Schistosoma in China. American Malacological Bulletin 7(2):131-133 (with Y.Y. Liu).

1990. Comparison of recent classifications of stylommatophoran land-snail families, and evolution of large ribosomal - RNA sequencing for their phylogenetics. Malacologia 31(2):327-352 (with K. Emberton, G. Kuncio, M. Phillips, K. Monderewicz, and Y. Guo).

Biographical Sketch
of
Bruce A. McPheron

Business Address:
The Pennsylvania State University
Entomology Department
003 Pesticide Lab
University Park, PA 16802

Education:
The Ohio State University - B.S., 1976
University of Illionis - M.S., 1980
University of Illinois - Ph.D., 1987

Positions Held:
University of Illinois, Teaching Assistant - 1976-80
Ohio Cooperative Extension Service, County Extension Agent - 1980-83
University of Illinois, Teaching Assistant - 1984-87
Louisiana State University - Postdoctoral Research Associate - 1987-88
The Pennsylvania State University, Intercollege Program in Genetics
Member, Graduate Faculty, Biotechnology Institute-1988-present
Assistant Professor of Entomology - 1988 - present
Member, Institute of Molecular Evolutionary Genetics - 1990 - present

Invited Presentations:
1989 Entomological Society of America national meeting (2 papers)
Society for the Study of Evolution national meeting
1988 Entomological Society of America, Pacific Branch meeting
Penn State University
USDA-ARS, Beltsville, MD
Entomoligical Society of America national meeting
1987 Louisiana State University
Southern Illinois University at Edwardsville
University of Illinois at Urbana-Champaign
1986 Entomological Society of America national meeting

Contributed Papers:
1989 Entomological Society of America national meeting
Entomological Society of America, Eastern Branch meeting
International Symposium on Molecular Insect Science
1988 Entomological Society of America national meeting (2 papers)
1987 Entomoligical Society of America national meeting
Genetics Society of America national meeting
1986 Entomological Society of America national meeting
Genetics Society of America national meeting
1985 Entomological Society of America national meeting

Graduate Student Advising:

Edward Carlini, M.S.
Cathryn Cego, Ph.D.

Professional societies:

American Association for the Advancement of Science
Entomological Society of America
Genetics Society of America
Society for the Study of Evolution
Society of Systematic Zoology

Other Activities:

Organizer, Formal Conference on Genetics and Molecular Biology, Entomological Society of America national meeting, 1990
Co-editor (with K.C. Kim), Evolution of insect pests: The Pattern of Variations, John Wiley & Sons, in preparation.

Colleagues:

S. H. Berlocher
D. P. Pashley
G. L. Bush
J. L. Feder
W. S. Sheppard
D. C. Smith

Publications:

Bouton, C. E., B. A. McPheron, and A. E. Weis. 1980. Parasitoids and competition. Amer. Natur. 116:876-881.

Price, P. W., C. E. Bouton, P. Gross, B. A. McPheron, J. N. Thompson, and A. E. Weis. 19870. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annu. Rev. Ecol. Syst. 11:41-65.

McPheron, B. A. and S. H. Berlocher. 1985. Segregation and linkage of allozymes of *Rhagoletis tabellaria*. J. Hered. 76:218-219.

Sheppard, W. S. and B. A. McPheron. 1986. Genetic variation in honey bees from an area of racial hybridization in western Czechoslovakia. Apidologie 17:21-32.

McPheron, B. A., C. D. Jorgensen, and S. H. Berlocher. 1988. Low genetic variability in a Utah cherry-infesting population of *Rhagoletis pomonella* (Walsh)(Diptera: Tephritidae). Entomol. Exp. Appl. 46:155-160.

McPheron, B. A., D. C. Smith, and S. H. Berlocher. 1988. Microgeographic genetic variation in the apple maggot fly, *Rhagoletis pomonella*. Genetics 119:445-451.

McPheron, B. A., D. C. Smith, and S. H. Berlocher. 1988. Genetic differences between host races of *Rhagoletis pomonella*. Nature 336:64-66.

Bush, G. L., J. L. Feder, S. H. Berlocher, B. A. McPheron, D. C. Smith, and C. A. Chilcote. 1989. Sympatric origins of *R. pomonella*. Nature 339:346.

McPheron, B. A. 1990. Genetic structure of apple maggot fly (Diptera: Tephritidae) populations. Ann. Entomol. Soc. Amer. 83:568-577.

McPheron, B. A. 1990. Implications of genetic variation in western apple maggots for understanding population biology. *In* Apple Maggot in the West: History, Biology, and Control. R. V. Dowell, L. T. Wilson and V. P. Jones (eds). Univ. of California Press, in press.

Biographical Sketch
of
Robert F. Carline

Business Address:

The Pennsylvania State University
School of Forest Resources
7 Ferguson Building
University Park, PA 16802

Education:

Rutgers University - B.A., June 1965
Oregon State University - M.S., December 1967
University of Wisconsin, Madison - Ph.D., May 1975

Positions Held:

Wisconsin Department of Natural Resources
- Research Biologist, 1967-1976

Ohio State University
- Assistant Leader, Cooperative Fishery Research Unit
- Leader, Cooperative Fishery Research Unit
- Assistant Professor, 1976-1984

Pennsylvania State University
- Leader, Cooperative Fish and Wildlife Research Unit
- Associate Professor, 1984-1990
- Professor, 1990-

Students Advised During Last Five Years:

Thomas Beard, MS
Amy Benson, MS
Francis Fiss, MS
Charles Gagen, PhD
Gerrit Jobsis, MS
William Krise, MS
M. Jon Siemien, PhD
Reginald Smith, MS
Mark Stopyro, MS

Total Number of Graduate Students Advised: 24

Number of Postdoctoral Scholars Sponsored: 1

Colleagues:

David DeWalle
James Meade
William Sharpe
Roy Stein

Graduate Advisors:

James Hall
John Magnuson

Selected Publications:

Carline, R.F., and J.D. Hall. 1973. Evaluation of a method for estimating food consumptions rates of fish. J. Fish. Res. Bd. Canada 30:623-629.

Carline, R.F. 1977. Production by three populations of wild brook trout with emphasis on influence of recruitment rates. Fish. Bull. 75(4):751-765.

Kempinger, J.J., and R.F. Carline. 1977. Dynamics of the walleye population and changes in structure of the sport fish community in Escanaba Lake. J. Fish. Res. Bd. Canada 34:1800-1811.

Riley, L.M., and R.F. Carline. 1982. Evaluation of scale shape for the identification of walleye stocks from western Lake Erie. Trans. Am. Fish. Soc. 111:736-741.

Carline, R.F., B.L. Johnson, and T.J. Hall. 1984. Estimation and interpretation of proportional stock density for fish populations in Ohio impoundments. North Amer. J. Fisheries Manage. 4:139-154.

Tomcko, C.M., R.A. Stein, and R.F. Carline. 1984. Use of bluegill forage by tiger muskellunge: Effects of predator experience, vegetation, and prey density. Trans. Am. Fish. Soc. 113:588-594.

Carline, R.F., and M.V. Lawal. 1985. Contaminants and bilateral asymmetry in yellow perch. Environmental Toxicology and Chemistry 4:543-547.

Carline, R.F. 1986. Indices as predictors of fish community traits. Pages 46-56 in G.E. Hall and M.J. Van Den Avyle, editors, Reservoir Fisheries Management: Strategies for the 80's. Reservoir Committee, Southern Division American Fisheries Society.

Carline, R.F., R.A. Stein, and L.M. Riley. 1986. Effects of stocking time, season, largemouth bass predation, and forage abundance on survival of tiger muskellunge. Pages 151-167 in G.E. Hall, editor. Managing muskies. Am. Fish. Soc. Spec. Public 15.

Carline, R.F. 1987. A simplified method based on bioenergetics modeling to estimate food consumption by largemouth bass and northern pike. Trans. Am Fish. Soc. 116:224-231.

Proposed Budget Submitted by The Pennsylvania State University
to the National Science Foundation
January 1, 1991 - December 31, 1993

	Year 1 1/1/91- 12/31/91	Year 2 1/1/92- 12/31/92	Year 3 1/1/93- 12/31/93	Total 1/1/91- 12/31/93
A. Senior Personnel				
Co-Prin. Inv. J. R. Stauffer, 5%, 36 mos.	3,328	3,528	3,740	10,596
Co-Prin. Inv. B. McPheron, 5%, 36 mos.	2,199	2,331	2,471	7,001
Co-Prin. Inv. R. Carline, 5%, 36 mos.	0	0	0	0
Total Category I	5,527	5,859	6,211	17,597
B. Other Personnel				
Total Category II	0	0	0	0
Wages, technician	5,000	5,000	5,000	15,000
Total Category III	5,000	5,000	5,000	15,000
Total Salaries and Wages	**10,527**	**10,859**	**11,211**	**32,597**
C. Fringe Benefits				
Category I @ 30.7%	1,697	1,799	1,907	5,403
Category II @ 11.2%	0	0	0	0
Category III @ 8.3%	415	415	415	1,245
Total Fringe Benefits	**2,112**	**2,214**	**2,322**	**6,648**
Total Salaries, Wages, & Fringe Benefits	12,639	13,073	13,533	39,245
D. Equipment				
1. Pipetmen	1,200	0	0	1,200
2. Sequencing Gel Reader	0	3,000	0	3,000
3. Microcentrifuge	0	0	3,000	3,000
4. Sequencing Rig & Power Supply	3,000	0	0	3,000
5. Artificial Streams	16,000	1,000	1,000	18,000
6. Underwater Video	7,500	1,200	1,200	9,900
Total Equipment	**27,700**	**5,200**	**5,200**	**38,100**
E. Travel				
1. Domestic - Data Collection	1,700	2,000	2,300	6,000
2. Domestic - Meeting with Co-PIs	1,000	1,000	1,000	3,000
3. Domestic - Professional Meetings		1,000	1,000	2,000
4. Foreign	0	0	0	0
Total Travel	**2,700**	**4,000**	**4,300**	**11,000**
F. Participant Support Costs	**0**	**0**	**0**	**0**

	Year 1 1/1/91- 12/31/91	Year 2 1/1/92- 12/31/92	Year 3 1/1/93- 12/31/93	Total 1/1/91- 12/31/93
G. Other Direct Costs				
G.1. Materials & Supplies				
a. Miscellaneous Supplies (enzymes, primers, nucleotides, consumables, radiochemicals, autoradiography supplies, preservatives, nets, jars, etc.)	7,000	5,000	5,000	17,000
Total Materials & Supplies	**7,000**	**5,000**	**5,000**	**17,000**
G.2. Publication Costs	**0**	**500**	**500**	**1,000**
G.3. Consultant Services	**0**	**0**	**0**	**0**
G.4. Computer Costs	**0**	**0**	**0**	**0**
G.5. Subcontracts	**11,744**	**11,744**	**11,744**	**35,232**
G.6. Other				
a. Postage, Telephone	350	350	350	1,050
b. Photocopying	150	150	150	450
Total Other	**500**	**500**	**500**	**1,500**
Total Other Direct Costs	**19,244**	**17,744**	**17,744**	**54,732**
H. Total Direct Costs	**62,283**	**40,017**	**40,777**	**143,077**
I. Indirect Costs @ 45.4%	15,701	15,807	11,507	43,015
1. Less PSU Cost Sharing	780	558	523	1,861
Total Indirect Costs Requested from NSF	**14,921**	**15,249**	**10,984**	**41,154**
Total Project Costs	77,984	55,824	52,284	186,092
PSU Cost Sharing, 1% (from Indirect Costs Category)	780	558	523	1,861
J. Total Amount Requested	**77,204**	**55,266**	**51,761**	**184,231**

Budget Notes:

Dr. Carline is a U.S.D. I. employee. No salary recovery is requested.

In addition to the above personnel, two PhD graduate assistants will work on this project. The associated costs for stipend, fringe benefits, and tuition will be contributed by the College of Agriculture. This amounts to approximately $35,000/year.

Fringe Benefits: Rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency. Rates for the period of July 1, 1989 through June 30, 1990 are fixed at 26.4% applicable to Category I salaries and 8.1% applicable to Category III wages. Rates for July 1, 1990 and forward are proposed at 30.7% applicable to Category I salaries, 11.2% applicable to Category II graduate assistant salaries, and 8.3% applicable to Category III wages and are pending final negotiation.

Indirect Costs: Rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency. Rates for the period of July 1, 1989 through June 30, 1990 are fixed at 44.4% of modified total direct costs (MTDC). Rates for July 1, 1990 and forward are proposed at 45.4% of MTDC and are pending final negotiation.

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING) Year 1 01/01/91-12/31/91

SUMMARY PROPOSAL BUDGET

APPENDIX IV

FOR NSF USE ONLY

ORGANIZATION	PROPOSAL NO.	DURATION (MONTHS) Proposed	Granted
The Pennsylvania State University			
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR	AWARD NO.		
J. R. Stauffer			

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)	NSF FUNDED PERSON MOS. CAL.	ACAD	SUMR	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT)
1. J. R. Stauffer	0.6			$ 3,328	$
2. Bruce McPheron	0.6			2,199	
3. Robert Carline				0	
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. (3) TOTAL SENIOR PERSONNEL (1-5)	1.2			5,527	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. ( ) GRADUATE STUDENTS					
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL-CLERICAL					
6. (1) OTHER				5,000	
TOTAL SALARIES AND WAGES (A+B)				10,527	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				2,112	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				12,639	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000:) 1. Pipetmen - 1,200 2. Sequencing Rig & Power Supply - 3,000 3. Artificial Streams - 16,000 4. Underwater Video - 7,500					
TOTAL PERMANENT EQUIPMENT				27,700	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				2,700	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				7,000	
2. PUBLICATION COSTS/PAGE CHARGES				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER (ADPE) SERVICES				0	
5. SUBCONTRACTS				11,744	
6. OTHER				500	
TOTAL OTHER DIRECT COSTS				19,244	
H. TOTAL DIRECT COSTS (A THROUGH G)				62,283	
I. INDIRECT COSTS (SPECIFY) The difference between total indirect costs and the amount requested represents cost sharing.					
TOTAL INDIRECT COSTS				14,921	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				77,204	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 77,204	$

PI/PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION: Date Checked / Date of Rate Sheet / Initials - DGC / Program
INST. REP. TYPED NAME & SIGNATURE*	DATE	

NSF Form 1030/4-87 Supersedes All Previous Editions

*SIGNATURES REQUIRED ONLY FOR REVISED

(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)

Year 2
01/01/92-12/31/92

SUMMARY PROPOSAL BUDGET

APPENDIX IV

FOR NSF USE ONLY

PROPOSAL NO.	DURATION (MONTHS) Proposed	Granted
AWARD NO.		

ORGANIZATION: The Pennsylvania State University

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: J. R. Stauffer

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)	NSF FUNDED PERSON-MOS. CAL.	ACAD	SUMR	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT
1. J. R. Stauffer	0.6			$ 3,528	$
2. Bruce McPheron	0.6			2,331	
3. Robert Carline				0	
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. (3) TOTAL SENIOR PERSONNEL (1-5)	1.2			5,859	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. ( ) GRADUATE STUDENTS					
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL-CLERICAL					
6. (1) OTHER				5,000	
TOTAL SALARIES AND WAGES (A+B)				10,859	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				2,214	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				13,073	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000:)					
1. Sequencing Gel Reader - 3,000 2. Artificial Streams - 1,000 3. Underwater Video - 1,200					
TOTAL PERMANENT EQUIPMENT				5,200	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				4,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,000	
2. PUBLICATION COSTS/PAGE CHARGES				500	
3. CONSULTANT SERVICES				0	
4. COMPUTER (ADPE) SERVICES				0	
5. SUBCONTRACTS				11,744	
6. OTHER				500	
TOTAL OTHER DIRECT COSTS				17,744	
H. TOTAL DIRECT COSTS (A THROUGH G)				40,017	
I. INDIRECT COSTS (SPECIFY) The difference between total indirect costs and the amount requested represents cost sharing.					
TOTAL INDIRECT COSTS				15,249	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				55,266	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 55,266	$

PI/PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY
		INDIRECT COST RATE VERIFICATION
INST. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked / Date of Rate Sheet / Initials - DGC / Progra

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(SEE INSTRUCTIONS ON REVERSE BEFORE COMPLETING)

Year 3
01/01/93-12/31/93

SUMMARY **PROPOSAL BUDGET**

APPENDIX IV

FOR NSF USE ONLY

PROPOSAL NO. | DURATION (MONTHS) Proposed | Granted

AWARD NO.

ORGANIZATION: The Pennsylvania State University

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: J. R. Stauffer

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)	NSF FUNDED PERSON-MOS. CAL.	ACAD	SUMR	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT)
1. J. R. Stauffer	0.6			$ 3,740	$
2. Bruce McPheron	0.6			2,471	
3. Robert Carline					
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. (3) TOTAL SENIOR PERSONNEL (1-5)	1.2			6,211	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. ( ) GRADUATE STUDENTS					
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL-CLERICAL					
6. (1) OTHER				5,000	
TOTAL SALARIES AND WAGES (A+B)				11,211	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				2,322	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				13,533	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000:)					
1. Microcentrifuge - 3,000					
2. Artificial Streams - 1,000					
3. Underwater Video - 1,200					
TOTAL PERMANENT EQUIPMENT				5,200	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				4,300	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,000	
2. PUBLICATION COSTS/PAGE CHARGES				500	
3. CONSULTANT SERVICES				0	
4. COMPUTER (ADPE) SERVICES				0	
5. SUBCONTRACTS				11,744	
6. OTHER				500	
TOTAL OTHER DIRECT COSTS				17,744	
H. TOTAL DIRECT COSTS (A THROUGH G)				40,777	
I. INDIRECT COSTS (SPECIFY) The difference between total indirect costs and the amount requested represents cost sharing.					
TOTAL INDIRECT COSTS				10,984	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				51,761	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 51,761	$

PI/PD TYPED NAME & SIGNATURE* | DATE

INST. REP. TYPED NAME & SIGNATURE* | DATE

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INDIRECT COST RATE VERIFICATION

Date Checked | Date of Rate Sheet | Initials - DGC / Progra

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Summary
01/01/91-12/31/93

SUMMARY PROPOSAL BUDGET

APPENDIX IV

FOR NSF USE ONLY

PROPOSAL NO.	DURATION (MONTHS) Proposed	Granted
AWARD NO.		

ORGANIZATION: The Pennsylvania State University

PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR: J. R. Stauffer

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title; A.6. show number in brackets)	NSF FUNDED PERSON-MOS. CAL.	ACAD	SUMR	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT)
1. J. R. Stauffer	1.8			$ 10,596	$
2. Bruce McPheron	1.8			7,001	
3. Robert Carline					
4.					
5. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET EXPLANATION PAGE)					
6. (3) TOTAL SENIOR PERSONNEL (1-5)	3.6			17,597	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. ( ) POST DOCTORAL ASSOCIATES					
2. ( ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)					
3. ( ) GRADUATE STUDENTS					
4. ( ) UNDERGRADUATE STUDENTS					
5. ( ) SECRETARIAL-CLERICAL					
6. (1) OTHER				15,000	
TOTAL SALARIES AND WAGES (A+B)				32,597	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				6,648	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				39,245	
D. PERMANENT EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $1,000.)					
1. Pipetmen - 1,200 2. Sequencing Gel Reader - 3,000 3. Microcentrifuge - 3,000 4. Sequencing Rig & Power Supply - 3,000 5. Artificial Streams - 18,000 6. Underwater Video - 9,900					
TOTAL PERMANENT EQUIPMENT				38,100	
E. TRAVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				11,000	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $					
2. TRAVEL					
3. SUBSISTENCE					
4. OTHER					
TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				17,000	
2. PUBLICATION COSTS/PAGE CHARGES				1,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER (ADPE) SERVICES				0	
5. SUBCONTRACTS				35,232	
6. OTHER				1,500	
TOTAL OTHER DIRECT COSTS				54,732	
H. TOTAL DIRECT COSTS (A THROUGH G)				143,077	
I. INDIRECT COSTS (SPECIFY) The difference between total indirect costs and the amount requested represents cost sharing.					
TOTAL INDIRECT COSTS				41,154	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				184,231	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPM 252 AND 253)					
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$184,231	$

PI/PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY
INST. REP. TYPED NAME & SIGNATURE*	DATE	INDIRECT COST RATE VERIFICATION: Date Checked / Date of Rate Sheet / Initials - DGC / Progra

*SIGNATURES REQUIRED ONLY FOR REVISED

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BUDGET JUSTIFICATION

A-C. Salaries and Fringe Benefits

Five percent salary for each year is requested for Jay R. Stauffer, Jr. and Bruce A. McPheron to conduct this research. No salary support is requested for George Davis and R. Carline. Two Ph.D. students will be pursuing their dissertation research on facets of this study and will be supported by Penn State. The sum of $5000/year is requested for wage/payroll monies to hire assistants to aid in the collection of specimens and assist in the laboratory studies.

D. Equipment

1. Four underwater videos. We will require four Sony 8mm video recorders contained in an Amphibico underwater housing to record the *in situ* observations and the observations made in the artificial streams. We have used this system previously to record fish behavior in Lake Malawi, Africa and darter interactions in streams throughout Pennsylvania. Replacement of the internal camcorders is requested in years two and three.

2. Eight artificial streams. We will need to construct eight artificial streams for a portion of this study. The streams will be constructed from plexiglass (Figure 1) and the current maintained by an electric trolling motor. Temperature in the stream will be controlled by a refrigeration unit located beneath the stream. We currently have one such system constructed at Penn State in which we can investigate interactions between riffle-dwelling fishes. Replacement of chillers is requested in years two and three.

3. Equipment money is requested to purchase an additional sequencing rig and power supply in year one to relieve pressure on existing units in McPheron's laboratory. Also to be purchased in year one will be two sets of Pipetmen to be dedicated to extraction and PCR in this project. Equipment money in year two will be used for a sequencing gel reader and software. Equipment money in year three will be used to purchase a new microcentrifuge to compensate for normal use of existing equipment.

E. Travel

Funds are requested to support travel between Penn State and French Creek and to one scientific meeting per year for each investigator.

G. Other direct costs

1. Materials and Supplies. Funds are requested for each year of this proposal to purchase the following:

a. nets, formalin, alcohol, collecting gear, video tapes;
b. aquarium supplies to maintain live fish and mussels;

c. normal consumable items and supplies for PCR and sequencing. A major expense in the first year will be development of primers, which will be synthesized at the Penn State University Biotechnology Institute on their Milligen 7500 oligonucleotide synthesizer, at a substantial savings over commercial rates.
d. copying and telephone charges

2. Publication costs. We anticipate that an average of three papers a year by the co-PI's and the graduate assistants will be published as a direct result of this project. Including the cost of reprints, the amount requested is modest.

CURRENT AND PENDING SUPPORT FOR RESEARCH

J. R. Stauffer, Jr.

Source and Project Title	Amount Awarded	Months Committed per year	Duration	Loc.
Identification of Macroinvertebrate Samples National Park Service	37,282	0.5 mon	10/88-6/94	PA
Factors Influencing Biotic Diversity of Streams in the Upper Allegheny River Systems Wild Resources Cons. Bd.	140,666	1 mon	1/90-12/92	PA
Prediction of Resistance and Resilience in Stream Ecosystems National Park Service	61,032	0.8 mon	5/90-4/93	PA
Bower - building: New Characters for Analysis of Relationships Among Lake Malawi Cichlids NSF	507,539	1 mon	pending	MALAWI
Reconstruction of Biotic Communities: Historical Analysis of Changes in Stream Invertebrates EPA	197,575	1 mon	pending	PA
Extent and Pattern of Genetic Variation Between Color Morphs Within the Species <u>Melanochromis auratus</u> (Pisces: Cichlidae) NSF	26,600	--	pending	MALAWI
Taxonomic Revision of the <u>Pseudotorpheus zebra</u> (Teleostei: Chichlidae) of Lake Malawi National Geographic	14,600	0.6 mo	pending	MALAWI

Identification of Commercially Important Fishes of Lake Malawi USAID	48,235	0.6 mo	pending	MALAWI
Role of Fish Host Specificity in Reproductive Ecology of Mussels NSF	184,231	0.6 mo	pending	PA

B. A. McPheron

Source and Project Title	Amount Awarded	Months Committed per year	Duration	Loc.
Honeybee breeding for Tracheal Mite Resistance in the Northeast U. S. USDA	40,000	0.6 mon	6/90-5/91	PA
Vector Potential of Ticks in Lyme Disease Epidemiology PSU Biomedical Res. Grant	8,000	--	4/90-3/91	PA
The Implementation of Rational Management Management Strategies to Reduce Pesticide Use for Tufted Apple Bud Moth: Pheromone-mediated Mating Disruption, Ground Cover Management and Molecular Genetic Approaches to Resistance Management. PA Dept. of Agric.	98,532	0.6 mon	1/89-12/90	PA
Development of an Integrated Approach to Tufted Apple Bud Moth Management: Understanding Pest/Predator Ground Cover Dynamics, Mating Disruption, and Resistance Development. PA Dept. of Agric.	64,913	0.3 mon	7/89-6/91	PA

Role of Fish Host Specificity in Reproductive Ecology of Mussels	184,231	0.6 mo	pending	PA

G. M. Davis

Source and Project Title	Amount Awarded	Months Committed per year	Duration	Loc.
Creation of Computerized Baseline Data for Number, Distribution, and Rarity of Freshwater Unionidae of Pennsylvania PA Wild Res. Cons. Fund	56,409	--	1/90-12/92	PA
Analysis of Molluscan Faunas and Asian Schistosomiasis NIH	284,067	--	1/90-12/93	
Support for the Curation, Improvement, and Service Function of a Major Collection of Recent Mollusks NSF	417,727	--	1/90-12/93	PA
Role of Fish Host Specificity in Reproductive Ecology of Mussels	184,231	0.6 mo	pending	PA

R. F. Carline

Source and Project Title	Amount Awarded	Months Committed per year	Duration	Loc.
Responses of aquatic fauna to episodic pH depressions US EPA	450,000	3 mon	7/88-12/90	PA

Response of brown trout to altered thermal regions. PA Fish Commission	75,000	2 mon	7/90-6/93	PA
Migration of Atlantic salmon smolts - US Forest Service	45,000	3 mon	7/88-12/90	PA
Role of Fish Host Specificity in Reproductive Ecology of Mussels	184,231	0.6 mo	pending	PA