

Zebra-Mussel-Specific Containment Protocols

ASSP - 12/29/93

This document has been approved (12/29/93) by the Aquatic Nuisance Species Task Force, Research Protocol Committee, as an Approved Species-Specific Protocol (ASSP) for zebra mussels. Use of the procedures and protocols contained herein will minimize the requirements for Protocol Committee review of zebra mussel research proposals. However, use of these protocols is voluntary.

Researchers desiring to submit their own protocols, or desiring to modify the protocols contained herein, will have to submit the new or modified procedures to the Research Protocol Committee for review and approval, which may add significant delay to the proposal process. The funding agency can provide you with information about how to do this.

This document was developed to meet the requirements of the ANS Task Force Research Protocol Committee by the following individuals under grants from both the NOAA National Sea Grant Program and the U.S. Environmental Protection Agency:

Dr. David F. Reid
NOAA
Great Lakes Environmental Research Laboratory
2205 Commonwealth Boulevard
Ann Arbor, MI 48105

Mr. Joseph Bidwell
Department of Biology
Virginia Polytechnical Institute
and State University
Blacksburg, VA 24061

Dr. James Carlton
Dr. Ladd Johnson
Maritime Studies Program
Williams College
Mystic Seaport, CT 06355

Dr. Ellen Marsden
Illinois Natural History Survey
Lake Michigan Biological Station
Box 634
Zion, IL 60099

Ms. S. Jerine Nichols
National Biological Survey
Great Lakes Center
1451 Green Road
Ann Arbor, MI 48105

Communications about this document should be sent to David Reid.

GLERL Contributin #890

Zebra-Mussel-Specific Containment Protocols

ASSP - 12/29/93

Foreword.....		i
Documentation Checklist.....		iii
Part I:	Background and Rationale for Requiring Containment Protocols.....	I-1
Part II:	The General Evaluation Protocol (GEP).....	II-1
	II-A. Statement of Institutional Responsibility	II-3
	II-B. Statement of Principal Investigator Responsibility.....	II-5
	II-C. GEP Risk Assessment for Zebra Mussel Research Proposals.....	II-7
Part III:	Containment Protocols.....	III-1
	III-A. Training Protocol.....	III-3
	III-B. Field Equipment Decontamination Protocol.....	III-5
	III-C. Disinfectant Procedures and Solutions Protocol.....	III-7
	III-D. Protocol for Treatment of Accidental Discharges.....	III-9
	III-E. Transportation and Shipping Protocol.....	III-11
	III-F. Facility Containment Protocol	III-13
	III-G. Research Termination Protocol.....	III-19
Part IV:	Additional Considerations - All Proposals.....	IV-1
Appendix A:	<u>Protocol for Evaluating Research Proposals Concerning Nonindigenous Aquatic Species</u> , ANS Task Force, Research Protocol Committee, 1993	
Appendix B:	<u>Life History and Ecological Requirements of the Zebra Mussel - North American Experience Through 1992</u> , by S. J. Nichols.	

Foreword

This document was prepared to assist researchers proposing to conduct research on the zebra mussel in meeting the requirements of the ANS Task Force, Research Protocol Committee's "Protocol for Evaluating Research Proposals Concerning Nonindigenous Species." For the purposes of this document, the ANS Task Force protocol will hereafter be referred to as the General Evaluation Protocol, or GEP. Appendix A is the September 1993 revised version of the GEP. This is provided here for information and reference only, and may be subject to future changes.

When reviewing the present document (*Zebra-Mussel-Specific Containment Protocols*), the researcher should bear in mind that some sections are for information only and require no action. Other sections require specific actions and the addition of information. Specifically, the Research Protocol Committee requires written documentation of the following:

1. Evidence that the principal researchers are knowledgeable about the biology and life history of the target organism. While this document does not specifically address this requirement, Appendix B is provided for information and as a starting point for those who may not be fully versed relative to the zebra mussel. The references at the end of Appendix B will provide substantial additional information. We suggest that all proposals include a short cv for each principal researcher, and that each cv include a statement of expertise to meet the Committee's requirements.
2. A **SIGNED** statement that the institution(s) where the research will be conducted have reviewed and approved the proposal, the risk assessment, and the proposed preventative measures, and accept(s) responsibility for assuring that the research is conducted as described, and that the preventative measures are carried out. Part IIA, page II-3, is an example statement, and this or a similar document must be included with the proposal.
3. A similar **SIGNED** statement is required from each principal investigator on the project. See Part IIB, page II-5 for an example. This or a similar document must be included with the proposal.
4. Each research proposal will have to have a completed written risk assessment. The risk assessment is a major part of the General Evaluation Protocol and contains twelve questions that must be answered. Section IIC (pages II-7 - II-11) works through the risk assessment for the general case of the zebra mussel; some suggested answers, or at least discussion of considerations, are provided where applicable, but some questions are specific to the research or the facility and must be answered by the researcher.
5. Depending on the outcome of the risk assessment, research proposals may require either a subset or the full set of containment protocols contained in Section III of this document. There are seven individual protocols in Section III. **The Facility Containment Protocol (III-F) requires specific facility descriptions and location information from the researcher as part of the proposal.** In addition, some of the protocols offer more than one procedure or choice for a particular action. The researcher must provide the necessary information and identify which procedures or choices will be used during the conduct of the proposed research. This information

may be provided on separate sheets of paper. If you wish to propose your own, or in any way modify the approved zebra-mussel-specific protocols contained in this document, your proposed protocols or modifications will have to be reviewed and approved by the research protocol committee.

6. The research protocol committee also requires that the principal investigator(s) must provide evidence, such as a copy of a letter or other written communication, that the appropriate agencies of each state in which live zebra mussels will be used during the conduct of the proposed research have been notified about the research and the use of live zebra mussels. Part IV of this document discusses additional information and provides a starting place for investigators to determine if state or local jurisdictions have their own regulations concerning possession and transport of zebra mussels. Several states do have restrictions on possession and transport of zebra mussels. **However, researchers are warned that the information contained herein may be out of date, so they should contact their state and local officials for updated information.**

The checklist on the next page is meant to be removed or copied, completed, and attached to the protocol section of your research proposal. If you complete and/or attach everything listed, your protocol documentation should meet all the requirements of the Research Protocol Committee.

Questions, clarification, or correction of the material contained herein may be referred to:

Dr. David Reid
(address on front cover)
313-741-2019

CONTAINMENT PROTOCOL DOCUMENTATION CHECKLIST

(COMPLETE THIS SHEET IN FULL AND ATTACH IT TO THE FRONT OF THE PROTOCOL SECTION OF YOUR PROPOSAL)

Principal Investigator(s): _____

Proposal Identification: _____

THE FOLLOWING IS PROVIDED TO ASSIST YOU IN ASSEMBLING THE REQUIRED DOCUMENTATION FOR THE CONTAINMENT PROTOCOL PACKAGE :

- _____ 1. Provide evidence of knowledge & expertise about biology, life history, environmental requirements of the target organism? See Foreword, Page i.
- _____ 2. Attach a signed statement from Research Institution(s). See Section II-A, Page II-2.
- _____ 3. Attach a signed statement from PI(s). See Section II-B, Page II-3
- _____ 4. Attach the completed risk assessment. See Section II-C, Page II-4.
- _____ 5. As a result of the risk assessment, are Preventive Measures/Containment Protocol(s) needed?
 Yes No
- _____ 6. If answer to "5" is YES, check required protocols and attach a copy of each.
 Training Protocol (See III-A, Page III-3)
 Field Equipment Decontamination Protocol (See III-B, Page III-5).
 Disinfectant Procedures and Solutions Protocol (See III-C, Page III-7).
 Protocol for Treatment of Accidental Discharges (See III-D, Page III-9).
 Transportation and Shipping Protocol (See III-E, Page III-11).
 Facility Containment Protocol (See III-F, Page III-13).
 Research Termination Protocol (See III-G, Page III-19).
- _____ 7. For each of the attached protocols, indicate the specific options you will use when more than one approach or procedure is listed (can be provided on a separate sheet or directly in the protocols themselves).
- _____ 8. Include, as required in the Facilities Containment Protocol (Section III-F, Page III-13), a complete description of all facilities that will receive unpreserved samples outside range of organism, including geographic relationship and distance to surrounding surface water as well as nearest established population of target organism (can be provided on a separate sheet or directly in the protocol itself).
- _____ 9. Attach a copy of document(s) advising state authorities that research involving live or unpreserved samples of zebra mussels will be conducted in their jurisdiction. (Note: this is required within 30 days after receipt by the PI(s) of notification from the funding agency that the proposed research will be funded; however, it is recommended that such notification be sent when the proposal is first submitted, and a copy be attached with the submitted proposal).

This page intentionally left blank.

Part I. Background and Rationale for Requiring Containment Protocols

Background: The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Public Law 101-646, 104 STAT. 4671, 16 U.S.C. 4701-4741 approved November 29, 1990, hereafter referred to as "the Act") requires that an intergovernmental Aquatic Nuisance Species (ANS) Task Force, established under the Act, develop and implement a research protocol to ensure that research carried out under Subtitle C of the Act does not result in the unintentional introduction or dispersal of aquatic nuisance species to the navigable waters of the United States.

Rationale: The rationale behind the requirement for establishing containment protocols for research activities on nonindigenous aquatic species is based on 1) the probability of disruptive or costly economic or ecological impact by the organism (i.e., it is an aquatic nuisance) combined with 2) previous experience that research activities have been responsible, in some cases solely responsible, for the introduction of nonindigenous species into the waters of the United States (Table 1).

An aquatic organism is only declared a nuisance if, according to the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, it threatens "the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities dependent on such waters". The zebra mussel, for example, is adding millions of dollars per year to the maintenance costs of recreational boat owners, Great Lakes shippers, shoreline industries, and municipalities, and is considerably impacting the ecological dynamics and biological make-up of the lower food web in some areas of the Great Lakes. It is clear that human activity of various types has been primarily responsible for the initial introduction and most of the spread of nonindigenous aquatic species through confluent navigable waters in North America.

While the risk of release through scientific endeavors may be considered small relative to other potential spreading vectors (such as recreational boating and commercial shipping, the aquarium trade, private individuals), scientific possession and transportation of mussels is much more readily documented and thus easier to blame. Moreover, in contrast to most other vectors, researchers usually make serious attempts to keep their samples alive and well during their possession. Consequently, the potential for research activities to transport and release nonindigenous aquatic nuisance species, such as the zebra mussel, into new habitats is very real, especially over long distances (e.g., across the continental divide) and between unconnected waterways. It is imperative, both in perception and actuality, that the scientific community become part of the solution and not a continued contributor to species invasions.

Table 1: Examples of aquatic organisms released through research activities

Taxa	Location of Release (Date)	Origin	Remarks
------	-------------------------------	--------	---------

Chordata

California Seasquirt <i>Botrylloides diegensis</i>	Woods Hole, Massachusetts (1972-73)	California	Well established; now occurs from Maine to Connecticut
---	--	------------	--

Circumstances: A research biologist from California, seeking to maintain his animals between research summers, placed glass slides containing this ascidian into Eel Pond at Woods Hole at the end of each of two summers. It is now one of the most abundant and prolific fouling organisms of southern New England and Long Island Sound [Carlton, 1989; JTC, unpublished data].

Event Summary: Intentional release without anticipating introduction.

Platyhelminthes

Triclad Flatworm <i>Phagocata woodworthi</i>	Loch Ness, Scotland (1977)	Northeastern North America	Established
---	-------------------------------	----------------------------	-------------

Circumstances: In transporting underwater observation and surveying equipment from North America to Loch Ness, researchers simultaneously transported the cocoons of this freshwater turbellarian [Reynoldson *et al*, 1981].

Event Summary: Accidental transportation on research equipment.

Crustacea

American Lobster <i>Homarus americanus</i>	Bodega Bay, California (1970s)	Atlantic	Not established
---	-----------------------------------	----------	-----------------

Circumstances: In the course of extensive research on the American lobster, juvenile lobsters were occasionally found in open tidepools at the base of the University of California Bodega Marine Laboratory, having escaped from holding facilities. The laboratory's seawater system empties into the cove adjacent to these tidepools [Carlton, 1992; JTC, unpublished data].

Event Summary: Escape from a research facility through drain pipes.

Mollusca

Giant California Sea Hare <i>Aplysia californica</i>	Woods Hole, Massachusetts (early 1980s)	Southern California	Not established
---	--	---------------------	-----------------

Circumstances: For several research seasons, sea hares were raised and used experimentally at the Marine Biological Laboratory in Woods Hole. Adult sea hares were found in open waters at Woods Hole, having apparently gone through the seawater system of the laboratory [Carlton, 1992; JTC, unpublished data].

Event Summary: Escape from a research facility through drain pipes.

Table 1: (continued)

Taxa	Location of Release (Date)	Origin	Remarks
------	-------------------------------	--------	---------

Invertebrata

Many soft-bottom infaunal species	Elkhorn Slough in Monterey Bay, California (1990)	Coos Bay, Oregon, and other west coast localities	Status not known
-----------------------------------	--	---	------------------

Circumstances: In the course of research, scientists transported eelgrass *Zostera marina* from various localities along the Pacific coast of the United States to Elkhorn Slough in Monterey Bay, California. The work focused on identifying the best ecophenotypes of the eelgrass to use for the re-establishment of *Zostera* in the Slough. The method of transportation was to collect and transport the eelgrass in the original sediments to reduce root trauma, and then replant the eelgrass, with its accompanying sediments, in the Slough. In doing so, living invertebrates of potentially many species were also transported and released [JTC, unpublished data].

Event Summary: Inadvertent transportation with research materials, without anticipating introduction.

Fishes

Nile Tilapia <i>Oreochromis niloticus</i>	Alabama (1980s)	Africa	Not yet established in the wild
--	--------------------	--------	---------------------------------

Circumstances: One specimen was found in the wild, having escaped from experimental facilities at Auburn University in Alabama, where the species was under study for its aquaculture potential [Courtenay and Williams, 1992].

Event Summary: Escape from a research facility.

Pike Killifish <i>Belonesox belizanus</i>	Dade County, Florida (1957)	Mexico or Central America	Well established; has eliminated populations of mosquitofish (<i>Gambusia affinis</i>).
--	--------------------------------	---------------------------	---

Circumstances: The pike killifish was released into a canal after a research grant was terminated [Courtenay *et al*, 1986]

Event Summary: Intentional release (without anticipated introduction?).

References Cited

Carlton, J. T. 1989. Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. Conservation Biology 3: 265 - 273.

Carlton, J. T. 1992. Dispersal of living organisms into aquatic ecosystems: the mechanisms of dispersal as mediated by aquaculture and fisheries activities. In, A. Rosenfield and R. Mann, editors, Dispersal of Living Organisms into Aquatic Ecosystems, University of Maryland, Sea Grant Program.

Courtenay, W.R., Jr., D.A. Hensley, J.N. Taylor, and J.A. McCann. 1986. Distribution of exotic fishes in North America. In, C.H. Hocutt and E.O. Wiley, editors, The Zoogeography of North American Freshwater Fishes, pp. 675-698. John Wiley & Sons, New York.

Courtenay, W.R., Jr., and J.D. Williams. 1992. Dispersal of exotic species from aquaculture sources, with emphasis on freshwater fishes. In, A. Rosenfield and R. Mann, editors, Dispersal of Living Organisms into Aquatic Ecosystems, University of Maryland, Sea Grant Program.

Reynoldson, T.B., B.D. Smith, and P.S. Maitland. 1981. A species of North American triclad (Paludicola; Turbellaria) new to Britain found in Loch Ness, Scotland. Journal of Zoology 193: 531 - 539.

(This page intentionally left blank)

Part II: The General Evaluation Protocol (GEP)

The ANS Task Force established a Research Protocol Committee in 1991 which developed a general "Protocol for Evaluating Research Proposals Concerning Nonindigenous Aquatic Species" (hereafter referred to as the General Evaluation Protocol, or GEP) that was adopted by the ANS Task Force in April, 1992, and revised in September 1993 after public comment. However, the GEP is generic because it must be applicable to all possible nonindigenous aquatic nuisance species. Rather than providing a specific research containment protocol, it establishes a process for evaluating the spreading risk posed by research activities as a means to identify that research for which specific containment protocols would be required.

The applicant must submit a narrative Risk Assessment as defined in Part I of the GEP. The results of this risk assessment determine whether research containment protocols are required. If such protocols are required, the researcher must provide specific containment protocols to the Research Protocol Committee for review and approval. This has the potential to add substantial delays in the proposal process. Therefore, under Part II of the GEP, approved species-specific protocols (ASSPs) can be adopted by the researcher in lieu of developing his/her own, and under this circumstance, further review by the Research Protocol Committee prior to release of funds will not be required. However, Part II is very firm in the requirement that:

1. The researcher and his/her institution must sign a statement (**see pages II-3 and II-5 of this document**) that the research shall fully comply with all provisions of the adopted ASSP.
2. No deviations from the ASSP as approved will be allowed without additional review and approval by the Research Protocol Committee.
3. The proposal must include all of the information used for or required by the risk assessment, as well as a written copy of the ASSP that will be used, and which options within each protocol included in the ASSP will be used.
4. A complete copy of all documentation related to the proposal, the risk assessment, and the confinement and containment protocols to be used must be sent to the Research Protocol Committee for reference purposes.

This page intentionally left blank.

II-A. Statement of Institutional Responsibility (one for each institution involved):

On behalf of _____
(Name of Institution Sponsoring Principal Investigator)

the following individual(s) OR committee(s) (must be one or more persons or committees with oversight and/or authority over the conduct of research and the actions of the Principal Investigators, such as an Institutional Biosafety Committee) has/have reviewed the attached proposal and research containment protocols, and accept responsibility for assuring that all required procedures and restrictions under the aforementioned protocols will be followed without modification in the conduct of the research described herein.

(Name and Title of Person and, if applicable, Committee represented)

Signature Date

(Name and Title of Person and, if applicable, Committee represented)

Signature Date

(Name and Title of Person and, if applicable, Committee represented)

Signature Date

(Name and Title of Person and, if applicable, Committee represented)

Signature Date

(Note: the number of signatories is determined by the institution and it's requirements; the GEP does not require more than one signature)

This page intentionally left blank.

II-B. Statement of Principal Investigator Responsibility:

I, _____, as a/the Principal Investigator
(Name of Principal Investigator(s))

of the attached research proposal, acknowledge that it is my responsibility to assure that the conduct of this research will be in strict accord with the requirements of the attached research containment protocols, and in addition, notwithstanding, it is my responsibility to take whatever additional steps may be appropriate to minimize the risk of and prevent the escape of live life stages of the zebra mussel into open waters of North America during the conduct of research related to this proposal.

Signature

Date

Note: for proposals with more than one actual Principal Investigator, a single sheet signed by all the PIs is acceptable in lieu of a separate sheet from each.

This page intentionally left blank.

II-C. GEP Risk Assessment for Zebra Mussel Research Proposals

This section provides the procedures and information needed by zebra mussel researchers to work through the General Evaluation Protocol (GEP). The applicant should be familiar with and follow the requirements associated with the GEP: "Protocol for Evaluating Research Proposals Concerning Nonindigenous Aquatic Species" published in the Federal Register (September 24, 1992), revised 9/93, and reproduced as Appendix A in this document. The following is the GEP assessment made specific for the zebra mussel, and is meant to provide assistance and act as a reference for zebra mussel researchers who must work through the GEP. Some parts still require specific information and/or answers that must be provided by the researcher him/herself.

1. Does the research concern a nonindigenous aquatic species as defined by the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990?

Yes. The zebra mussel, *Dreissena polymorpha*, meets the definition in the Act and was declared an aquatic nuisance by the Aquatic Nuisance Species Task Force in April, 1992. For the purposes and intent of the Act, all species of nonindigenous mussels similar to or associated with populations of *Dreissena polymorpha*, such as the so-called "Quagga" Mussel, are considered to be nonindigenous aquatic species and included under the generic name "zebra mussel".

2. Does the species carry any known nonindigenous diseases, parasites or any other nonindigenous species or viable biological material?

Answer "Yes", "No", or "Not Sure" and explain.

At present, if the source of zebra mussel specimens for the proposed research is from waters within the contiguous 48 states, the Great Lakes including Canadian waters of the Great Lakes, their connecting channels including Canadian waters of the connecting channels, or the St. Lawrence Seaway including Canadian waters of the Seaway, then the GEP allows the specimens to be considered free from the above, and the answer is "No". However, should any nonindigenous diseases, parasites, or other nonindigenous species or viable biological material become documented in zebra mussel populations within the above geographic area, the answer to this question may be "Yes" in the future. Researchers must keep abreast of developments along these lines. Note that this question concerns itself only with the nonindigenous organism itself; Question 3, which sounds similar, expands consideration to ancillary material, such as sediment, water, or biological samples.

IMPORTANT NOTE: If your research involves zebra mussel specimens or other environmental materials originating from waters or shipped from areas other than those just listed above, your proposed research is **NOT** covered by the approved zebra-mussel-specific containment protocols, and you will have to a) be able to certify that the samples you are dealing with are free of diseases and parasites **OR** b) develop a plan with adequate procedures to contain any diseases and parasites that might be included with your samples, and submit it to the ANS Task Force Research Protocol Committee for review and approval. You will also have to be concerned about regulations governing the importation of foreign biological or soil materials into the United States - contact the U.S. Department of Agriculture and the U.S. Fish and Wildlife Service for further guidance.

3. *Do or could transportation of waters, media, sediments, or sampling equipment carry any nonindigenous diseases, parasites, or other viable material (study or extraneous organisms)?*

Answer "Yes" or "No" and explain.

Zebra mussels collected in the waters specified above (Question 2) are assumed to be free of disease and parasites unless knowledge dictates otherwise. However, it must be assumed that most field samples contain a variety of organisms in addition to the zebra mussel, depending on the nature of the samples. Whether or not these would be nonindigenous aquatic nuisances depends on many factors, especially the location where the samples will be opened and processed, and this question will need to be considered and discussed by the researcher. Researchers are advised not to ignore this issue - see Part I, Table 1 for a perspective on unintentional release of extraneous nonindigenous organisms during research experiments.

Unless field samples are preserved and sampling equipment (nets, water samplers, boats, etc.) are disinfected at the collection site in such a manner as to kill everything that may be in the sample or on the equipment, the answer to this question will usually be "Yes".

4. *Are your answers to Questions 1, 2, and 3 all "No"? If so, then STOP - your research does not require containment protocols and you need go no further. However, you must attach a copy of your GEP answers up to this point to your proposal.*

The answer to Question 1 is "Yes", therefore, proceed to Question 5.

5. *Will live, viable, or fresh specimens be required?*

Answer "Yes" or "No" and explain; if Yes, proceed to Question 6.

If your research does **not** involve the collection or use of any field samples or the use of live zebra mussels, you may answer "No" and you do not have to use confinement and containment protocols.

Anyone answering "Yes" will automatically need, at a minimum, the **Training Protocol** (Page III-3), the **Field Equipment Decontamination Protocol** (Page III-5), the **Disinfectant Procedures and Solutions Protocol** (Page III-7), and the **Research Termination Protocol** (Page III-19), even if you are treating the samples at the collection site in such manner as to kill all living organisms.

6. *Will unpreserved zebra mussels or field samples be transferred away from the site where collected?*

Answer "Yes" or "No" and explain.

If your answer is "No", then you do not need any additional protocols beyond those listed under Question 5.

If your answer is "Yes", describe what kind of samples will be shipped to what facility (give precise location of the facility), for what purposes, and in what condition (alive, in water, in damp paper, etc.).

Go to Question 7.

7. Will the species be transported through areas which are free of the infestation?

Answer "Yes" or "No" and explain.

If your answer is "No", go on to Question 8.

If your answer is "Yes", identify the point(s) of origin and the point(s) of destination and the route and method of transport. You will need to add the **Transportation and Shipping Protocol** (Page III-11) to those listed under Question 5. Go on to Question 8.

8. Is the zebra mussel present within a one mile radius of the research facilities that will receive live organisms or other non-preserved field material that may contain live zebra mussels of any life stage? If research will be conducted at more than one facility, answer and document for each such facility separately.

Answer "Yes", "No", or "Not Sure" and explain.

A research facility is considered to be **within the range** of the zebra mussel if (a) it is within one mile of a body of water known to contain widespread **established populations** of zebra mussels *and* (b) one or more live **adult** zebra mussel populations are physically present (in that body of water) within 10 miles of the research facility.

If your answer is "Yes", give: a) the precise location and name of the facility; b) the distance to the nearest body of water in which zebra mussels are known to be present; and c) the over-water distance to the nearest known established zebra mussel population from the facility. Provide a reference to the source of the information in (b) and (c). The following are presently accepted as authoritative sources with respect to the zebra mussel:

U.S. Fish and Wildlife Service
National Fisheries Research Center - Gainesville
Gainesville, FL, 904-378-8181)

New York Zebra Mussel Clearing House
New York Sea Grant Extension
250 Hartwell Hall
SUNY College at Brockport
Brockport, NY 14420-2928

Research conducted entirely within the range of the zebra mussel will not require additional protocols beyond those listed in Question 5.

If your answer is "No" or "Not Sure", give the location and name of the facility and distance to the nearest navigable body of water.

Proceed to Question 9.

9. *Can the species survive in the waters surrounding the research facilities identified in Question 8?*

Answer "Yes", "No", or "Not Sure" and explain.

You must be familiar with the environmental conditions that facilitate zebra mussel survival, and the environmental conditions of the waters surrounding the research facility to properly address this question. Each researcher will have to develop a specific answer. See Appendix B of this document for a summary of what is known about the life history, basic habitat and environmental requirements of the zebra mussel.

"Surrounding waters" refers to any navigable body of water within 1 mile of the research facility, or any standing or flowing water within 1 mile of the research facility that connects to a navigable body of water.

If your answer is "No" and your discussion supports this answer, you do not need any additional protocols beyond those listed under Question 5 for the facilities to which this answer applies.

If your answer is "Yes" or "Not Sure", you will need the **Facility Containment Protocol** (Page III-13).

Proceed to Question 10.

10. *Is it absolutely certain that the species will not be a nuisance if it is released into the surrounding water?* [Note: a nuisance species threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities dependent on such waters.]

No - the zebra mussel was declared an aquatic nuisance species by the ANS Task Force.

Go to Question 11.

11. *Have you previously been approved for zebra mussel research at your present location(s) using the same facilities?*

Answer "Yes" or "No" and explain.

If you answer "Yes", explain how the work under this proposal differs from your previously approved studies (if at all) and attach a copy of previous protocol reviews and approvals; be sure to indicate if you propose to follow any protocol(s) approved for previous work (these count as ASSPs).

If you answer "No", it means that you have not previously conducted approved zebra mussel research, or the research proposed now involves major changes from earlier approved studies, or you are proposing to follow protocols that differ from those approved for your previous research. Go to Question 12.

12. *Will you use and adhere to the zebra-mussel specific containment protocols that have been approved by the Research Protocol Committee of the ANS Task Force?*

Answer "Yes" or "No" and explain.

If you answer "Yes", you must attach copies of the Zebra Mussel ASSPs you will follow and you must provide any additional details or specific information that is required under any of these ASSPs.

If your answer is "No", then you will have to develop your own written containment protocols and submit them to the Research Protocol Committee for review. This may take up to 90 days from receipt.

This page intentionally left blank.

PART III: ZEBRA-MUSSEL-SPECIFIC CONTAINMENT PROTOCOLS

Required Protocols

Approved containment protocols have been developed for the zebra mussel for each of the following research activities:

(1) **Training:** all research personnel working with or having access to areas containing live zebra mussels shall be provided with specific information and training as specified in the Training Protocol (Page III-3).

(2) **Field Equipment Decontamination:** field equipment (nets, boats, grab samplers, corers, water samplers, etc.) used for field sampling must be decontaminated before it can be used for other (not zebra-mussel-related) research, especially for sampling or monitoring in waters presently uninhabited by zebra mussels and for equipment that is shared with other users. The Field Equipment Decontamination Protocol (Page III-5) must be followed to decontaminate such equipment.

(3) **Disinfectant Procedures and Solutions:** a list of procedures and solutions (Page III-7) that may be used as a biocide to eliminate living zebra mussels (and other biota) from various media.

(4) **Treatment of Accidental Discharges:** this is a protocol (Page III-9) required for any research that will move unpreserved field samples (such as plankton samples, water samples for chemical analyses, etc.) from bodies of water known or suspected of containing live zebra mussels in any stage to a location outside the range. It covers the accidental discharge of material into areas outside the bounds of containment areas.

(5) **Transportation and Shipping:** if any live zebra mussels or unpreserved field samples (such as plankton samples, water samples for chemical analyses, etc.) from bodies of water known or suspected of containing live zebra mussels in any life stage are moved outside the range of the zebra mussel, the **Transportation and Shipping Protocol** (Page III-11) must be used. Investigators obtaining zebra mussels from other parties must ensure that the requirements of the **Transportation and Shipping Protocol** are met.

(6) **Facility Containment:** if live zebra mussels or unpreserved field samples are transported to a location outside of their current range, the **Facility Containment Protocol** (Page III-13) is required until the samples are preserved or destroyed. The **Facility Containment Protocol** applies **ONLY** to closed systems (i.e., water is recycled, not continuously discharged or released). **There is no ASSP for facilities containing "flow through systems" outside the range of the zebra mussel, due to the severe risk of accidental release.**

The use of open flow-through systems in areas not immediately adjacent to existing zebra mussel populations is NOT recommended and scientists proposing to do so will have to develop and submit for full review protocols appropriate for such high-risk work.

(7) **Research Termination:** procedures for decontaminating facilities and equipment upon termination of zebra mussel research, to make them available for other activities (Page III-19).

Range of the Zebra Mussel: The key factor in deciding what containment protocols are necessary for a particular research project is whether or not all activity for that project is conducted entirely within the current range of the zebra mussel. **To be entirely in the range:**

- (a) all research facilities that receive unpreserved field specimens must be (1) within one mile of a body of water known to contain widespread established populations of zebra mussels and (2) one or more live adult zebra mussel populations are established (in that body of water) within 10 miles of the research facility; AND**
- (b) all field sampling sites must be on bodies of water which are known to contain widespread established populations of live zebra mussels; AND**
- (c) samples must NOT be transported through areas not in the range in order to transfer them from the field site(s) to the research facilities.**

Both the New York Sea Grant Zebra Mussel Clearing House and the U.S. Fish and Wildlife Service, National Fisheries Research Center - Gainesville, Florida maintain data and maps depicting the most up-to-date confirmed distribution of zebra mussels. If all research will be conducted within the range of the zebra mussel as defined, then the **Training Protocol (Page III-3)**, **Field Equipment Decontamination Protocol (Page III-5)**, **Disinfectant Procedures and Solutions Protocol (Page III-7)**, and the **Research Termination Protocol (Page III-19)** will still be required.

If the project is not conducted entirely within the range of the zebra mussel, then the **Facility Containment Protocol (Page III-13)**, the **Protocol for Treatment of Accidental Discharges (Page III-9)** and the **Transportation and Shipping Protocol (Page III-11)** will also be required.

ZEBRA MUSSEL ASSP**III-A. TRAINING PROTOCOL**

- (1) All personnel participating in the research shall be qualified by virtue of scientific research experience or training, and/or shall be trained by the PI(s) as necessary to insure that they are knowledgeable about and/or familiar with:
 - (a) the life history, biology, and basic environmental needs of the zebra mussel at various life stages (see Appendix B);
 - (b) the possible environmental problems if live mussels are released into open waters;
 - (c) the correct procedures for handling specimens;
 - (d) the correct procedures for decontaminating surfaces and objects;
 - (e) the correct procedures for disposing of specimens and field materials;
 - (f) the security arrangements for zebra mussel work areas;
 - (g) the correct procedures for mitigating accidental discharges outside the bounds of containment areas;
 - (h) the correct procedures for routine termination of research
 - (i) emergency termination procedures.

(This page intentionally left blank)

ZEBRA MUSSEL ASSP

III-B. FIELD EQUIPMENT DECONTAMINATION PROTOCOL

- (1) After collecting field samples from bodies of water known or suspected to contain live zebra mussels at any life stage, **all field equipment used to collect those samples or that was in some way in contact with the body of water, will be sterilized before moving to another site (field or facility) that is outside the known current range of the zebra mussel.**
- (2) Whenever practicable the least infested (or least likely to be infested) sites will be sampled before the most infected sites to reduce the risk of accidentally infecting a new area during sampling,
- (3) If sampling is being performed to determine whether zebra mussels are present at a given site, we will assume that they are present and will sterilize all sampling equipment before moving to another site outside the known range.
- (4) Methods
 - (a) Small equipment (plankton nets, bottles, buckets, small benthic grabs, waders, boots, etc.)
 - (1) all field equipment will be visually surveyed and all visible mussels will be removed and killed or, if practical, returned to the original collection site.
 - (2) all field equipment will then be cleaned by soaking in, dipping in, or scrubbing with, one of the disinfectant solutions listed under the **Disinfectant Procedures and Solutions Protocol** (Page III-7). If one of these approaches is not possible, the equipment will be steam cleaned (first preference), or rinsed with water (second preference; hot and/or high pressure if possible) and allowed to dry completely before next use.
 - (3) particular attention will be paid to places where mussels could be accidentally trapped, such as the treads of boots and waders, hinges of benthic grabs, etc.
 - (b) Large equipment (boats, anchors, trailers, etc.)
 - (1) Compartments:
 - (a) Bilges, wet wells, live wells, outboard and inboard motor cooling systems, and any other compartment that could hold water from an infested field collection site will be drained of water at the field site, and, if possible, flushed with disinfectant solution or hot water and allowed to dry before next use. If appropriate, the field site water may be drained back into the original body of water, as long as conditions are such that this would not cause chemical or biological contamination. Otherwise such water will be drained into a suitable container for treatment prior to final disposal.
 - (b) If the water is drained and collected, it shall be disinfected using one of the methods listed under the **Disinfectant Procedures and Solutions Protocol** (Page III-7) and then disposed of by a suitable means. It will be up to the investigators to

determine how this wastewater can be properly disposed of without causing environmental damage or contamination.

(c) After draining contained water, all compartments shall be filled with a disinfecting solution from the **Disinfectant Procedures and Solutions Protocol** (Page III-7). Whenever feasible, the disinfectant will be retained in the compartment until arrival at the next site.

(d) If a compartment is too large to make filling practical, it shall be thoroughly rinsed, twice, with a disinfecting solution.

(2) Boat Hull Surfaces, Anchors, and Trailers

(a) Option 1: all surfaces will be scrubbed to remove any clinging material from the field site, then visually inspected and any remaining field site material removed, and finally, hosed down with hot and/or high pressure water.

(b) Option 2: all boats, anchors, and trailers used in field sampling will be cleaned using a self-service or automatic carwash.

(c) Option 3: a boat hull, anchor, or trailer will be assumed to be free of live mussels if it has been thoroughly scrubbed, visually inspected and any visible field site material removed, and then it has been allowed to dry thoroughly, and has remained dry and out of water for at least two weeks, or one week in dry, hot (>20°C) weather.

(d) Regardless of which option is used for cleaning, visual inspection will follow, with special attention paid to cracks and crevices in which mussels may become trapped, and aquatic macrophytes caught on trailers or propellers which may harbor juvenile mussels.

(e) Particular attention will be paid to trailer pads made of carpet and foam rubber which could trap tiny mussels - if possible, such material will be removed from trailers before doing work in zebra mussel-infested waters.

ZEBRA MUSSEL ASSP

III-C. DISINFECTANT PROCEDURES AND SOLUTIONS PROTOCOL

- (1) Accepted methods for disinfecting include:
 - (a) Chemical disinfecting: depends on concentration and contact time. Since adult zebra mussels can close-up and survive for extended periods of time under toxic external conditions, chemical disinfecting as a means to kill adult mussels may require a contact time of several days. Each PI should confirm that the solution he/she chooses is 100% effective in killing the target zebra mussel life stage within the contact time adopted. This determination should be completed at the beginning of the proposed research, and documentation of the test method used and observations confirming mortality, should be retained and made available upon requested. Suitable procedures for solution, contact time, and life-stage effectiveness documented in the peer-reviewed scientific literature may be adopted by reference without further testing.
 - (b) Heat: temperature and exposure time determine the effectiveness of temperature treatments. Live steam, autoclaving, or boiling are all believed to be 100% effective against all zebra mussel life stages, as well as potential parasites they may contain. However, the appropriate exposure time is uncertain, and until standards are established to the contrary, the Committee recommends an arbitrary minimum exposure time of 3 minutes at full heat for individual mussels, and 10 minutes for clusters.
 - (c) Freezing: adult zebra mussels have a relatively low tolerance to freezing. Clarke et al. (1993) reported 100% mortality when individual mussels were exposed to -10°C for as little as 1.3 hours. However, clusters of mussels were more tolerant than individuals and the corresponding freezing mortality exposure time at -10°C appears to be at least 4 hours.
 - (d) Physical: crushing is an effective way to kill adult mussels, but may not kill attached larval or juvenile stages. Therefore, crushed adult zebra mussel remains should also be exposed to a chemical disinfectant solution prior to final disposal.
 - (d) Desiccation: desiccation is effective if allowed to continue for a long enough period of time. There are reports that live adult zebra mussels have survived for up to 21 days out of water under ideal conditions in a controlled laboratory setting. However, complete desiccation and exposure to warm dry air and/or direct sunlight should be effective in a week or less, but this must be confirmed.

(2) Disinfectant Solutions

Precise concentrations and contact times necessary to kill zebra mussels at various life stages are not known for most chemicals. The following guidelines (see next page) are believed to be more than adequate to obtain 100% mortality for the veliger stage, and may also be adequate for other life stages including juvenile and adult mussels. However, researchers should test the procedure(s) they decide to use to assure that 100% mortality is achieved for the life stage of interest under the conditions of concentration and contact time chosen.

Disinfectant	Concentration	LIFE STAGES	Minimum Exposure Time
salt solution (iodized salt must be used)	saturated solution	V, CR	30 minutes
ethanol	>50%	V, CR	multiple flooding rinses or dip for 3 minutes
Lysol, other phenol-based	undiluted (i.e., as sold)	V, CR	multiple flooding rinses or dip for 3 minutes
fresh chlorine bleach (at least 5% sodium hypochlorite)	100 ml in 20 liters of solution (approx. 3 fl. ounces in 5 gallons of water)	V, CR	1 hour

V = veliger CR = juveniles and adults with fractured/crushed shells

Notes:

Frequent use of chlorine-based solutions are not recommended for use with plankton nets or rubber articles, due to its corrosive nature.

A brief dip in most disinfecting solutions is unlikely to kill mussels that are larger than a few millimeters.

References

Clark, M., R. F. McMahon, A.C. Miller, and Barry S. Payne, 1993. Tissue freezing points and time for complete freezing in zebra mussels (*Dreissena polymorpha*) with reference to dewatering during freezing conditions as a mitigation strategy. Abstracts, 3rd International Zebra Mussel Conference, Toronto, Canada, February 23-26.

ZEBRA MUSSEL ASSP**III-D. PROTOCOL FOR TREATMENT OF ACCIDENTAL DISCHARGES**

- (1) If water or unpreserved solid material is accidentally discharged outside the bounds of containment areas (such as into a facility sewer or drain, or into uninfested open waters in the environment), the following steps shall be taken:
 - (a) To the extent possible, all flow of water into the affected area will be shut off or diverted.
 - (b) All discharged material that can be recovered by mechanical means (vacuuming, scooping, using a shovel, sweeping, etc.) shall be removed and decontaminated.
 - (c) Depending on the practical value of doing so, a suitable disinfectant may also be added as soon as possible at the point of entry. It may be practical to attempt disinfection of a sewer line, a storm drain, or a stagnant drainage ditch; it is not practical to add disinfectants to an open body of water unless the discharge was into an area with very limited exchange with the rest of the system.

The volume of disinfectant to be used shall be determined by the size of the original discharge and the period of time that has elapsed since the discharge occurred. Federal, state and local laws may also regulate the discharge of chemicals into sewer systems, drainage ditches, and natural bodies of water. It is the responsibility of the investigators to obtain the necessary approvals from local municipal and environmental authorities prior to taking any action that adds chemicals to systems outside the containment area of the research facility. Most sanitary sewer systems already operate under significant loads of chlorine bleach, so this may be the chemical of choice. However, written contingency plans for such action should be established in conjunction with the local sewer authority before the onset of research so that there will be no delay should such action be necessary.

- (d) All discharges of zebra mussel contaminated material outside zebra mussel work areas shall be reported immediately to the Institutional Biosafety (or equivalent) Committee, appropriate state agencies if required, the sponsoring Grant or Program Manager of the agency funding the research, and the ANS Task Force Research Protocol Committee.
- (e) A written report detailing the nature and causes of the discharge, the clean-up and other steps taken to minimize the impact, and providing an assessment of whether the discharge resulted in the release of, or may result in the introduction of, any nonindigenous species into a new area, shall be provided to the funding agency within one month of the incident.

(This page intentionally left blank)

ZEBRA MUSSEL ASSP**III-E. TRANSPORTATION AND SHIPPING PROTOCOL**

- (1) All live zebra mussels and related unpreserved sample material to be shipped or transported outside the known range of the zebra mussel, shall be contained for shipping so as to minimize the possibility of release through leakage, spillage, or other accident.
- (2) Any shipment of live ZEBRA MUSSELS or unpreserved sample material via parcel post, express mail, commercial freight, or the like, shall be packaged according to this protocol AND APPLICABLE DOT and postal regulations.
- (3) FIELD SAMPLES THAT HAVE BEEN TREATED IN A MANNER THAT WILL KILL ALL CONTAINED LIFE FORMS REQUIRE NO FURTHER PRECAUTIONS UNDER THIS PROTOCOL. Examples include, but are not limited to materials treated with and stored in formalin or alcohol, or frozen to at least -10°C for at least 4 hours prior to onset of transportation or shipping.
- (4) No live zebra mussels at any life stage shall be transferred to persons not associated with an approved research program that has adopted and is following approved containment protocols for the zebra mussel. It is the responsibility of the person(s) supplying the live specimens to assure that recipients meet this requirement.
- (5) Containment of Samples
 - (a) Primary containment
 - (1) All unpreserved sample material shall be sealed in heavy-walled containers with positive sealing, water-tight tops; plastic containers are preferred, but glass containers may be used when the scientific requirements make their use essential.
 - (b) Secondary containment
 - (1) When shipping via a third-party, each primary container shall be bagged (solid materials with little water phase) or double bagged (samples with significant water phase), with each bag tightly sealed at the throat. All glass containers must be double bagged.
 - (2) When transportation is provided directly by the scientific party, primary sample containers shall be placed in a sturdy waterproof box with a positive closure, such as a plastic lined waxed heavy-wall cardboard carton or a plastic camping cooler. Bagging and/or double bagging under these conditions is recommended but optional, except for glass containers.
- (6) Shipping Containerization (for Third-Party/Commercial Shipping)
 - (a) Small Individual Samples: small individual sample containers, sealed as described in sections 5a and 5b above, shall be packed together inside a plastic bag liner within a waterproof shipping box, such as a large Coleman-type cooler, or sturdy cardboard or wood

box,. Spaces surrounding sample containers shall be filled with absorbent material - newspapers, paper towels, vermiculite, or the like - and the throat of the bag liner shall be sealed. The box shall be tightly sealed and double taped along all seams. Cardboard boxes shall be reinforced with fiberglas-stranded strapping tape.

(b) Large Individual Samples: large sample containers, such as drums and 10-liter or larger water jugs, that are too large for routine packing as described in section 5b shall be double bagged in large plastic heavy-walled bags and then crated in a manner that (1) provides absorbent material in case of leakage and (2) meets the requirements of the third party shipper.

7) Labeling

(a) All secondary containers shall be clearly marked as to contents, and shall contain a clearly visible label stating the following:

WARNING: CONTAINS LIVE ZEBRA MUSSELS, AN AQUATIC PEST THAT MAY, IF RELEASED, CAUSE ENVIRONMENTAL AND/OR ECOLOGICAL DAMAGE. DO NOT OPEN IF FOUND; LOCALIZE AND CONTAIN CONTENTS IF SPILLED. PLEASE PHONE THE FOLLOWING:

NAME 1	AFFILIATION	TELEPHONE #
NAME 2	AFFILIATION	TELEPHONE #

(b) The label must be firmly affixed to the main container, not a removable top.

(c) A complete inventory of all samples to be shipped shall be prepared in duplicate; one copy shall accompany the shipment, the other shall be held at the point of origin.

(8) Shipping

(a) Samples and materials packaged according to the above protocols and being transported under the supervision of the scientific party must be securely fastened to the vehicle, or placed in trays or box frames that are securely fastened. The shipment shall be reinventoried at the destination to account for all samples.

(b) For samples and material packaged according to the above protocols and being shipped under the supervision of a third party, such as a commercial postal service or freight carrier, the shipper shall:

1. use a shipping method that allows positive tracking of shipment and proof of delivery to destination,
2. check with the carrier for additional requirements under state, federal, or international law;
3. assure that upon receipt at destination, the shipment is compared to the point-of-origin inventory and that all samples are accounted for. **ZEBRA MUSSEL ASSP.**

III-F. FACILITY CONTAINMENT PROTOCOL

(1) General Description of Laboratory and Experimental Facilities

For each facility involved, describe in narrative text:

(a) Location of Facility Containing Zebra Mussel Work Spaces

(1) Give a precise and descriptive location of each facility that will house zebra mussel work space(s) and give the straight-line distance between the facility and the nearest navigable body of water (identify the body of water). For example: "This facility is in northeast Ann Arbor, Michigan at 2205 Commonwealth Boulevard, approximately 0.7 miles west of the intersection of US 23 and Plymouth Road. The nearest navigable body of water is the Huron River, located 5 miles south of the facility."

(2) Describe the location (including what body of water) and distance from the facility of the nearest known zebra mussel population(s). The following are presently accepted as authoritative sources with respect to confirmed populations of zebra mussels:

U.S. Fish and Wildlife Service
National Fisheries Research Center - Gainesville
7920 NW 71st Street
Gainesville, FL 32606 (904-378-8181)

New York Zebra Mussel Clearing House
New York Sea Grant Extension
250 Hartwell Hall
SUNY College at Brockport
Brockport, NY 14420-2928

(3) Briefly describe the general use of the building which contains the zebra mussel work spaces (e.g., "This building is dedicated to the Department of Biology and houses classrooms, office space, and research labs, all of which are related to Departmental activities"; "This is a research and office building occupied by the XYZ Institute for Broad-Based Science. It houses administrative and research staff offices, and labs of researchers working on a variety of topics including environmental science, molecular biology, solar chemistry, and automotive engineering"; etc.)

(4) Are other research activities not related to the zebra mussel conducted in this facility? If so, do they involve the collection of field samples from areas that are or might be infested with zebra mussels - please describe (e.g., "This Department supports a wide range of environmental research, many of which involves processing of water, sediment, and biota samples from various aquatic environments, including Western Lake Erie, and the Ohio River between Cincinnati, Ohio and Louisville, Kentucky, the latter of which are known to be infested with zebra mussels. Labs used for this work are adjacent to the proposed zebra mussel work spaces.")

(b) Zebra Mussel Work Spaces

(1) Identify and describe the use of each specific room where zebra mussel research will be conducted, samples stored, or live zebra mussels maintained (include a floor plan or diagram **if it will help**). Identify possible modes of entry (e.g., "This room has two lockable entry doors, and two permanently sealed windows"; "This space has no windows and one door that is kept under padlock.")?

(2) Will other activities **unrelated** to the zebra mussel research also be conducted in these spaces? If yes, describe.

(3) Does the space have one or more sinks or floor drains? If yes, what type of sewer system are they connected to (i.e., municipal sewer system of the city; site has it's own sewerage treatment plant; etc.)? Is there is any pretreatment of the lab or facility drain effluent prior to entering an external sewer system? If yes, briefly describe.

2. Laboratory Protocol

(a) Security and Confinement

(1) **UNESCORTED** access to all zebra mussel work spaces in the facility shall be limited to those personnel authorized and trained by the principal investigator.

(2) Zebra mussel work spaces shall be kept locked when no authorized personnel are present. Arrangements for cleaning and janitorial services shall be consistent with the aforementioned provisions.

(3) Zebra mussel work spaces will be clearly marked as restricted areas containing live aquatic nuisance organisms, such as:

<p>RESTRICTED AREA CONTAINS LIVE ZEBRA MUSSELS, AN AQUATIC NUISANCE ORGANISM</p> <p>AUTHORIZED PERSONNEL ONLY.</p> <p>FOR ACCESS, INFORMATION, OR IN CASE OF EMERGENCY, CONTACT: NAME 1 PHONE 1 NAME 2 PHONE 2 NAME 3 PHONE 3</p>		
---	--	--

(4) The names and telephone numbers of at least three persons to contact in the event of an emergency must be posted.

- (5) All sink drains and floor drains in zebra mussel workspaces shall be securely plugged to prevent loss of contaminated water or other material into the drain system. **Signs warning against discharge of untreated zebra mussel material shall be posted over all sinks.**
- (b) Experimental Chambers, Holding Tanks, Containers, Systems and Related Equipment
- (1) Experimental chambers, holding tanks, containers, or systems that house live zebra mussels in any life stage must be:
- a. static systems, or closed-loop recirculation, and
 - b. to the extent practical, all aquaria, tanks, and containers holding live zebra mussels shall be kept in trays or liners capable of holding all of the water if the container were to leak or be broken.
- (2) It is recommended (but not mandatory) that tanks/systems used to hold general stocks of live ADULT zebra mussels be fitted with a chiller to maintain water temperature below 10°C to prevent unregulated spawning of mussels.
- (3) Equipment and reusable supplies frequently used for zebra mussel research (nets, siphon tubing, selected glassware or buckets) shall be marked for zebra mussel use only and stored separately from other equipment and supplies.
- (4) Equipment and reusable supplies used infrequently, or which must remain available for general laboratory use, shall be disinfected before release for non-zebra mussel work. See **Field Equipment Decontamination Protocol (Page III-5)**.
- (5) All equipment, holding tanks, pumps and filter systems, and experimental chambers used shall be thoroughly disinfected at the end of the project
- (c) Handling and Disposal of Mussels
- (1) If disposable gloves are used during contact with mussels or associated wastewater, after use, the gloves shall be disinfected (see **Disinfectant Procedures and Solutions Protocol, Page III-7**) and then discarded in the trash.
- (2) Dead or unwanted mussels shall be crushed **AND** disinfected (see **Disinfectant Procedures and Solutions Protocol, Page III-7**), and then disposed of as trash unless the institution requires a different procedure under guidelines for the disposal of animal carcasses.
- (d) Disposal of Wastewater
- (1) All water which has been in contact with live mussels shall be disinfected in place, or shall be first be passed through a 1 mm mesh screen to remove any juveniles or adults and then transferred to a waste tank for disinfection before discharge.

(2) Wastewater shall be disinfected by an accepted method (see **Disinfectant Procedures and Solutions Protocol, Page III-7**).

(e) Disposal of Solid Material

(1) All solid disposable environmental materials, such as sediments and macrophytes that were in contact either in the field or in the lab with water containing zebra mussels shall be disinfected via an accepted method (see **Disinfectant Procedures and Solutions Protocol, Page III-7**) prior to disposal.

(f) Spill Containment

(1) The following procedure shall be posted conspicuously in all zebra mussel work spaces:

**IN CASE OF A SPILL OF MATERIAL POTENTIALLY CONTAINING
LIVE ZEBRA MUSSELS AT ANY LIFE STAGE:**

Liquid Spills:

Prevent or stop discharge into drains
Treat drains receiving discharge according to predetermined plan (to be developed and posted by PI in conjunction with sewer system authorities)
Wipe up remaining liquid with paper towels or mop
Ring excess water into waste tank
Allow towels to dry before disposal in trash
Treat mop with appropriate disinfectant, rinse, and allow to dry thoroughly.
Isolate area of floor receiving spill and treat with disinfecting solution.
Wash area and rinse with water; allow to dry

Solid Material

Recover as much of solid material as possible, with a brush, scoop, forceps, etc. and place in another container for reuse or disposal.
Isolate area of floor receiving spilled material and treat with disinfecting solution.
Wash area and rinse with water; allow to dry.

Disinfectant solution(s) to be used:

- (a) list solution, concentration, and how it is to be used for spills)
- (b) list solution, concentration, and how it is to be used for spills)
- (c) etc.

(g) Emergency Termination

If the integrity of the research facility is threatened (e.g. fire, flood, hurricane, etc.), and time allows (without threat to the safety of personnel) experiments shall be terminated and chlorine bleach shall be added to all systems and tanks containing live zebra mussels, at a volume:volume ratio of 1:50 (1 part bleach for every 50 parts water).

(This page intentionally left blank)

ZEBRA MUSSEL ASSP**III-G. RESEARCH TERMINATION PROTOCOL**

- (1) Upon termination of a zebra mussel research project , the following procedures will be carried out.
 - (a) All live zebra mussels shall be destroyed and unpreserved field samples will be disinfected by appropriate physical or chemical methods chosen from the **Disinfectant Procedures and Solutions Protocol** (Page III-7).
 - (b) All water that was in contact with live zebra mussels will be disinfected using one of the methods described in the **Disinfectant Procedures and Solutions Protocol**.
 - (c) All containers, equipment, and other materials that were in contact with either live zebra mussels or water that was in contact with live zebra mussels at the end of the project will be disinfected using one of the methods presented in the **Disinfectant Procedures and Solutions Protocol**.
 - (d) All remaining waste material that was not previously disinfected shall be disinfected using one of the methods presented in the **Disinfectant Procedures and Solutions Protocol**, and then disposed of by appropriate means.
 - (e) All warning signs may then be removed from the work spaces.

(This page intentionally left blank)

Part IV. ADDITIONAL CONSIDERATIONS - ALL PROPOSALS

Sewer Lines

It is not known whether zebra mussels can survive and reproduce in sewer systems. Given the difficulty in keeping zebra mussel veligers alive under controlled laboratory conditions, one would think this is of little concern. However, without more definitive evidence, all Principal Investigators should take the following simple but effective steps to avoid possible problems:

- (1) plug and seal all floor drains in work spaces containing live zebra mussels; and
- (2) keep sink drains in all zebra mussel work spaces securely plugged so that personnel working with zebra mussel material must physically unplug the drain in order to dispose of any waste material.
- (3) post signs warning against untreated discharges over all sinks.

Permit Requirements For Transport And Possession Of Zebra Mussels

The ANS Research Protocol Committee **REQUIRES** that persons proposing zebra mussel research demonstrate that they are cognizant of and have complied with federal, state, and local laws and regulations governing the possession and transport of nonindigenous species. A number of states have already implemented restrictions and requirements for approval to possess and work with live zebra mussels within their borders. In addition, the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 amended the Lacey Act (18 U.S.C. 42) to add the zebra mussel to the list of injurious species (see below; reference Federal Register, November 7, 1991, pages 56942).

Investigators are responsible for obtaining all permits which are required within the jurisdiction(s) in which they will be working with, collecting, or transporting mussels. The information given below is supplied to assist the investigator. Because state and local regulations can change, and are likely to do so as concern about the zebra mussels increases, this information should be regarded as up-to-date only to January 1993, and for information purposes only. Individual investigators should check with appropriate state and local officials as they plan their research.

The final general research protocol (1993) requires the PI to provide written evidence that appropriate state agencies have been notified of the proposed zebra mussel research to occur within their jurisdiction(s). A copy of letters or other written communications must be included with the protocol package for each proposal.

Federal

Congress amended the Lacey Act by adding the zebra mussel (*Dreissena polymorpha*) to the list of injurious fish, mollusks, and crustaceans. The U.S. Fish & Wildlife Service has since amended its regulations, contained in 50 CFR part 16, which implement the requirements of the Lacey Act.

Effective December 9, 1991, the importation into the United States, or transportation between the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, or any territory or possession of the United States by any means whatsoever

of live zebra mussels, veligers, or viable eggs thereof is prohibited except by permit for zoological, educational, medical, or scientific purposes. In addition, no live zebra mussel or other species of the genus *Dreissena*, viable eggs, or progeny thereof acquired under permit may be sold, donated, traded, loaned, or transferred to any other person unless such person has a permit issued by the Director of the U.S. Fish and Wildlife Service.

Permits take approximately 60 days to process after receipt of the application form, which is available from:

USF&WS,
Permit Branch, Office of Management Authority
Mailstop 430, ARLSQ
1849 C St. NW
Washington DC 20240
1-800-358-2104

States

The following list is not complete and is only up-to-date to December, 1992. It is provided as a starting point for potential zebra mussel investigators. However, it is the responsibility of the investigators to determine the state and local requirements and restrictions that will apply to their proposed work, and to obtain all necessary permits.

Delaware: No restrictions which specifically pertain to zebra mussels

Illinois: Importation of species not on the approved species list is prohibited without a permit. Permit requests should include information about how the species to be imported will be contained.

Contact: Rod Horner
RR # 4, Box 54
Manito, IL 61546

Indiana: A permit is specifically required for any possession of zebra mussels.

Contact: Indiana Department of Natural Resources
Division of Fish and Wildlife
Room W-273, 402 W. Washington St.
Indianapolis, IN 46204

Kentucky: Under state regulation 301 KAR 1:122, a permit is required to import and conduct research with the zebra mussel.

Contact: David Pelren
Kentucky Department of Fish and Wildlife Resources
1 Game Farm Rd
Frankfurt, KY 40601
(502) 564-5448

Maryland: Importation of zebra mussels for any reason is currently prohibited pending the establishment of a Chesapeake Bay regional research protocol

Contact: Ron Klauda
Maryland DNR
Chesapeake Bay Research and Monitoring Division
Tawes State Office Building
580 Taylor Avenue
Annapolis, MD 21401
(410) 974-2680

Michigan: Information has not been received as of December, 1992.

Minnesota: Information has not been received as of December, 1992.

New Jersey: As of December, 1992, no restrictions which specifically pertain to zebra mussels.

New York: Senate Bill S. 5616, effective May 14, 1991, amends the state environmental conservation law to restrict the importation, transportation, possession, and sale of zebra mussels without a permit.

Contact: Bill Culligan
NY Department of Environmental Conservation
50 Wolf Rd.
Albany, NY 12233-0001
518-457-5430

North Carolina: As of December, 1992, no restrictions which specifically pertain to zebra mussels.

Ohio: Information has not been received as of December, 1992

Pennsylvania: Information has not been received as of December, 1992

Tennessee: As of December, 1992, no restrictions which specifically pertain to zebra mussels.

Virginia: Under state regulation VR 325-03-1, a permit is required to import and conduct research with the zebra mussel.

Contact: David Whitehurst
Virginia Department of Game and Inland Fisheries
4010 West Broad St.
Box 11104
Richmond, VA 23230
(804) 367-1000

Wisconsin: State law NR 1905 makes the importation or release of exotic species unlawful without a permit.

Contact: Michael Talbot
Chief of Fisheries Management Section
Wisconsin Department of Natural Resources
Box 7921, Madison WI 53707
608-267-7503

West Virginia: As of December, 1992, no restrictions which specifically pertain to zebra mussels.

APPENDIX A

**PROTOCOL FOR EVALUATING RESEARCH PROPOSALS
CONCERNING NONINDIGENOUS AQUATIC SPECIES
(GENERAL EVALUATION PROTOCOL)**

**AQUATIC NUISANCE SPECIES TASK FORCE
SEPTEMBER 1993**

Introduction

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Act; Public Law 101-646, 104 STAT. 4671, 16 U.S.C. 4701-4741 approved Nov. 29, 1990) requires that an intergovernmental Aquatic Nuisance Species Task Force (Task Force) develop and implement a protocol to ensure that research carried out under Subtitle C of the Act does not result in the introduction or dispersal of nonindigenous aquatic nuisance species to the waters of the United States. This protocol fulfills the requirements of the Act. The Task Force intends to develop the research protocol further based on experience gained through implementation of this protocol. This protocol will supplement other existing Federal protocols established to control activities with specific major classes of organisms, such as those already established for plants and insects under the Plant Quarantine Act of 1912 and the Federal Plant Pest Act of 1952, and for research involving recombinant DNA molecules under the Public Health Service Act of 1944.

This protocol must be used when research is carried out under Subtitle C of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990. Individuals, states, corporations, and institutions not required by the Act to follow this protocol are encouraged to do so to prevent introductions and dispersal of nonindigenous aquatic nuisance species through research activities. Prevention of unintentional introductions through means other than research is addressed in the Task Force's proposed Aquatic Nuisance Species Program (which addresses prevention, detection, monitoring, and control of nonindigenous aquatic nuisance species). Intentional introductions are addressed in the Task Force's Report to Congress entitled "Findings, Conclusions, and Recommendations of the Intentional Introductions Policy Review".

A Research Protocol Committee (Appendix III) composed of representatives from the Task Force members was established to develop the required research protocol. The committee met in Gainesville, Florida, on June 25, 26, and 27, 1991, drafted the protocol, and prepared policy recommendations to the Task Force concerning implementation of the protocol. The draft protocol was circulated to all Task Force agencies for review. A second draft was presented to the Task Force on September 27, 1991. Following a meeting of the Research Protocol Committee on April 1 and 2, 1992, and receipt of additional comments from Federal and non-Federal sources, a final draft was prepared and presented for Task Force approval on April 21, 1992. The research protocol was adopted by the Task Force on April 22, 1992 as an interim working protocol until the protocol had completed a public review. The availability of the Research Protocol for public review was announced in the Federal Register on September 24, 1992.

Research Protocol

The research protocol consists of two parts: a risk assessment (Part I) and a set of guidelines outlining preventative containment and confinement procedures (Part II). The risk assessment requires the Principal Investigator and the Research Institution to evaluate the risk that the species, if it escapes or is released, will be a nuisance, and to determine if preventative measures must be taken to prevent the species from escaping or being released. Research may be conducted with minimal special preventative measures if 1) the research site is within the present established or historic range of the species, 2) the species is free of nonindigenous diseases, parasites or other extraneous viable material, 3) the species is not likely to be a nuisance if released, and 4) the species cannot survive in the waters adjacent to the research location, or 5) only non-viable forms are used, or 6) the research does not involve actual handling or transfer of the species (e.g. computer modelling and in situ data collection). The evaluation of the proposal by the risk assessment will determine if preventative measures must be taken.

The second part of the protocol is a detailed set of preventative containment and confinement guidelines that the Principal Investigator may be required to follow to prevent the escape or release of any research species that fails to meet one or more of the conditions listed above. If directed by the risk assessment, the Principal Investigator must develop preventative measures that will contain or confine the species to the research facility or location(s).

Appendix I is a list of some of the presently existing guidelines and protocols that may be used as resources by investigators to identify the types of precautions that can be taken to prevent unintentional releases of organisms used in research or to guide research on aquatic nonindigenous species. The specific precautions needed (which include procedural and facility design and use elements) will depend on the species to be studied, its life stage and size (e.g. macroscopic and/or microscopic, and size range within each), the scope of the project, the characteristics of the research location(s) with regard to the species' critical environmental factors, and the potential of the species to survive in that locale(s) and to be a nuisance. If the species is a disease-causing organism or a parasite, or the species or the source of the species under consideration is not free of nonindigenous diseases or parasites, extra precautions may be necessary. Most of the guidelines listed require that test species be contained or confined by some combination of physical, biological, chemical, and/or environmental barriers, or by limiting the scope of the research. The number and types of barriers needed depends on the species and the potential problems the species could create if it escapes or is released from the research site(s).

Procedures to Process Research Proposals

1. The Principal Investigator

The Principal Investigator shall determine that the research proposal complies with all applicable local, state, and national laws and regulations. The Principal Investigator will submit all research proposals concerning nonindigenous aquatic species to their Research Institution for review -- usually the Research Institution will establish a committee similar in membership, roles and responsibilities to the Institutional Biosafety Committee (IBC) described in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (Federal Register 51, Number 88, page 16959 (51 FR 16959)). In the proposal the Principal Investigator must demonstrate a knowledge of the life history and biology of the species, provide all information necessary for preparation of a risk assessment, and provide citations for all supporting data. If the species is found to present any possibility of being a nuisance, as determined by the risk assessment, the proposal must clearly demonstrate that 1) adequate confinement and containment procedures will be in place during research and throughout the time that the species is held, and 2) the Principal Investigator has incorporated into the study plan procedures, facility design elements, and other preventative measures analogous to those in guidelines developed by NIH for research within recombinant DNA molecules, and the U.S. Department of Agriculture for research in agricultural biotechnology (49 FR 50856, 51 FR 23302, and 56 FR 4134), which are adequate to contain and confine the species and any pathogens or parasites it may contain or be infested with. Within 30 days of being notified by a Funding Agency that a nonindigenous species research proposal will be funded, the Principal Investigator must notify the appropriate state authorities in writing that the research is going to be carried out, and must submit a copy of that written notification to the Funding Agency by the end of the thirty day period. The Funding Agency will be responsible for sending a copy of the state notification document to the Research Protocol Committee before the research is initiated.

2. The Research Institution

The Research Institution accepts and reviews the research proposal, reviews and approves the risk assessment and preventative measures, agrees to support the research and signs a statement that it will ensure that the research will be conducted as planned and the preventative measures will be carried out. The Research Institution may establish an Institutional Biosafety Committee (IBC) and a Biosafety Officer (BO) position to assist it to meet its obligations. The use of an IBC or a BO is optional but the Principal Investigator and the Research Institution should have a system in place to demonstrate that the proposal has been reviewed by a qualified independent group before submitting it to the Funding Agency. The Research Institution must determine that the proposal is complete, and that it includes an accurately completed risk assessment, all required life history and biological data, and adequate and detailed containment and confinement measures, if needed. The Research Institution should also determine that the proposal

complies with all applicable local, state, and national laws and regulations. The Research Institution should determine if a species-specific containment/confinement protocol has been approved by the Research Protocol Committee for the species and if so, whether the proposal fully meets all requirements of that approved species-specific protocol (ASSP). If an ASSP exists and the Principal Investigator is proposing to deviate from that ASSP, the Research Institution should ensure that the differences and the substituted preventative measures are clearly described, since further review and approval of the proposal by the Research Protocol Committee will be required. If no ASSP exists, the Research Institution must be assured that the Principal Investigator has conducted a thorough literature review on the species, is knowledgeable of its life history, biology and ecology, and has developed and described preventative measures to adequately contain and confine the species if necessary. Proposals not conforming to an ASSP or for which no ASSP exists will require a full review by the Research Protocol Committee, and should follow guidelines similar to that outlined in Appendix I. The proposal, with the appropriate findings and a certification of compliance statement signed by the Principal Investigator and the Research Institution that states that the Principal Investigator and the Research Institution will adhere to the proposed containment and confinement procedures, must be transmitted to the Funding Agency. If the Research Institution or the IBC does not have the expertise to evaluate a particular proposal, the proposal should be transmitted to the Funding Agency accompanied by a request for a review by the Research Protocol Committee. The Principal Investigator is still responsible for providing all the information needed to fully evaluate the species.

3. The Funding Agency

The Funding Agency provides technical and programmatic review, determines if the proposal is complete and that it complies with the requirements of the National Environmental Policy Act (NEPA) and other applicable laws and regulations (Appendix IV). The Funding Agency makes all funding decisions; prioritizes and selects proposals for funding, submits the proposals to be funded to the Research Protocol Committee, and after receipt of the Research Protocol Committee's review, determines what steps must be taken, if any, before the proposals will be funded. The Funding Agency may require that the Principal Investigator make changes in the proposal before submittal to the Research Protocol Committee for initial or re-review. All proposals selected for funding will be transmitted to the Research Protocol Committee within 15 days after the proposal has been selected for funding, either for review, if the Research Institution has not already certified that the proposal is in compliance with an ASSP, or for informational purposes, if the Research Institution has certified compliance with an ASSP. The Research Protocol Committee will eventually review all proposals, but proposals following an ASSP do not have to be reviewed prior to funding.

4. The Research Protocol Committee

All proposals concerning nonindigenous aquatic species (including the risk assessment and preventative measures to be used to prevent escape or

inadvertent release) selected for funding by a Funding Agency will be submitted to the Research Protocol Committee within 15 days of selection for funding. Research proposals requiring preventive/containment measures and for which the Principal Investigator and Research Institution have certified that one or more ASSPs will be followed without modification, will not have to be reviewed by the Research Protocol Committee prior to funding. However, such proposals will still be sent to the Research Protocol Committee by the Funding Agency for review to verify the risk assessment and ASSP(s), to verify compliance with the intent and provision of the Research Protocol, to obtain information that may be used to revise the Research Protocol or the ASSP(s) as appropriate, and to obtain information necessary for reporting purposes. For all other proposals, the Research Protocol Committee will review in detail the completed risk assessment, the research proposal, and the proposed containment and confinement procedures to insure that the proposed procedures are adequate to prevent the species from escaping or being released during the research. The Research Protocol Committee will review and provide comments and recommendations to the Funding agency within 90 days of receipt of the research proposals from the Funding Agency. Proposals requiring major changes must be resubmitted to the Research Protocol Committee for review. The Research Protocol Committee may call on outside expertise when necessary or may establish subcommittees to review multiple proposals for work on the same species. The Research Protocol Committee will advise the Funding Agency and make recommendations: (1) the proposal (including the completed risk assessment and preventative measures) appears to be adequate and thus funding is appropriate; (2) the proposal is not adequate in all aspects and needs to be resubmitted to the Research Protocol Committee after deficiencies identified are addressed and appropriate changes made to the proposal; or (3) the proposal has serious inadequacies that require major changes, and should not be funded until these changes are made and the proposal has been resubmitted to the Research Protocol Committee and the Research Protocol Committee has deemed the revised proposal to be adequate.

All proposals (both those complying with an ASSP and those with individualized containment and confinement plans) will be reviewed by the Research Protocol Committee to determine if there are problems in the use of the risk assessment and to improve both this research protocol and the ASSP. The Research Protocol Committee will provide an annual report to the Task Force detailing the proposals reviewed, the species involved, the number of proposals needing detailed confinement and containment procedures, the location of the research sites by species, the problems encountered, and announce the availability of ASSP's and recommend changes to the Task Force as needed.

The Research Protocol Committee will serve as an advisor to the Funding Agencies, providing comments and recommendations on the risk assessment and adequacy of preventative measures being taken by the researcher. The responsibility of ensuring NEPA compliance, and of selecting and funding the research belongs entirely to the Funding Agency.

At every level of the processing of the proposals every effort will be taken to protect the confidentiality of the research. Genetically altered species, unless they are also nonindigenous species, should not be processed through this protocol. Research involving genetically altered species should be processed through other appropriate protocols (See Appendix I).

PART I

Risk Assessment

Completed risk assessments must be submitted in narrative form to the Funding Agency along with the preventative measures, if needed. The reasoning behind each answer must be stated. The submittal of the complete research proposal to the Research Protocol Committee is not necessary, however, the Principal Investigator is responsible for providing enough information to enable the Research Protocol Committee to understand the research, and to evaluate the risk assessment and the effectiveness of the preventative measures, if needed.

- I. Does the research concern a nonindigenous aquatic species as defined by the Nonindigenous Aquatic Nuisance Species Prevention and Control Act of 1990 (Act)? Nonindigenous aquatic species means any species or other viable biological material that enters an ecosystem beyond its presently established or historical range, including transfers from both domestic and foreign sources. [Historical range is the territory occupied by a species at the time of European colonization of North America.]

ALL ANSWERS: go to II.

- II. Does the species carry any known nonindigenous diseases, parasites or any other nonindigenous species or viable biological material? Unless there is knowledge or evidence to the contrary (e.g., oysters being transferred from an area where MSX or dermo or imported oyster drills exist, salmonid transfers from areas where IHN and VHS viruses occur, or warmwater species transfers from areas where the Asian tapeworm occurs) species transfers within the continental U.S. can be considered free of nonindigenous diseases or parasites. Any species recently imported directly or indirectly into the continental U.S., Hawaii, Alaska or a territory of the U.S. from a foreign country, or from Alaska, Hawaii, or a territory of the U.S. into the continental U.S. or the reciprocal should be considered to have nonindigenous diseases or parasites unless proven otherwise; appropriate preventative measures must be taken (see Part II, Guideline of Preventative Measures).

YES or NOT SURE: go directly to Part II (Guideline of Preventative Measures) and to III.

NO: go to III.

- III. Do or could transportation waters, media or sediments or sampling equipment carry any nonindigenous diseases, parasites, or other viable material (study or extraneous organisms)?

YES or NOT SURE: transfer species to clean water and container, treat waste water to kill all organisms, disinfect original container. If this is sufficient to rid the shipment (transfer) of all extraneous organisms, go to IV; if not, go to Part II (Guideline of Preventative Measures).

NO: go to IV.

- IV. If the research does not concern a nonindigenous aquatic species under the Act and the research could not spread nonindigenous diseases, parasites or other viable material, this protocol does not apply, however, some precautions may be necessary to avoid the spread of nonindigenous species by incidental means such as contaminated equipment. If the species falls under the Act, continue on to V.

If answers to I, II, and III are all NO: the protocol does not apply to your research organism.

If any answer to I, II, and/or III above is YES or NOT SURE: the species falls under the Act; go to V.

- V. Will live, viable, or fresh specimens be required?

NO (specimens must be preserved in a manner to kill the organisms immediately to assure no possibility of infestation if the specimens are released): no additional procedures may be necessary.

YES: go to VI.

- VI. Will the species be transferred away from the site where collected?

NO: The spread of the organism is unlikely therefore environmental concerns are minimal. Some precautions to avoid the incidental spread of the organism by contaminated sampling equipment may be needed. If the research will not result in the spread of live organisms the remainder of the protocol does not apply.

YES: go to VII.

- VII. Will the species be transported through areas which are free of the infestation?

YES: adequate preventative measures must be taken to prevent escape or release during transportation; go to VIII.

NO or NOT SURE: go to VIII.

- VIII. Is the species under investigation presently established within one mile of any facility which will receive live nonindigenous species or other nonpreserved field material which may be contaminated with a nonindigenous species? Studies may be conducted in more than one research laboratory (including field laboratories). List each laboratory in which the research will be conducted, and discuss and document for each laboratory.

YES (The species is found within one mile of a research facility or its effluent discharge point): the study may not require more

than minimal measures at this facility to prevent the species' introduction. It may however require precautionary measures to ensure that nonindigenous species are not spread between collection sites, from one facility to another facility, or from a facility to noninfested sites by means of equipment or supplies used at more than one study site or used for more than one study.

NO (the species is not found within one mile of a research facility which will receive live nonindigenous species or other nonpreserved field material which may be contaminated with a nonindigenous species, or within one mile of the facility's effluent discharge point): the researcher should report the nearest known population of the species from each facility and go to IX.

IX. Can the species survive in the surrounding waters?

NO: only minimum preventative measures may be needed.

YES or NOT SURE: go to X.

X. Is it absolutely certain that the species will not be a nuisance if it escapes or is released into surrounding waters? [Note: A nuisance species threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities dependent on such waters.]

YES: only minimum preventative measures may be needed.

NO or NOT SURE: go to XI.

XI. Have you previously been approved for research with this species at your present location(s) using the same facilities?

YES: explain the changes, if any, between this proposal and previous funded studies and attach a copy of previous approval letter and submit to the Funding Agency for review by the Research Protocol Committee. Explain any changes in detail.

If major changes exist from earlier funded study or the answer is NO: go to XII.

XII. Is there a Research Protocol Committee approved species-specific protocol (ASSP) for the nonindigenous species that is (are) the subject(s) of your research proposal, and will this ASSP be used by you for this proposal?

YES (an ASSP exists and will be adhered to in every particular): attach the ASSP and list specifics (e.g. options to be used) that are to be used in your research. Submit to Funding Agency for review by Research Protocol Committee.

NO (no ASSP exists, or an ASSP exists but will not be used): go to **XIII**.

NO (An ASSP exists but will not be exactly adhered to, i.e. additional or different methods will be used, or parts of the ASSP will not be used): describe in detail any deviation from the ASSP, specify if any part of the ASSP will be used, and describe preventative methods to be used that differ from those in the ASSP. If any part of the ASSP is to be used, attach the ASSP: go to **XIII**.

XIII. If the proposal has reached this point in the risk assessment, a preventative containment/confinement plan must be developed and described in detail which will ensure that the species or any diseases or parasites it might carry cannot escape or be released into the surrounding waters. The species under consideration is a live or viable nonindigenous aquatic species, a nonindigenous pathogen or parasite of aquatic species, or might be carrying nonindigenous diseases or parasites of aquatic species, is not present in the waters surrounding the research site, could survive if released, and could be a nuisance. The researcher must document knowledge of the literature concerning the species and the problems which could result if released. A plan must be developed to ensure that the research does not result in the release, escape, or dispersal of the species. The investigator will be required to develop a preventative plan (PART II) and submit it with the risk assessment to the Funding Agency who will forward it to the Research Protocol Committee for review. The investigator and the supporting Research Institution must agree to comply with the preventative plan, and this protocol or an approved species-specific protocol. The Funding Agency and the Research Institution will ensure compliance.

Every investigator conducting research on a live or viable nonindigenous aquatic species which could be a nuisance, and is conducting the research outside the species' present established or historic range, is required to develop containment and confinement procedures and have a secure facility. Reference to guidelines already available (Appendix I) can be of assistance in developing a containment and confinement plan. Table I is an outline of the information and containment and confinement procedures required in most existing guidelines. In the future species-specific protocols may be developed for high visibility species (like the zebra mussel) whose life history, biology, and impacts are known and for which there are multiple studies under consideration. When reviewed and approved by the Research Protocol Committee, ASSPs may be used by investigators, however, compliance to all points of the ASSP will be mandatory if the Investigator elects to use an ASSP. Any or all protocols may be changed by the Research Protocol Committee as new knowledge is accumulated. Deviations from an ASSP will require case by case approval of research proposals and their preventative plans. Research on nonindigenous species which may also have nonindigenous diseases and parasites will require maximum security for the species and for any diseases or parasites the species may carry. Every effort should be made to conduct research on nonindigenous species in facilities located within the existing established range of the species; in this case only one level of preventative measures may be required.

PART II

Guideline of Preventative Measures

The Research Protocol Committee cannot develop a detailed set of guidelines for every nonindigenous species under research. Investigators and Research Institutions must develop containment and confinement plans taking into consideration the species, its characteristics, diseases and parasites, and critical environmental factors, its capabilities to be a nuisance, the design of the research facilities, and the location of the test site in relationship to the species' present range. Appendix I lists guidelines which have already been developed for groups of organisms. Table I is an outline of the informational needs and preventative measures to contain or confine test species found in most guidelines. The appendix and table are included as reference materials for investigators.

If the investigator determines that live specimens must be used, that the research must be conducted in an area where the species is not already present, that the species could survive if released into surrounding waters, and that the species or its diseases or parasites could be a nuisance, major preventative measures would be required to prevent escape or release.

The preventative plan should use a combination of physical, biological, environmental, and/or chemical barriers to contain or confine all life stages of the organism. Reducing the scope of the research should also increase the safety of the research.

For containment of diseases, parasites, small species, or the early life stages of larger species, the procedures outlined in the NIH guidelines (FR 51 No. 88, May 7, 1986, pg. 16959) or guidelines developed by the U.S. Department of Health and Human Services (see references) are the most comprehensive.

For containment or confinement of larger forms, the guidelines developed for whole plants or animals by the Office of Agricultural Biotechnology, USDA, are the most appropriate, especially if the research is to be conducted outside the laboratory (see Appendix I).

Preventative measures should address all life stages present or possible during the research phase. Where feasible, use of juvenile specimens, monosex populations, or sterile individuals is recommended.

Species-Specific Confinement and Containment Protocols

The Research Protocol Committee expects to receive many research proposals on a few high profile, high risk species, such as zebra mussels. A subcommittee of the Research Protocol Committee or one of the Funding Agencies may submit a species-specific confinement/containment protocol for review by the Research Protocol Committee. When such a proposed species-specific protocol is submitted, the Research Protocol Committee will review the adequacy of proposed containment procedures to insure that the species or any associated diseases, parasites, or any other nonindigenous species or viable biological

materials cannot escape or be released during research. The Research Protocol Committee will complete its review and provide a response to the appropriate Funding Agency or subcommittee within 90 days. The form of the Research Protocol Committee's response will be either: 1) the species-specific protocol is adequate as proposed and is approved for general use by the research community (i.e., the protocol has become an ASSP); or 2) the species-specific protocol is not adequate as proposed and is not approved. If the proposed species-specific protocol is not approved, the Research Protocol Committee will state reasons and may suggest modifications to correct problems seen. Since these protocols will only be prepared for species which are considered nuisance species, the risk assessment section can be reduced and the preventative plan can be standardized. Research proposals adhering to an ASSP will not need to be reviewed by the Research Protocol Committee prior to funding.

Compliance with all provisions of an ASSP must be fully accepted in writing by the Principal Investigator and the Research Institution by submitting a signed statement (certification of compliance) to that effect. Specific preventative measures to be used by the Principal Investigator must be documented in the research proposal. If all aspects of the ASSP are accepted, the Research Institution can approve confinement and containment procedures and monitor the research. All documentation, including the proposal, completed risk assessment, and preventative measures to be used, will be forwarded to the Research Protocol Committee by the Funding Agency. Any deviations from the requirements of an ASSP will require that the research proposal and confinement and containment plan be reviewed by the Research Protocol Committee before funding is approved.

The Research Protocol Committee will use the information in all research proposals (using both species-specific and non-standard protocols), to improve future protocols and to establish the location of research on nonindigenous aquatic species.

The Research Protocol Committee will report annually to the Task Force the number of proposals requiring confinement/containment measures, the species involved, and the location of research sites. Problems will be identified and recommendations for correcting them provided to the Task Force.

Until a research proposal is funded and becomes public property the confidentiality of the contents of the proposal must be maintained at all levels. All levels of review before funding must be made aware of the legal and ethical responsibilities not to discuss, copy, or share proposals with anyone not directly involved or authorized to assist in the review.

Compliance, Inspection, Reporting

All proposals which are required to follow a confinement and containment protocol must include certification by the Principal Investigator and the Research Institution that they will comply with the requirements of the protocol, and within the proposal must document the specific containment and confinement measures to be used. The Research Institution or The Institutional Biosafety Committee and/or the Biological Safety Officer, if

appointed by the Research Institution (see NIH guidelines 51 FR 16963 for specific duties), will monitor the conduct of the research and verify compliance with the containment and confinement procedures agreed to by the Principal Investigator and the Research Institution.

The Funding Agency, the Research Protocol Committee, and appropriate state agencies may inspect the facilities and containment and confinement procedures at any time. The Research Institution should inspect its research at least twice yearly.

Failure to comply with the protocol, or the escape or release of a nonindigenous aquatic species must be reported to the Funding Agency, the appropriate State agencies and the Research Protocol Committee immediately. Penalties for noncompliance with the protocol will be administered by the Funding Agency and could include suspension of research funding. The major responsibility for compliance with the protocol falls to the Principal Investigator and the Research Institution.

APPENDIX I

Existing Guidelines and Protocols

Guidelines for Recombinant DNA Molecule Research:

The following is a list of guidelines and protocols used to confine or contain nonindigenous species or organisms involved in recombinant DNA research. These can also be applied to nonindigenous aquatic species proposals. Consulting one or more of these will help investigators to identify physical, biological, chemical, and/or environmental preventative measures that may be used to confine or contain the nonindigenous aquatic species during research, transportation and storage. (Federal Register 51 No. 8, pg. 16958; FR 51 No. 123, pg. 23367; FR 52 No. 154, pg. 29800; FR 56 No. 22, pg. 4134; FR 51 No. 88, pg. 16959)

Guidelines for Microorganisms

National Institutes of Health (NIH). 1968. Guidelines for Research Involving Recombinant DNA Molecules. Published in Federal Register May 7, 1986 (51FR 16958-16961) with additional major actions August 24, 1987 (52FR 31838); July 29, 1988 (53FR 28819); October 26, 1988 (53FR 43410); March 13, 1989 (54FR 10508); March 1, 1990 (55FR 7438); and August 11, 1987 (52FR 29800) with appendix P for plants and Q for animals.

Guidelines for Whole Plants and Animals

U.S. Department of Agriculture (USDA). 1984. Coordinated Framework for Regulation of Biotechnology. Federal Register December 31, 1984 (49FR 50856) and June 26, 1986 (51FR 23302+).

USDA. 1986. Advance Notice of Proposed USDA Guidelines for Biotechnology Research. Federal Register June 26, 1986 (51FR 23367-23393) and February 1, 1991 (56FR 4134-4149).

USDA. 1986. Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or for Which There is Reason to Believe are Plant Pests. Federal Register June 26, 1986 (51FR 23352-23366) and June 16, 1987 (52FR 22892-22915).

Coulson, J. R., and R. S. Soper. 1989. Protocols for the Introduction of Biological Control Agents in the U.S. Chapter I, pages 2-35 In: Kahn, R. P. (ed.). Plant Protection and Quarantine. Volume III Special Topics. CRC Press, Inc., Boca Raton, Florida.

USDA, Office of Agricultural Biotechnology. 1988. USDA Guidelines for Research Outside the Laboratory Involving Biotechnology, also Federal Register June 26, 1986 (51FR 23367-23313) and February 1, 1991 (56FR 4134-4149).

International Guidelines and Protocols:

European Inland Fisheries Advisory Commission. 1988. Code of Practice and Manual of Procedures for Consideration of Introductions and Transfers of Marine and Freshwater Organisms. FAO. EIFAC. Occasional paper No. 23. 52 pages.

International Council for the Exploration of the Sea. 1982. Proposed Guidelines for Implementing the ICES Code of Practice Concerning Introduction and Transfer of Marine Species. 23-page manuscript.

Disease Related Guidelines and Protocols:

Anonymous. 1989. Operating Procedures for the Alma Quarantine Facility. Prepared for the Alma Research Station, Guelph, Ontario, Canada. 16 pages typewritten.

Horner, R. W., and R. L. Eschenroder. 1991. Protocols to Minimize the Risk of Introducing Salmonid Disease Agents with Importation of Salmonid Fishes. Draft manuscript. 11 pages. Prepared for Great Lakes Fish Disease Control Committee. Pages 27-37.

U.S. Department of Health and Human Services. 1984. Biosafety in Microbiological and Biomedical Laboratories. 1st Edition (March 1984). U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, and National Institutes of Health, Bethesda, Maryland 20892.

An additional 17 references on laboratory disease and pathogen control methods can be found listed in the Federal Register, May 7, 1986 (51FR 16965).

Other Guidelines and Protocols:

Klingman, D. L., and J. R. Coulson. 1983. Guidelines for Introducing Foreign Organisms into the United States for Biological Control of Weeds. Bulletin of Entomological Society of America. Fall 1983:55-61.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Arthropod-Parasitic Nematodes into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Interstate Movement and Export of Foreign and Native Arthropod-Parasitic Nematodes for Research on Biological Control of Such Pests.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Microbial Pathogens (Fungi, Bacteria, Rickettsia Viruses, Protozoa) into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Export of Foreign and Native Arthropod Pathogens for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of

Foreign Arthropods and Nematodes into the United States for Biological Control of Weeds, and for the Interstate Movement and Export of Foreign and Native Arthropod and Nematode Natural Enemies of Weeds.

Guidelines for the Importation, Interstate Movement, and Field Release in the United States of Foreign Microbial Pathogens for Biological Control of Weeds, and for the Interstate Movement and Export of Foreign and Native Pathogens of Weeds for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Beneficial Organisms (Microbial Pathogens and Antagonists) into the United States for Biological Control of Plant Nematodes and Plant Pathogens, and for the Export of Such Organisms (Foreign and Native) for Research.

Southeastern Cooperative Wildlife Disease Study. 1985. Model for State Regulations Pertaining to Captive Wild and Exotic Animals. University of Georgia, Athens, Georgia. 48-page manuscript. Prepared in response to Resolution #9. U.S. Animal Health Association, Milwaukee, Wisconsin 10/27-11/1/85.

Jennings, D. P., and J. A. McCann. 1991. Research Protocol for Handling Nonindigenous Aquatic Species. National Fisheries Research Center, U.S. Fish and Wildlife Service, Gainesville, Florida. 43-page manuscript.

Brown Tree Snake Protocol:

Pacific Basin Development Council. 1991. Recommended Protocol for Transport of Live Brown Tree Snakes (*Boiga irregularis*). Prepared for Plant Quarantine Branch, State of Hawaii Department of Agriculture and Biological Survey, and the U.S. Fish and Wildlife Service.

Guidelines for Animal Care and Welfare:

Guidelines for Use of Live Amphibians and Reptiles in Field Research. American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL), and the Society for the Study of Amphibian and Reptiles (SSAR). 1987.

Interagency Research Animal Committee's Report. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Published in the Federal Register. May 20, 1985.

Guidelines for the Use of Fishes in Field Research. American Society of Ichthyologists and Herpetologists (ASIH), American Fisheries Society (AFS), and American Institute of Fisheries Research Biologists (AIFRB).

APPENDIX II

Definitions

Aquatic Nuisance Species - a nonindigenous species that threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural or recreational activities dependent on such waters. Aquatic nuisance species include nonindigenous species that may occur in inland, estuarine and marine waters and that presently or potentially threaten ecological processes and natural resources. In addition to adversely affecting activities dependant on waters of the United States, aquatic nuisance species adversely affect individuals, including health effects.

Biological Safety Officer (BSO) - an individual who is a member of the IBC who has the direct responsibility (after the PI) to ensure the activities and precautions stated in the research proposal are followed. See NIH guideline FR 51 No. 88, pg. 16963, for other roles and responsibilities.

Confinement - a term used primarily in the USDA guidelines meaning organisms restricted to research field facilities such as outside experimental pond areas and involving whole plants and animals.

Containment - a term used primarily in the NIH guidelines to mean restricted to laboratory environments and is usually in reference to micro-organisms, recombinant DNA molecules, or whole plants (Appendix P) or whole animals (Appendix Q).

Established - when used in reference to a species, this term means occurring as a reproducing, self-sustaining population in an open ecosystem, i.e. in waters where the organisms are able to migrate or be transported to other waters.

Institutional Biosafety Committee (IBC) - see NIH guidelines FR 51 No. 88, pg. 16962, for membership, roles, and responsibilities.

Nonindigenous Species - any species or other viable biological material that enters an ecosystem beyond its historic range, including any such organisms transferred from one country to another. Nonindigenous species include both exotics and transplants. [Note: Historic range is interpreted to mean the territory occupied by a species at the time of European colonization of North America.]

Pathogen - as defined in USDA guidelines, is a virus or micro-organism (including its viruses and plasmids, if any) that has the ability to cause disease in another living organism.

Principal Investigator (PI) - see FR 51 No. 88, pg. 16963, for roles and responsibilities.

Research Institution - means any public or private entity (including Federal, state, or local government agencies) conducting the research.

Research Protocol Committee (RPC) will be comprised of one or more representatives from each Federal Task Force agency who are qualified to evaluate nonindigenous species research proposals. Knowledgeable experts from other Federal, state, or private groups with different areas of expertise might be asked to assist the committee.

Surrounding Waters - means any free flowing or standing waters in the immediate vicinity of the research facility that are connected with public waters either directly or indirectly.

Survival - organism able to live in an ecosystem during its normal life span but not necessarily able to reproduce itself.

Unintentional Introduction - an introduction of nonindigenous species that occurs as a result of activities other than the purposeful or intentional introduction of the species involved, such as the transport of nonindigenous species in ballast or in water used to transport fish, mollusks or crustaceans for aquaculture or other purpose. Involved is the release, often unknowingly, of nonindigenous organisms without any specific purpose. The virtually inevitable escapement, accidental release, improper disposal (e.g., "aquarium dumping") or similar releases of intentionally introduced nonindigenous species do not constitute unintentional introductions.

Waters of the United States - the navigable waters and the territorial sea of the United States. Since aquatic nuisance species can move or be transported by currents into navigable waters, all internal waters of the United States, including its territories and possessions, are included. The Territorial Sea of the United States is that established by Presidential Proclamation Number 5928 of December 27, 1988.

APPENDIX III

Membership of the Research Protocol Committee

- Dr. James A. McCann, National Fisheries Research Center-Gainesville, U.S. Fish and Wildlife Service - Chairman, May 1991-Present
- Dr. Althaea Langston, Animal and Plant Health Inspection Service - Policy and Program Development, U.S. Department of Agriculture - Member, May 1991-Present
- Dr. David F. Reid, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration - Member, May 1991-Present
- Dr. Edwin A. Theriot, Environmental Laboratory, Waterways Experiment Station, U.S. Army Corps of Engineers - Member, August 1991-Present
- Dr. J. David Yount, Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency - Member, March 1993-Present

APPENDIX IV**Other Legislation or Executive Orders Related
to the Nonindigenous Aquatic Species Act**

Applicable State Laws, Regulations, Permit and Notification Requirements -
Must be determined on an individual basis by Principal Investigators and
Research Institutions.

Lacey Act of 1900 - 16 USC 3371-3378 and 18 USC 42 Item 2,58

Endangered Species Conservation Act of 1973-16 USC 1531-1543 plus Convention
on International Trade in Endangered Species of Wild Fauna and Flora
(CITES)-16 USC 1531-1543.

Executive Order #11987 dated March 1977 - Exotic Organisms

Plant Quarantine Act of 1912 (7 USC 151 et seq.)

Federal Plant Pest Act of 1957 (7 USC 150aa et seq.)

Federal Noxious Weed Act of 1974 (Public Law 93-629-Jan. 3, 1975) (7 USC 2801
et seq. + 21 USC 111 et seq.)

National Environmental Policy Act of 1969 (NEPA)

Occupational Safety and Health Act of 1970 - Federal Register April 12, 1984
(50FR 14468) (29 USC et seq.)

Animal Welfare Act. 7 USC 2131-2155; 80 STAT.350, 84 STAT.1560, 90 STAT.417,
99 STAT.1645.

TABLE I**Outline of Information Required by Reference Guidelines**

Identification of Principal Investigator and Research Institution

Identification of Species and Source of Research Specimens

Justification for Research

Complete Description and Exact Location of Research Facility

Discussion of the Life History, Biology, Critical Environmental Factors, Ecology, Performance in Areas where Previously Introduced, Present Distribution and Status of the Study Species

Biosafety Level Based on Risk Assessment and Possible Impacts if Species Escapes or is Released

Diseases and Parasites
Identification
List of All Known Diseases and Parasites Found in Waters Where Species Were Taken
Quarantine Facilities/Procedures

Complete Description of Methods used for Physical, Biological, Chemical, and Environmental Containment and/or Scope Limitations

Fate of Surviving Specimens - Close Out Procedures

Required Permits and Related Laws and Regulations

Shipping and Transportation Precautions

Training and Qualifications of Personnel

Security

Emergency Plan and Procedures for Termination of Study

Administrative Control, Roles, Responsibilities

Frequency of Inspections, Monitoring, Compliance Evaluations and Reporting

Life History and Ecological Requirements of the Zebra Mussel - North American Experience Through 1992

by

S.J. NICHOLS
National Biological Survey
1451 Green Rd
Ann Arbor MI 48105
313-994-3331

The rapid spread of zebra mussels (*Dreissena polymorpha*) across the United States is due to their ability to grow and reproduce in a wide range of environmental conditions, coupled with a free-living, planktonic larvae (veliger). When zebra mussels were first discovered in the United States, predictions concerning their habitat requirements were based on the European experience with these bivalves. However, zebra mussel populations in this country have consistently exceeded all expectations and predictions as to how fast they could grow, reproduce, and expand their range. Although many research projects are currently underway to delineate the ecological needs of zebra mussels in the United States, much of these results are not yet published.

The information presented below represents what is currently known about the life history and ecological requirements of zebra mussels. The primary purpose of this information is to emphasize specific features that increase the risk of accidental escape of zebra mussels from research facilities. Data from both on-going research and findings presented in the European literature has been used, although as mentioned earlier, European results have not always been applicable here. The recent discovery of the second type of Dreissenidae, the **quagga**, may complicate the situation since the ecological needs of this mussel are unknown. Based on available information and experience, we have assumed that the basic environmental needs of quaggas are similar to those of zebra mussels.

ADULT MUSSELS: Life History

Mobility. Mussels less than 15 mm in length are very mobile, capable of crawling, drifting, and floating for some time in the water column. Movement is believed to be in response to environmental conditions.

Risk Assessment: Severe. Mussels will crawl into any small crack or crevice, into filter floss, water intake systems, and even up out of the water. The narrowness of their shells enables mussels to pass through small openings. For example, 5-mm-long mussels have been known to crawl through 0.5-mm mesh netting. Extra precautions are needed to prevent contamination of all equipment that is in contact with zebra mussels or water in which zebra mussels are known to be present. Do not assume that netting or coarse filters can prevent escape of small mussels.

Reproduction. Zebra mussel fertilization is external, and spawning can continue over a period of several weeks. Mussel reproduction starts when water temperatures are above 12°C. In most temperate regions, water temperature limits the spawning season to May through September. However, reports from Russia and laboratory studies conducted in this country indicate that spawning continues year-round in areas where water temperatures remain above 12 °C. About 10-15% of zebra mussels will reach sexual maturity at a ventral shell length of 2-3 mm. Most become sexually mature at a ventral shell length of 6 mm.

Risk Assessment: Severe. Laboratory colonies held at water temperatures above 12°C can and will spawn continually, increasing the risk of veligers being present in all wastewater.

Food Supply. Mussels are filter feeders and were initially reported by the Europeans to feed and survive only on live algae. However, research done in this country indicate that zebra mussels consume all types of food, including detritus and zooplankton, as well as their own young, and can therefore grow during periods of time when live algae are unavailable. Also note that mussels can survive for up to 11 months without food under laboratory conditions at 4° C.

Risk Assessment: Moderate. Mussels can colonize areas where live algae is limited or areas where the food supply is intermittent (such as drainage pipes).

Growth. Juvenile mussels are capable of rapidly growing to sexual maturity. Juvenile mussels average only 0.4 mm in ventral shell length just after undergoing metamorphosis, and under optimal conditions can reach 13 mm in less than 3 months. Growth begins when water temperatures are over 3°C.

Risk Assessment: Low. Small mussels will grow to sexual maturity under laboratory conditions even if held at less than 10° C, although spawning has not been reported at such temperatures.

ADULT MUSSELS: Special Handling Problems.

Handling small mussels. Juvenile mussels (less than 1 mm long) are difficult to detect visually without using a microscope. The easiest way to determine if these mussels are present under field conditions is to feel them--they feel like sand grains. They also "stick" to everything, lodging under fingernails, in net handles, on clothing, etc., increasing the risk of accidental release. Extra precautions should be taken to insure proper "decontamination" of all gear, etc. that may have been exposed to juveniles less than 1 mm in shell length.

How to determine if mussels are dead. When mussels die, the shells remain open with body parts exposed. A dull probe can be used to touch mussel tissue to determine if animal is alive or dead. Mussels that float when they are placed in water are not necessarily dead. Live quaggas frequently retain air in the shell valves during handling and will float for hours.

Risk Assessment: Severe. Assume mussels are alive, unless body tissue has sloughed off from the shell.

ADULT MUSSELS: Habitat Needs.

Zebra mussels are very tolerant of a wide range of environmental conditions if certain basic needs are met. The following basic needs and tolerances have been noted in Europe and in the Great Lakes region:

Calcium needs. European research indicates that mussels require 30 ppm dissolved calcium for shell growth and 50 ppm for reproduction. However, laboratory studies done in this country indicate that some growth can occur at 20 ppm and reproduction at 35 ppm. Quagga calcium needs have not been tested, but their shells are noticeably thinner than zebra mussels.

Dissolved oxygen. Oxygen needs of zebra mussels have not been documented. However, mussels have been reported from lakes in Europe where summer oxygen levels are less than 2.0 ppm.

pH. In Europe, zebra mussels usually occur in areas where the pH is over 7.5. The degree of acidity in the water that will be tolerated by zebra mussels will in part be related to calcium levels, and is at this time unknown.

Salinity. European studies indicate that zebra mussels will not live in sea water, but can tolerate estuarine conditions. However, Russian literature indicates that some of the other Dreissenidae are more salt-water tolerant than zebra mussels. At this time, salinity tolerance of the quagga mussel is unknown.

Water temperatures. Mussels can survive in temperatures ranging from below 0° to 35°C, if they are submerged. Mussels exposed to the air have a much narrower temperature range (about 6-28°C). To date, spawning has only been seen when water temperatures are over 12°C.

Water velocity. Mussels are positively attracted to water current and will colonize areas with water velocities up to 2 meters per second.

VELIGERS: Life History.

The physical requirements necessary to insure survival of the free-living larvae or veliger are poorly understood. Much of the information available from the European literature relates to distribution and abundance data rather than physiological studies run under laboratory situations, in part due to the difficulties in handling larvae in the laboratory.

Development. When water temperatures rise above 12° C, adult mussels release eggs and sperm into the water column. After fertilization, developing embryos remain in the water column, and can drift for some distance from the parent colony. The time required to develop from egg to juvenile mussel varies according to water temperature, but averages about 2 weeks under laboratory conditions at 22°C. Studies in Europe have documented the presence of veliger in the water column for up to one year. Initial size at shell formation is approximately 100 microns (some quaggas are smaller at D-shell, under 70 microns), and 300 to 450 microns at metamorphosis.

Risk Assessment: Severe. Since larvae are microscopic, their presence or absence on sampling gear or in samples cannot be determined unless examined under a microscope. Assume that veligers are present if water temperatures are over 12° C.

Mobility. Young larvae have a ciliated organ called a velum that is used for swimming. Older larvae, just before metamorphosis, also have a foot that can be used for crawling. Since the larvae are so small, they are readily picked up by water currents, and can be transported some distance.

Risk Assessment: Severe. Assume that veligers are present if water temperatures are over 12° C. Although veligers are described as planktonic, any object collected in a zebra mussel area during spawning season will have veligers of various ages crawling on it.

VELIGERS: Habitat Needs.

Very little is known about the habitat needs and food requirements of veligers. European literature describes veligers as being very intolerant of a wide range of conditions, and mortality rates of over 99% under field conditions are common. However, since specific habitat needs are not known for this life stage, assume that veligers can survive under the same conditions that are suitable for adult mussels.

Food. Veligers begin to feed just after shell formation. They are filter feeders, consuming algae, bacteria, and detritus. Initially, veligers feed off of particles less than 4 microns in size.

Settling substrate. Proper substrate must be present during the time veligers under metamorphosis, or the larvae will die. Veligers settle on filamentous material first, undergo metamorphosis, and then move to a hard substrate.

Water temperature. Veligers tolerate the same temperature regime as do the adults. Development rate is directly correlated to water temperature. Live larvae have been held at 4° C for up to one week without food.

Water velocity. Water velocities over 2 meters per second discourage the settling of veligers.

FOR FURTHER INFORMATION:

Griffiths, R. Kovalak, W., and Schloesser, D. 1989. The zebra mussel, Dreissena polymorpha (Pallas, 1771), in North America: Impact on raw water users. In Symposium: Service Water System Problems Affecting Safety-related Equipment, held in Charlotte, NC., November 6-8, 1989. sponsored by Nuclear Power division, Electric Power Research Institute, Palo Alto, CA.

Mackie G., Gibbons W., Muncaster B., Gray I. 1989. The zebra mussel, Dreissena polymorpha: A synthesis of European experiences and a preview for North America. Ontario Ministry of the Environment. Toronto, Canada. 76pp.

Nalepa T. and Schloesser D. eds. 1992. Zebra Mussels: Biology, Impacts, and Control. Lewis Publishers. Chelsea Mi.

Ramcharan C., Padilla D., Dodson S. 1992. A multivariate model for predicting population fluctuations of Dreissena polymorpha in North American lakes. Canadian Journal Fisheries and Aquatic Sciences. 49:150-158.

Stanczykowska A. 1977. Ecology of Dreissena polymorpha (Pall.) (Bivalvia) in lakes. Polskie Archiwum Hydrobiologii. 24(4):461-530.