NEWHALL LAND

November 26, 2012

[Via electronic mail]
Mr. Samuel Unger, Executive Officer
California Regional Water Quality Control Board,
Los Angeles Region
320 West Fourth Street, Suite 200
Los Angeles, California 90013
losangeles@waterboards.ca.gov

RE: SUBMITTAL OF PLAN FOR HAZARD ANALYSIS AND CRITICAL CONTROL POINTS for the PREVENTION & CONTROL OF AQUATIC NUSANCE SPECIES
NEWHALL LAND AND FARMING COMPANY
RESOURCE MANAGEMENT AND DEVELOPMENT PLAN
WDR Order No. R4-2012-0139 (File No. 11-168)

Dear Executive Officer Unger:

Transmitted herewith is the first draft of the Plan for Hazard Analysis and Critical Control Points (HACCP) for the Prevention and Control of Aquatic Nuisance Species as required by the Newhall Land & Farming Company Clean Water Act Section 401 Water Quality Certification and Waste Discharge Requirements (WDR) No. R4-2012-0139. The attached HACCP discusses the threat of invasive aquatic nuisance species, specifically the New Zealand Mudsnail (NZMS) which currently threatens waters of the State, and the critical control points where prevention measures are most effective in preventing infestation on the Santa Clara River from the development of Newhall Ranch.

As the control of NZMS is relatively new in our region and has to date focused on recreational users of waters of the State, this plan primarily includes information previously discussed and adopted with the California Department of Fish and Game through the Newhall Ranch RMDP master streambed alteration agreement. As such, this plan is intended to be a working document, incorporating new information from resource agencies as that information is available and applicable to the project activities. Agencies currently implementing education and control measures include the LA Regional Water Quality Control Board, the State Water Quality Control Board, California Department of Fish and Game, and the U.S. Fish and Wildlife Service. Information from other states in the western United States has been reviewed and incorporated where appropriate.

I look forward to working with the Regional Board staff in further developing the HACCP for Aquatic Nuisance Species on the Newhall Ranch development. Should you have any questions regarding this transmittal please do not hesitate to contact Sam Rojas at (661) 255-4283.

Sincerely,

Samuel Rojas
Manager, Environmental Resources
The Newhall Land and Farming Company

Attachment: **DRAFT Plan for Hazard Analysis and Critical Control Points (HACCP) for the Prevention & Control of Aquatic Nuisance Species** (File No. 11-168)

Certification:

"I declare under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who managed the system or those directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations."

Executed on the 26th day of November, 2012 at 25124 Springfield Court, Suite 300, Valencia, California 91355.

(Signature)

Sr. Vice-President, Community Development (Title)

CC: Valerie CarrilloZara, Section 401 Program, LARWQCB
Aaron Allen, US Army Corps of Engineers
Karen Drewe, California Department of Fish and Game

ORDER No. R4-2012-0139
CLEAN WATER ACT SECTION 401 WATER QUALITY CERTIFICATION
AND WASTE DISCHARGE REQUIREMENTS (WDR) FOR:
NEWHALL LAND & FARMING COMPANY
PROPOSED RESOURCE MANAGEMENT AND DEVELOPMENT PLAN AND SPINEFLOWER CONSERVATION PLAN, SANTA CLARITA, LOS ANGELES COUNTY
(File No. 11-168)

DRAFT

Plan For Hazard Analysis And Critical Control Points (HACCP)

For

The Prevention & Control Of Aquatic Nuisance Species

Prepared by: Newhall Land and Farming Company

Dated: November 26, 2012

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1.0 INTRODUCTION

This Hazard Analysis and Critical Control Points (HACCP) plan has been developed in compliance with Condition 15 of Section 3.0 of the Newhall Ranch Resource Management and Development Plan (RMDP) Clean Water Act Section 401 Water Quality Certification and Waste Discharge Requirements (WDR) Order No. R4-2012-0139 (File No. 11-168). This plan is specific for the prevention and control of aquatic nuisance species that are a threat to the Santa Clara River and which could be introduced to the environment during construction activities as described in the WDR. This document is intended to be a DRAFT for discussion with the regulatory agencies having oversight of the RMDP development as well as the statewide effort to control and contain the New Zealand Mudsnail (NZMS).

As documented on Fish and Game's website, NZMS is known to occupy many watersheds in Southern California (Trabuco Creek, Malibu Creek, Piru Creek, several creeks along Hwy 101 corridor in Los Angeles Ventura County Calabasas to Camarillo, and the CA Aqueduct at Lone Pine). The spread of NZMS is primarily attributed to recreational boating and fishing, however, all potential pathways for this invasive nuisance species should be evaluated and controls implemented. Additional information regarding NZMS is available from the California Department of Fish and Game at http://www.dfg.ca.gov/invasives/mudsnail/.

2.0 REGULATORY / PERMITTING BACKGROUND

The Los Angeles Regional Water Quality Control Board included Condition 15 in Section 3.0 of WDR No. R4-2012-0139 (File No. 11-168). This condition specifically requires an HACCP for the prevention and control of aquatic nuisance species that are a threat to the Santa Clara River. The methods of an HACCP are intended to address the release of a contaminant into the environment, more specifically related to the Federal Food and Drug Administration (FDA), however the methods and concepts can be directly applied to the evaluation of pathways and control of the New Zealand Mudsnail that currently threaten beneficial uses of waters of the State. The Master Streambed Alteration Agreement (MSAA No. 1600-2004-0016-R5) issued by Fish and Game for the Newhall Ranch RMDP also contains specific mitigation measures for the prevention and control of New Zealand Mudsnails and other invasive exotic species and weeds. These permit conditions provide education to project personnel informing them of the NZMS threats to the aquatic environment. Biological monitors are required to implement inspection and control requirements on contractor equipment operations to ensure such activities do not

introduce NZMS to the Santa Clara River. These controls include establishing clear project boundaries, restricting work in flowing water (and only under the direction of a biological monitor and with Fish and Game authorization), inspecting equipment for debris, pooled water and leaking petroleum products, in addition to documenting site conditions and maintaining equipment lists. The existing permit conditions have been incorporated into the HACCP process described below.

3.0 HACCP PROCESS

3.1 Task 1 - Establish a HACCP team

The HACCP is to be comprised of internal Newhall Land and Farming personnel, 3rd Party biological monitors, and Agency personnel, specifically:

- Newhall Land & Farming:
  - Sam Rojas, Manager of Environmental Resources
  - Bob Brazell, Vice-President Operations
- Biological Monitors
  - To Be Determined by Project
- California Department of Fish and Game
  - Karen Drewe
- US Fish and Wildlife Service
  - Chris Dellith
- Los Angeles Region Water Quality Control Board
  - Valerie Zara-Carrillo

Team members bring specific expertise in various aspects of project implementation, construction methodologies, risk/threat analysis, control methodologies, and project monitoring and documentation. This plan is intended to be updated as necessary as more information becomes available on this, and other, invasive aquatic species. Information from LA County Sanitation District, Geosyntec Consultants, and other field monitoring consultants has been considered in this plan.

3.2 Task 2 - Describe Construction Industry Vectors

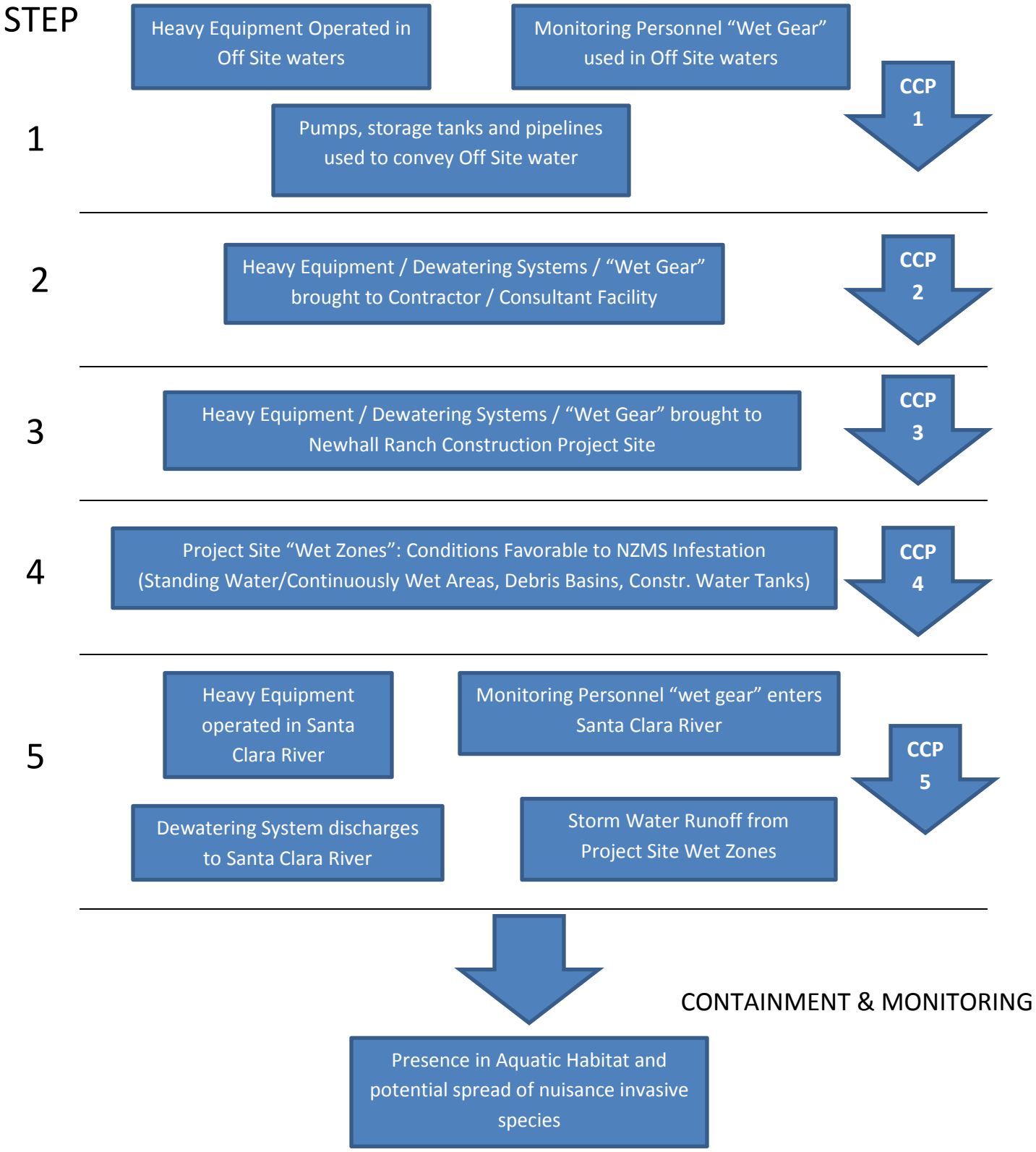
Heavy equipment, personal vehicles, pumps, tanks and pipelines, monitoring personnel "wet equipment" and sample coolers are capable of harboring nuisance invasive species,

specifically New Zealand Mudsnails, and allowing their transport from an infested Off Site location to the Santa Clara River.

3.3 Task 3 – Typical Use of these Vectors on Project Site

Equipment will primarily be operated in upland areas, away from aquatic resources. Barriers are established and maintained at construction sites to inform construction personnel where entry is not permitted. Heavy equipment is used as necessary to construct the RMDP facilities, including bank protection, bridges, storm drain outfalls, water quality control systems and utilities. In addition, subsequent to development of the RMDP project sites, long term maintenance and operation of these facilities may be required. In some instances, equipment will travel or be operated in wetted portions of the Santa Clara River, or in wet environs that are tributary to the Santa Clara River. Pumps, tanks, and water pipelines may be used for construction dewatering, distribution of construction water, or pumping of nuisance ponded water for maintenance work or after storm events. Other activities, not traditionally considered as construction but which occur due to the construction, include surveys for sensitive aquatic species, water sampling, BMP or general site inspections, and other foot traffic by monitoring personnel may require access to aquatic habitats using "wet gear" (such as waders, floats, nets, coolers and other containers).

3.4 Tasks 4 & 5- Flow Diagram (verified)



3.5 Task 6 - Identify / Analyze Hazards - (Principle 1)

The Heavy Equipment, if operated in an area infested with NZMS, could contain mud and pooled water that harbor the nuisance species in transport. NZMS can survive for extended periods in damp soils, therefore, unless NZMS are either dried completely for 48 hours, subjected to heat (10 minute water bath of 120 degrees or flash heat of 140 degrees), treated with chemicals, or kept in freezing temperatures for 6 hours, they may be viable upon reintroduction into a wet environ. Pumps, storage tanks and pipelines, if used to transport or contain NZMS infested waters, present the highest threat of contamination as pump heads and pipe fittings may not be fully emptied of water prior to use on a new project site. These systems may require disassembly to fully inspect for pooled water. Monitoring personnel "wet gear" is also a high threat of contamination due to the potential that "Wet Gear" is likely to be used in Off Site waters for similar survey or monitoring activities. The spread of NZMS by recreational water users (fishing, boating, hiking) is well documented, and infestation in many cases is attributed to "Wet Gear".

Threats from the RMDP are limited to construction activities that actually occur in wet environs with an actual pathway for discharge to the Santa Clara River. Storm water runoff can become a

(1) The risk from Heavy Equipment is very low. Heavy Equipment is generally delivered to a Project Site free of debris. Furthermore, Heavy Equipment is likely to be staged on the project site for several days prior to use. As nearly all construction occurs in dry upland areas, Heavy Equipment transporting viable NZMS into an aquatic habitat would be rare occurrence on the Project Site.

(2) Pumps, tanks, pipelines and fittings used for conveying construction water or for construction dewatering present the highest risk of spreading NZMS. Lack of due diligence during inspection of such equipment could result in the use of dewatering systems that are harboring NZMS and could result in direct discharge to the Santa Clara River. Water conveyance systems used for construction water could result in the spread of NZMS. If the pumps or pipelines contain NZMS they could be transported to water trucks used for dust control and could be spread over the Project Site during routine road and excavation watering operations. Any continuously moist/damp areas of the project site could then harbor NZMS. If wet areas of the Project Site are allowed to become infested with NZMS, storm events could then transport the invasive species into the Santa Clara River. Although not likely to be used at the Project Site, improper containment of debris and water from equipment cleaning presents a very high risk of spreading NZMS. Failure to properly contain wash water or by not controlling debris containing viable NZMS

could result in infestation on the Project Site and subsequent transport of the species to the Santa Clara River.

California Department of Fish and Game identifies several areas in the general vicinity of Newhall Ranch that have NZMS infestations ongoing. The closest location is Piru Creek, below Pyramid Lake. Other locations near Malibu, Long Beach, Mission Viejo, the Aqueduct in Lone Pine and locations in northern California are also potential sources. There are also areas that have yet to be surveyed for NZMS or where infestation has not yet been reported on CDFG's map (see Link below):

http://nas.er.usgs.gov/taxgroup/mollusks/newzealandmudsnaildistribution.aspx

Most efforts by regulatory agencies are directed at anglers and boating, and the outdoor enthusiasts have been well informed of the NZMS threat. Biologists and water quality monitoring consultants are well aware of the threat NZMS present to the environment and most have "wet gear" cleaning procedures to prevent spread of NZMS.

At this time, the biggest threat of NZMS presented by the construction industry is a lack of awareness of the issue. Using a bulldozer to clean a debris basin infested with NZMS in Calabasas and then using that same machine a day or two later on a similar wet environment in the Santa Clara Valley could result in introduction of the invasive species. Newhall Land, in conjunction with Fish and Game staff, brought the issue of NZMS to the RMDP with the MSAA in 2010. Newhall Land has already implemented an information and training program for consultant and construction personnel that access Newhall Ranch for surveys, monitoring, and geotechnical investigation work. This training includes a discussion of prevention and control of invasive nuisance species such as NZMS.

3.6 Task 7 - Determine the critical control points (CCPs) - (Principle 2)

The Critical Control Points (CCPs) represent the locations where vectors and pathways can be identified. For NZMS these are generally represented by a Vector coming in contact with NZMS infested waters and then being transported to an un-infested watershed. Five (5) CCPs have been identified. Others may be identified as this plan is implemented.

CCP1 represents the transport Heavy Equipment, Pumps, or "Wet Gear" from a project with NZMS (such as a biologist using waders to monitor in Trabuco Creek). As travel is likely along highways and roads, spreading of NZMS is remote. This stage of the life cycle is also entirely out of Newhall Land Project Site influence or control.

CCP2 represents the return of equipment to a controlled location, such as storage yard or consultant's office. As shown in the flow chart above, CCP2 is the best opportunity to control the spread of NZMS. At this point, Heavy Equipment and Pumps could be thoroughly cleaned at a construction yard, remote from any natural environs. Pumps, tanks and pipeline materials could be thoroughly drained of all water, and could be chemically treated if use was in a location known to have NZMS infestation. Existing Newhall Ranch permit mitigation measures promote control at this stage (see BIO-52). With regard to monitoring personnel, many consulting firms have implemented NZMS protocols, ensuring "Wet Gear" is decontaminated prior to reuse. Established methods of cleaning, cryo-freezing, hot water baths, and use of chemical are readily known and available. Newhall Land has also implemented a training program to ensure Consultants accessing Newhall Ranch have a control plan in place prior to accessing wet environs. Contractor Construction Specifications and Consultant's Scope of Work can easily incorporate BIO-52, and other related permit conditions, to enact this initial point of control. A verification program for heavy equipment, pumps, tanks and pipelines would still be needed.

CCP3 represents the delivery of Heavy Equipment, Pumps and other water conveying equipment to the Project Site. It is the first opportunity for trained monitoring personnel to inspect the equipment prior to its use. At this stage control of nuisance aquatic species is documented by the biological monitor. Heavy Equipment inspections document the presence of any mud caked on the undercarriage, blade, bucket, and wheels as well as determining if any pooled or standing water is present. Construction personnel vehicles will also be inspected for mud on a routine basis as an additional precautionary measure (weekend trail enthusiasts). Pumps, tanks, pipelines and fittings would be visibly inspected for pooled water and presence of NZMS or larvae. When necessary, pump heads may require removal to inspect the pump housing for pooled water, although verification of other treatment may be preferred (such as heating to 140 degrees F, or chemical treatment, other method to verify free of moisture for 24 hours). These visual inspections represent the primary control for CCP3.

Monitoring personnel "Wet Gear" inspection is performed by the respective consultant as such personnel are trained specifically for awareness and control of NZMS, and may not access the river during hours of general construction (alternative access points, and night/ evening/early morning work hours by biological surveys and water quality monitors). Each consultant must adhere to protocols for work in the Santa Clara River established in consulting agreements.

CCP4 represents a "lying in wait" status of a Project Site area that has conditions favorable to the harboring of NZMS (such as a wet area near construction water filling stations, storm water debris basins, low points, subdrain outlets, and nuisance flows at post development

storm drain outlets). These areas represent potential breeding areas for NZMS which in a storm event could be transported to the Santa Clara River. The easiest method of control for such environments is to prevent their establishment or eradicate them when they do develop.

CCP5 represents the point of introduction of NZMS to a wet environment at the Project Site. All Heavy Equipment proposed to be used in wet portions of the Project Site are subject to additional inspection by the monitoring biologist to verify equipment is free of debris and petroleum product leaks. This additional inspection represents the most critical point in vector pathway and is the last point of control to prevent NZMS entry into an environment where it can survive via Heavy Equipment.

For any work within wet areas of the Santa Clara River, additional biological monitoring is required, placing more inspection of equipment accessing the wetted area. The obvious exception is the use of construction dewatering systems that discharge directly to a surface water. Once installed, portions of these systems are not easily inspected (such as down well pumps, pipelines and fittings). Additional inspections with Fish and Game are required of the dewatering system prior to discharge to the river, which represents another opportunity for inspection and documentation of the absence of NZMS. The use of contaminated "wet gear" by monitoring personnel represents the highest risk at this stage as personnel would directly access the Santa Clara River for survey or sampling purposes.

3.7 Task 8 - Establish critical limits for each ccp - (Principle 3)

Upon delivery of equipment to a RMDP project site, the visual inspection will determine if mud or water is present on the equipment. All equipment will be subject to inspection, including monitoring personnel (ie., waders and sampling equipment) and construction personnel vehicles. Access by vehicles and personnel during non-construction periods must rely on the training and protocols established for "wet gear". No construction vehicles are allowed to access wet areas without biological monitor being present. Vehicles with mud cake will be sent for cleaning and/or treatment. The criteria for entry into the project will be no mud and no collected water (such as in pump heads or pipelines). Prior to utilizing equipment in flowing water of the Santa Clara River, additional visual inspections will be performed and equipment, even if already operated at the site and cleared for initial use, will be subject to inspection for presence of mud and cleaned if mud is present.

3.8 Task 9 - Establish a monitoring procedure - (Principle 4)

RMDP projects require a biologist to monitor construction. Monitor(s) will be instructed in this control plan and trained to visually inspect Heavy Equipment and Pumping equipment, such as pumps, tanks, fittings and pipelines. As a component of daily construction monitoring and recordkeeping, on site equipment inventory, results of new equipment inspections, inspections of perimeter fencing and other project site conditions are documented. Logging in of equipment delivery to the project site is the most critical control point for documentation. Documentation that the equipment being used on the site is free of NZMS is paramount to an effective control plan. Once such a determination is made, or equipment has been adequately treated for NZMS, there is no potential for that equipment to contain NZMS and therefore should present no threat. Keeping a correct inventory of equipment used on the site and whether that equipment leaves the site and comes back without re-verification is critical to using "Cleared for Project Entry" recordkeeping as the primary control.

3.9 Task 10 - Establish corrective action - (Principle 5)

Routine review of the implementation of the control plan will inform future corrective actions. During the Critical Control Points 1 through 3, corrective action is simply one of directing equipment to cleaning / decontamination and documenting "cleared for project entry". The adequacy of entry inspections can be verified through spot checking by supervisory staff and review of the process with the HACCP team members. In the event of a documented release of NZMS to a wet environ, several options are available, all of which are aimed at containment. No current method of eradication is available, although further information regarding containment is available at:

(http://cisr.ucr.edu/new_zealand_mud_snail.html)

If NZMS is determined to have been present on equipment that accessed a wet environ, this is the point where containment, agency notification, monitoring and other additional response actions would be necessary. In swift moving waters, transport of NZMS downstream would be likely and few control options would be viable. If debris from equipment entered could be collected from the ground, that is the immediate response action to be taken.

1) ***If a wet environment on the Project Site is discovered with NZMS, Immediately isolate any flowing water from entering or existing the area. Contain the area with berms if necessary to preclude storm water run-on or run-off from occurring. Notify Fish and Game. Remove all ponded water and wet soils to a location remote from the Santa Clara River. Bury the materials in a deep construction fill or otherwise spread and fully dry the material in an upland area secure from storm water run-on or run-off. Document the occurrence and inspect all equipment on site for NZMS presence.***
2) ***If release is from Heavy Equipment not directly to Santa Clara River, to the extent feasible, hydraulically isolate the area from connection with the Santa Clara River. Collect debris if possible. Contain and remove water if feasible and acceptable to Fish and Game and US Fish and Wildlife Service.***
3) ***If release is from Heavy Equipment or Dewatering discharge directly to swift moving water of the Santa Clara River, Immediately move the equipment to upland area and notify Fish and Game and US Fish and Wildlife Service.***
4) ***If release is from dewatering system, Immediately suspend pumping operations. To the extent feasible and without disrupting river flow, collect any surface water that may be present at the discharge location (ie., stilling basin, discharge channel). Slow moving water is less likely to carry NZMS further downstream, and those areas should be isolated and dewatered.***

If a release is documented as likely to have occurred (NZMS found in equipment after entry has taken place), the portion of the Santa Clara River where the release is likely to have occurred shoud be monitored for the presence of Mudsnail in consultation with Fish and Game and US Fish and Wildlife Service. Newhall Land would implement containment methods determined appropriate for the environment in consultation with Fish and Game, US Fish and Wildlife Service and LA Regional Water Quality Control Board. The Fish and Game MSAA includes permit conditions for the monitoring and control of other aquatic invasive species (such as the African Clawed Frog). Such measures could be augmented to include NZMS in the event of an infestation.

3.10 Task 11 - Verify the HACCP plan - (Principle 6)

Water quality monitoring required by existing NPDES permits in the vicinity of Newhall Ranch (Valencia WWTP, Newhall Ranch WWTP, Newhall Ranch Specific Plan) include Benthic macroinvertebrate monitoring of the Santa Clara River. An infestation of NZMS would be identified during such monitoring. Monitoring for the spring snail (***Pyrgulopsis***

castaicensis) required by the Fish and Game MSAA (Middle Canyon Spring) may also be a vehicle to document presence/absence of NZMS.

3.11 Task 12 - Keep record - (Principle 7)

As required by the Fish and Game MSAA, biological construction monitoring of each project completed under the RMDP is required. Daily records of project activities, including results of equipment inspections and details of any work completed in wet areas or flowing water of the Santa Clara River are kept and provided to Fish and Game staff. These records will include the documentation of heavy equipment, dewatering systems, and consultant "wet gear" described above. Training pursuant to these requirements is also further described and will be documented in accordance with BIO-52 (Worker Environmental Awareness Program or WEAP).

4.0 SUMMARY / CONCLUSIONS

This document has been prepared to document the process by which certain vectors and pathways were evaluated and specific controls determined for implementation for the prevention and control of aquatic nuisance species and instruct construction and maintenance personnel in accordance with an HACCP Plan process. The plan is considered a working document and subject to revision and augmentation as necessary.

The plan determined in the process above will be implemented during all aspects of development of the Newhall Ranch RMDP and will reduce the potential for the spread of NZMS. The threat of introduction of NZMS during project clearing and construction and future maintenance activities will primarily be controlled through contractor education and heavy equipment and dewatering system (pumps, tanks and pipeline) inspections prior to use. Newhall Land contractors are being instructed to clean all heavy equipment (including wheels, tracks, undercarriages, and bumpers, as applicable) before delivery to the Project Site. Inspections of equipment will be documented and include the following:

(1) vegetation clearing equipment (skid steer loaders, loaders, dozers, bacld1oes, excavators, chippers, grinders, and any hauling equipment, such as off-road haul trucks, flat bed, or other vehicles);

(2) earthmoving equipment (scrapers, dozers, excavators, loaders, motor-graders, compactors, bacld1oes, off-road water trucks, and off-road haul trucks);

(3) all Project-associated vehicles (including personal vehicles) that, upon inspection by the project biologist, are deemed to present a risk for spreading Mudsnails;

(4) dewatering system components (pumps, tanks, fittings, and pipelines); and

(5) surveying and monitoring consultant "Wet Gear" (Waders, floats, nets, coolers).

Various methods of determining equipment is free of NZMS are presented above with the primary being visual inspection. Certification by the contractor that equipment was treated off site would still require visual confirmation upon delivery to the Project Site. In this manner, equipment caked with mud or containing standing water is simply assumed to contain NZMS and is directed for further cleaning.

Although not a preferred method (see discussion above), equipment may be cleaned on site. A designated area with containment, collection of wash water and directing wash water to legal point of disposal (eg., WWTP), and proper handling of debris are all If equipment is washed on site, a written daily log shall be kept for all vehicle/equipment washing that states the date, time, location, type of equipment washed, methods used, and location of work. Records of equipment inspections will be kept by the monitoring biologist and provided to Fish and Game in accordance with the MSAA. Issues of non-compliance with these requirements are required to be reported to the RWQCB (Executive Officer) and Fish and Game.

Attachment A
Excerpt, Section 3.0, Condition No. 15
Order No. R4-2012-0139
Section 401 WQ Certification and WDR
(File No. 11-168)

15. Aquatic Nuisance Species Control.

Newhall Land shall develop and implement a Plan for Hazard Analysis and Critical Control Points (HACCP Plan) in order to implement prevention and control of aquatic nuisance species and instruct construction and maintenance personnel in HACCP Plan provisions. The draft HACCP Plan shall be submitted to the Regional Board 401 Certification Unit staff within two months after issuance of this Order.

To reduce the potential for the spread of New Zealand Mudsnails, or other aquatic nuisance species of concern, during Project clearing and construction, all heavy equipment proposed for use on the Project site shall be verified cleaned (including wheels, tracks, undercarriages, and bumpers, as applicable) before delivery to the Project site. Equipment must be documented as Mudsnail free upon delivery to the Project site initial staging area, including:

(1) vegetation clearing equipment (skid steer loaders, loaders, dozers, bacld1oes, excavators, chippers, grinders, and any hauling equipment, such as off-road haul trucks, flat bed, or other vehicles);

(2) earthmoving equipment (scrapers, dozers, excavators, loaders, motor-graders, compactors, bacld1oes, off-road water trucks, and off-road haul trucks); and

(3) all Project-associated vehicles (including personal vehicles) that, upon inspection by the project biologist, are deemed to present a risk for spreading Mudsnails.

Equipment shall be cleaned at existing construction yards or at a wash station and equipment that has been in Mudsnail impacted areas shall be required to dry out in the sun for a period of no less than 48 hours prior to use in other areas.

The biological monitor shall document that all construction equipment (as described above) has been properly cleaned and dried prior to working within the Project work site. Any equipment/vehicles determined to not be free of Mudsnails shall immediately be sent back to the originating construction yard for washing and proper drying, or wash station where rinse water is collected and disposed of in either a sanitary sewer or other legal point of disposal. Equipment/vehicles moved from the site must be inspected, and re-washed and re-dried as necessary, prior to re-engaging in construction activities in the Project work area.

A written daily log shall be kept for all vehicle/equipment washing that states the date, time, location, type of equipment washed, methods used, and location of work.

Attachment B
California Department of Fish and Game
MSAA Notification No. 1600-2004-0016-R5
Exhibit 2. MSAA Implementation Plan

Newhall Ranch RMDP Mitigation Measure BIO-52

(1) Prior to grading and construction activities, a qualified biologist shall be retained to conduct a Worker Environmental Awareness Program (WEAP) for all construction/contractor personnel. A list of construction personnel who have completed training prior to the start of construction shall be maintained on site and this list shall be updated as required when new personnel start work. No construction worker may work in the field for more than five days without participating in the WEAP. Night work and use of lights on equipment shall not be allowed unless CDFG approves of the night work and use of lights. Lighting shall not be used where threatened or endangered species occur. Lights shall be directed from natural areas and remain 200 feet away from natural areas unless otherwise approved by CDFG.

(2) The qualified biologist shall provide ongoing guidance to construction personnel and contractors to ensure compliance with environmental/permit regulations and mitigation measures. The qualified biologist shall perform the following:

- Provide training materials and briefings to all personnel working on site. The material shall include but not be limited to the identification and status of plant and wildlife species, significant natural plant community habitats (e.g., riparian), fire protection measures, and review of mitigation requirements.
- A discussion of the federal and state Endangered Species Acts, Bald and Golden Eagle Protection Act, Migratory Bird Treaty Act, other state or federal permit requirements and the legal consequences of non-compliance with these acts;
- Attend the pre-construction meeting to ensure that timing/location of construction activities do not conflict with other mitigation requirements (e.g., seasonal surveys for nesting birds, pre-construction surveys, or relocation efforts);
- Conduct meetings with the contractor and other key construction personnel describing the importance of restricting work to designated areas. Maps showing the location of special-status wildlife or populations of rare plants, exclusion areas, or other construction limitations (e.g., limitations on nighttime work) will be provided to the environmental monitors and construction crews prior to ground disturbance. This applies to preconstruction activities, such as site surveying and staking, natural resources surveying or reconnaissance,
- Discuss procedures for minimizing harm to or harassment of wildlife encountered during construction and provide a contact person in the event of the discovery of dead or injured wildlife;
- Review/designate the construction area in the field with the contractor in accordance with the final grading plan;

Attachment B
(cont)

• Ensure that haul roads, access roads, and on site staging and storage areas are sited within grading areas to minimize degradation of vegetation communities adjacent to these areas (if activities outside these limits are necessary, they shall be evaluated by the biologist to ensure that no special status species habitats will be affected);

(3) Conduct a field review of the staking (to be set by the surveyor) designating the limits of all construction activity;

• Flag or temporarily fence any construction activity areas immediately adjacent to riparian areas;

• Ensure and document that required pre-construction surveys and/or relocation efforts have been implemented; establishment of water quality BMPs, and geotechnical or hydrological investigations;

(4) To reduce the potential for the spread of exotic invasive invertebrates (e.g. New Zealand Mudsnails) and weeds (including weed seeds) during Project clearing and construction, all heavy equipment proposed for use on the Project site shall be verified cleaned (including wheels, tracks, undercarriages, and bumpers, as applicable) before delivery to the Project site. Equipment must be documented as exotic-invasive-invertebrate (e.g. Mudsnail)- and weed-free upon delivery to the Project site initial staging area, including:

(a) vegetation clearing equipment (skid steer loaders, loaders, dozers, backhoes, excavators, chippers, grinders, and any hauling equipment, such as off-road haul trucks, flat bed, or other vehicles);

(b) earth-moving equipment (scrapers, dozers, excavators, loaders, motor-graders, compactors, backhoes, off-road water trucks, and off-road haul trucks); and

(c) all Project-associated vehicles (including personal vehicles) that, upon inspection by the monitoring biologist, are deemed to present a risk for spreading exotic invasive invertebrates (e. g. Mudsnails) or weeds. Equipment shall be cleaned at existing construction yards or at a wash station.

(5) The biological monitor shall document that all construction equipment (as described above) has been cleaned prior to working within the Project work site. Any equipment/vehicles determined to not be free of exotic invasive invertebrates (e. g.Mudsnails) and weeds shall immediately be sent back to the originating construction yard for washing, or wash station where rinse water is collected and disposed of in either a sanitary sewer or other legal point of disposal. Equipment/vehicles moved from the site must be inspected, and re-washed as necessary, prior to re-engaging in construction activities in the Project work area. A written daily log shall be kept for all vehicle/equipment washing that states the date, time, location, type of equipment washed, methods used, and location of work;

• Be present during initial vegetation clearing and grading; and

• Submit to CDFG an immediate report (within 72 hours) of any conflicts or errors resulting in impacts to special-status biological resources.

Attachment C
California Department of Fish and Game
MSAA No. 1600-2004-0016-R5
Appendix A – RMDP Maintenance Manual of Exhibit 1. Newhall Ranch RMDP

RMDP Maintenance Manual Procedure A-18:

To reduce the potential for the spread of New Zealand Mudsnails and weeds (including weed seeds) during Project clearing and maintenance, all heavy equipment proposed for use on the Project site shall be verified cleaned (including wheels, tracks, undercarriages, and bumpers, as applicable) before delivery to the Project site. Equipment must be documented as Mudsnail and weed free upon delivery to the Project site initial staging area, including:

(1) vegetation clearing equipment (skid steer loaders, loaders, dozers, backhoes, excavators, chippers, grinders, and any hauling equipment, such as off-road haul trucks, flat bed, or other vehicles);

(2) earth-moving equipment (scrapers, dozers, excavators, loaders, motor-graders, compactors, backhoes, off-road water trucks, and off-road haul trucks); and

(3) all Project-associated vehicles (including personal vehicles) that, upon inspection by the monitoring biologist, are deemed to present a risk for spreading Mudsnails or weeds. Equipment shall be cleaned at existing construction yards or at a wash station. The biological monitor shall document that all construction equipment (as described above) has been cleaned prior to working within the Project work site. Any equipment/vehicles determined to not be free of Mudsnails and weeds shall immediately be sent back to the originating construction yard for washing, or wash station where rinse water is collected and disposed of in either a sanitary sewer or other legal point of disposal.

Equipment/vehicles moved from the site must be inspected, and re-washed as necessary, prior to re-engaging in maintenance activities in the Project work area. A written daily log shall be kept for all vehicle/equipment washing that states the date, time, location, type of equipment washed, methods used, and location of work (BIO-52).

Appendix 1

National Management and Control Plan

for New Zealand Mudsnail (2007)

National Management and Control Plan for the New Zealand Mudsnail (*Potamopyrgus antipodarum*)

Photo by Dan Gustafson, Montana State University

Prepared for the Aquatic Nuisance Species Task Force by the New Zealand Mudsnail Management and Control Plan Working Group

May 2007

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Executive Summary

The New Zealand mudsnail (*Potamopyrgus antipodarum*) (NZ mudsnail) is indigenous to New Zealand and its adjacent islands. In New Zealand, the snails have been found in nearly every aquatic habitat including large river, forested tributary streams, thermal springs, ponds, glacial lakes and estuaries. Over the past 150 years, NZ mudsnails have spread in three continents.

Three different clones of New Zealand mudsnails have been identified in the United States: the first clone is found in nine western States, having spread out from an initial population in the Snake River in Idaho; the second clone is found in Lakes Ontario, Erie and Superior and is the same as Clone A found in Europe; and the third has recently been identified in the Snake River near Bliss, Idaho. It is speculated that the eastern U.S. clone came in ballast water from Europe and the western U.S. clones came from the commercial movement of aquaculture products such as trout eggs or live fish from Australia or New Zealand.

The introduced populations of these tiny snails (up to 6 mm) are mostly all female and the snails are live bearers. Males are present only rarely in North America. Densities of NZ mudsnails fluctuate widely, reaching 500,000 snails/ m^2 in some locations.

A database established on the New Zealand Mudsnail in the Western USA web site (http://www.esg.montana.edu/aim/mollusca/nzms/) is being used to track new populations and keep people informed about the latest research. A map showing affected watersheds is kept current by the Department of Ecology at Montana State University-Bozeman.

In 2003, the Aquatic Nuisance Species Task Force (ANSTF) established the NZ Mudsnail Management Plan Working Group (Working Group) to create a national management and control plan. The Working Group met three times in Bozeman, Montana in August of 2003, 2004 and 2005. The goal of the National Management Plan for NZ mudsnails is to prevent and delay the spread to new areas, reduce the impacts of existing and new populations, and continue developing information to meet this goal. The Working Group developed the following objectives:

1. Identify foci, pathways and vectors
2. Develop methods of detecting new populations
3. Develop strategies and methods to control and manage populations
4. Develop further understanding of ecological and economic impacts
5. Increase public understanding of the need to deal with NZ mudsnails and gain political support for implementing national plan objectives.

The Stop Aquatic Hitchhikers and Habitattitude campaigns developed by the ANS Task Force are outreach tools to prevent the spread of NZ mudsnails. Hazard Analysis and Critical Control Point – Natural Resource Management (HACCP-NRM) planning is another general tool for managing invasive species pathways. HACCP-NRM plans identify potential pathways of introduction of invasive species and identify how the pathways can

be broken to prevent the introduction. Development and implementation of HACCP-NRM plans for activities likely to transport NZ mudsnails can significantly reduce spread.

Research to better understand the ecological impacts of NZ mudsnails on macroinvertebrates and higher tropic levels is moving forward. Research on possible control and containment methods continues. Because one of the major pathways of spread appears to be anglers, additional effort has concentrated on the best ways to eliminate the snails from fishing gear. It is clear that management decisions need to be made to prevent the spread of this invasive species prior to completely understanding the impacts.

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I. Purpose and Organization of the New Zealand Mudsnail National Management and Control Plan

The purpose of this National Management and Control Plan (NMP) is to guide the Aquatic Nuisance Species Task Force (ANSTF) and other interested parties in managing the New Zealand mudsnails (*Potamopyrgus antipodarum*) (NZ mudsnail) already present in U.S. waters as well as to prevent and delay the spread of NZ mudsnails to new areas. The ANSTF is an intergovernmental entity established under the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Act, 6 USC. 4701-4741), as amended by the National Invasive Species Act of 1996 (NISA). The ANSTF is co-chaired by the U.S. Fish and Wildlife Service (FWS) and the National Oceanic and Atmospheric Administration (NOAA). The ANSTF is responsible for coordination of national efforts to prevent the introduction and spread of aquatic invasive species. Among these responsibilities is the development of management plans for specific high-risk invasive species.

Following an initial NZ mudsnail meeting in Yellowstone National Park in 1998, three biologists sent a report about the research on the species to the ANSTF. They made a recommendation to create a national management plan to include specific research and public education objectives. The response in a November 2, 1998 letter from the ANSTF executive secretary, Robert Peoples, was that "based on available information, the ecological concerns raised in the report do not clearly indicate that the New Zealand mudsnail threatens or is likely to threaten the diversity or abundance of native species or the ecological stability of infested waters or human water dependent on those waters." At that time no action was taken by the ANSTF, although research and education activities in agencies and at universities continued.

In 2003, the ANSTF requested that a NZ mudsnail Management and Control Plan Working Group (Working Group) be established to create a national management and control plan. The Working Group met three times in Bozeman, Montana in August of 2003, 2004 and 2005. In 2003 and 2005, the Working Group met immediately following the annual NZ Mudsnail in the Western USA conferences.

The Working Group, composed of people from universities, state and federal agencies, private industries and non-profits, worked together to research available information regarding biology, distribution, and ecological impacts of the mudsnails. The goal of the NZ mudsnail NMP is to prevent and delay the spread of NZ mudsnails to new areas, reduce the impacts of existing and new populations, and continue developing information to meet this goal. The Working Group developed the following objectives for the Plan:

1. Identify foci, pathways and vectors
2. Develop methods of detecting new populations
3. Develop strategies and methods to control and manage populations
4. Develop further understanding of ecological and economic impacts

5. Increase public understanding of the need to deal with NZ mudsnails and gain political support for implementing national plan objectives.

The plan has four sections. Section I is the Introduction, which describes the NZ mudsnail clones and summarizes their biology and ecology. The last part of the Introduction includes a summary of state and federal regulations and state invasive species plans that affect what can be done or must be done regarding NZ mudsnails.

Section II includes a broad discussion of the five objectives expanding on prevention, detection, impacts, eradication and control. Education and outreach, implementation tasks and research needs are integrated into the discussion of each objective.

Section III summarizes and prioritizes management actions, education and outreach, research needs and funding that have been covered in the previous sections.

The Appendices give more extensive information about NZ mudsnail distribution, life history, biology and ecological impacts, further details on state and federal regulations regarding NZ mudsnails, an example of a risk assessment for a federal fish hatchery fisheries operation, and instructions for cleaning wading and other gear. The last appendix is a bibliography.

On May 24, 2006, the ANSTF approved publishing the Draft New Zealand Mudsnail NMP for public comment. The Federal Register of October 19, 2006 notified the public of the availability of the document and requested comments on it. The comment period ended December 4, 2006. A final draft, incorporating the comments received during the comment period, was presented to the ANSTF at its May 2007 meeting in Erie, PA and approved by the ANSTF.

II. Introduction

The New Zealand mudsnail (*Potamopyrgus antipodarum*) (NZ mudsnail) is spreading rapidly in the western United States with several new populations being discovered every year. The snails first appeared near Hagerman Idaho, and were documented by S.W. Taylor in 1987 (Bowler 1991). A separate population was first discovered in Lake Ontario in 1991. The western and eastern clones are different and probably arrived in the U.S. through different pathways. This species, which is indigenous to New Zealand and its adjacent islands (Winterbourn 1970b), is now found in Australia and is widespread in Europe where it was misidentified for many decades. This species is found in the literature under the various names of *Potamopyrgus antipodarum*, *P. jenkensi*, *P. niger* and *Hydrobia jenkensi.*

A. Description

The NZ mudsnail shell is normally horn-colored, but ranges from light to dark brown (similar to most freshwater snails). The shell is rather elongate compared to many western North American species. Like most snails, its whorls are dextral (opening to the animal's right). The shell of a full-grown NZ mudsnail normally has 5 or 6 whorls, a higher number than most western North American snail species. Almost all western populations reach a maximal size very near 5 mm. One population in Idaho (Cassia Creek of the Raft River) regularly reaches 6 mm. Other populations which are not monitored as closely may achieve similar sizes.

Figure 1. Like all prosobranchs, the NZ mudsnail has an operculum or covering to block the shell opening when the animal is withdrawn into its shell. (Photo from Dan Gustafson)

Preliminary identification of introduced populations is facilitated by noting that NZ mudsnail populations are mostly all female and the snails are live bearers. In populations that have been examined, males are present only rarely in western North America. The developing young are easily observed within a brood pouch inside the first whorl of the shell of most adult snails. The embryos are normally well-developed in the summer and fall.

Many clones of *Potamopyrgus antipodarum* are known from New Zealand. Prior to September 2005, all western U.S. NZ mudsnails were thought to be identical, representing

a single introduction from New Zealand or Australia (US 1). However, a second clone is known from the eastern U.S. (US 2) and a third clone is now known from a short section of the Snake River of Idaho (US 3).

US 3 represents a second introduction in the western U.S., but very little is known at this time. The snail has been in the Snake River area from several years at least. Preliminary genetic work by Mark Dybdahl verifies that it is a separate genotype, but he did not find a match to any other known invasive genotype from around the world (pers. comm.). Work continues in this area. Ecologically, the two clones look like two species. They overlap in range and where they co-occur, US 3 dominates US 1.

Figure 2. Two western U.S. NZ mudsnail clones. The right picture is the second clone (US 3) discovered in 2005 in the Snake River, Idaho. (Photo from Dan Gustafson)

The two western North American NZ mudsnails clones are about the same size (5-6 mm). US 3 (right) is distinctly broader than US 1. It also has a relatively larger last whorl. The aperture is also relatively larger and the basal lip is more extended. US 3 is normally paler in shell color and therefore more transparent to internal structures. In mixed samples (and all samples with the second clone are mixed), the two clones are easily separated as there are no intermediates.

Figure 3. Shell carina. Note the difference in raised carinas in the two clones. (Photo from Dan Gustafson)

On US 1 (left), there is a slightly raised carina on the shell of some individuals in some populations. On US 3 (right), the carina, if present, is much raised and broken into isolated scales, like the triangular points on a simple crown. The carina is present in some US 3 individuals at all known collection sites. The location of the carina is about the same in both clones. All native western U.S. hydrobiids lack such a carina entirely.

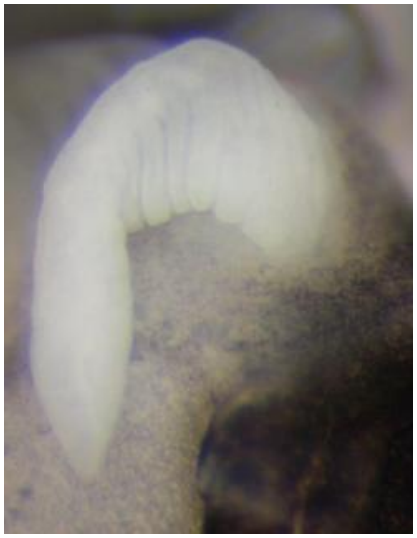

Figure 4. Penis of a male NZ mudsnail. The origin and role of the males is not known, but normal sexual reproduction seems unlikely. The penis is about the same in both of the western clones. (Photo from Dan Gustafson)

In the US 1 clone, males are very rare. Visual identification under a stereomicroscope is used to determine sex (Figure 4). Many populations seem to lack males entirely. In the US 3 clone, males are much more common. Finding a dozen or more is normal at all locations. Within the range of US 3, no males of US 1 have been seen. Preliminary results by Mark Dybdahl suggest that the US 3 is triploid, just like US 1.

The Eastern U.S. clone of New Zealand mudsnails (US 2) is the same as European clone A (Mark Dybdahl, pers. comm.). The clone has been found in three of the Laurentian Great Lakes as well as in parts of the St. Lawrence River. Morphologically, the clone is very similar to US 1 (Figure 5). The average length of adults is about 4.4 mm in one location (Wilson, NY) in Lake Ontario (Levri, unpublished data). About 10% of individuals collected at one site (Wilson, NY) in Lake Ontario show short spines (Levri unpublished data).

Figure 5. NZ mudsnail clone from the Great Lakes. (Photo from Ed Levri)

B. Summary of Biology and Ecology

In New Zealand the NZ mudsnail is dioecious (separate male and female sexes) and bears live young (Winterbourn 1970a, b, Wallace 1978). Ova develop within the female's brood pouch and emerge into the environment as fully functional snails. Female mudsnails in New Zealand may be either sexual or asexual. Asexual females develop eggs that can grow without fertilization and produce cloned genetically identical offspring. Therefore, one female is sufficient to initiate a new population. Although NZ mudsnails reproduce both sexually and asexually in New Zealand, introduced populations are entirely clonal (Zaranko et al 1997, M. Dybdahl unpublished data).

Figure 6. Female NZ mudsnail with brood. (Photo from Dan Gustafson)

NZ mudsnails have great potential for wide-spread colonization because they have a broad environmental tolerance. Although the species occurs in a wide range of aquatic habitat types, including diverse ranges of temperature, osmotic concentrations, flows, substrates and disturbance regimes, clonal lineages may have either narrow or broad ecological tolerances. In New Zealand, narrow preferences often result in distinctive habitat utilization among clones (Dybdahl and Lively 1995a, Fox et al. 1996, Jokela et al. 1999, Jokela et al. 2003), while one of the clones that is widely spread in Europe is broadly tolerant (Jacobson and Forbes 1997). Thus, the invasiveness and success of this species is likely to be a function of the clone present and local environmental conditions.

Densities of NZ mudsnails can fluctuate widely. In Australia, densities ranged between highs of 50,000 snails/m^2 during the summer and lows of 1,800 snails/m^2 during the winter (Ponder 1988, Schreiber et al. 1998). Similarly, densities often undergo broad fluctuations in Europe (Siegismund and Hylleberg 1987, Dorgelo 1987, van den Berg 1997, Savage 1996) where water bodies freeze in winter and are re-colonized the following spring. In the Greater Yellowstone Ecosystem, NZ mudsnails reach densities approaching 300,000 snails/m^2 at some locations (Kerans et al. 2005) and as high as 500,000 snails/m^2 in others (Hall et al. 2003), but fluctuate seasonally, reaching highest densities in July or September, and very low levels in March (Kerans et al. 2005). This fluctuation of density also occurs in the Owens Valley of California, and most likely any invaded areas where winter freezing occurs.

In the wild, NZ mudsnails are sometimes ingested by mountain whitefish (*Prosopium williamsoni*) (W. Dwyer, USFWS, personal observation), sculpin (*Cottus sp.*) and brown trout (*Salmo trutta*) (Cada 2004). Laboratory studies show that rainbow trout and steelhead will volitionally feed on NZ mudsnails regardless if fish are starved for a period of time or maintained on feed. However, in both cases, rainbow trout appear to exhibit a more aggressive behavior ingesting more snails than steelhead (Moffitt, pers. comm.). Unfortunately, studies have shown fish derive little or no energy value from eating snails because the snails are capable of passing through the fish's digestive system alive and intact. (Bondesen and Kaiser 1949, Haynes et al. 1985, Vinson, pers. comm., Moffitt, pers. comm.). In addition, energy contents of the NZ mudsnail was determined to be extremely low and variable by seasons (Ryan 1982). NZ mudsnails are grazers of attached periphyton and consumers of decaying plant and animal material (Haynes and Taylor 1984).

More detailed information on the biology and ecology of NZ mudsnails can be found in Appendix A.

C. Summary of Applicable State and Federal Policies and Regulations

This summary addresses federal and state laws, regulations, plans, and policies that directly relate to NZ mudsnails, either specifically or generally. It does not include other ANS prevention requirements, such as ballast water regulations, that relate to NZ mudsnails indirectly. More specific information on state provisions can be found in Appendix B.

At this time, NZ mudsnails are not listed as "injurious wildlife" in the federal Lacey Act regulations under 50 CFR Part 16. However, under certain conditions, transport of NZ mudsnails between states that restrict possession of this species can constitute a Lacey Act violation. The other key federal provision that has been applied to NZ mudsnail invasions is Executive Order 13112, signed by President Clinton in 1999. This policy prevents federal agencies from authorizing, funding, or carrying out actions that are "likely to cause or promote the introduction or spread of invasive species" (except under certain conditions). It has been the basis for NZ mudsnail prevention and control programs at federal fish hatcheries, federally-funded state fish hatcheries, and other facilities.

In the western U.S., California, Colorado, Kansas, Montana, Utah, Washington, and Wyoming are among those states that specifically prohibit importation, possession and transport of NZ mudsnails. Alaska, Hawaii, Idaho, Nevada, and Oregon are among those states that do not specifically list NZ mudsnails as prohibited, but nonetheless do not allow this species to be imported, possessed, or transported without prior authorization through a state permit system. States such as Colorado and California have used quarantine and fishing access closure authority to deal with NZ mudsnail infestations. NZ mudsnails are also specifically addressed in state aquatic nuisance species management plans developed by Alaska, Hawaii, Indiana, Kansas, Montana, Oregon, and Washington.

Minnesota has proposed that the NZ mudsnail be a prohibited invasive species, which will prohibit import, possession, transport and introduction into the wild. In the eastern U.S., neither New York nor Pennsylvania has specific laws or regulations pertaining to NZ mudsnails. Both States have general provisions for a permit system controlling species that are transported into their waters.

III. Objectives

A. Objective 1: Identify Foci, Pathways and Vectors

1. Introductions and Dispersal of NZ mudsnails in North America

The NZ mudsnail is indigenous to New Zealand and its adjacent islands (Stewart and Chatham Islands, Winterbourn 1970b, Ponder 1988). In New Zealand, the snails have been found in nearly every aquatic habitat including large rivers, forested tributary streams, thermal springs, ponds, glacial lakes, and estuaries (Winterbourn 1970b, 1978, Towns 1979, 1981b, Rounick and Winterbourn 1982, Talbot and Ward 1987, Winterbourn and Ryan 1994, Scott et al. 1994). Two other species of *Potamopyrgus* (*P. estuarinus* and *P. pupoides*) are also known from New Zealand; however these are confined to brackish waters (Winterbourn 1970b).

Over the past 150 years, NZ mudsnails have spread in 3 continents. These populations originated from either the North Island of New Zealand (Stadler et al. 2005), or from Australia (Figure 7). During the nineteenth century, NZ mudsnails were introduced to Europe. The first recorded occurrence in Europe dates to 1859 in Great Britain (Bondesen and Kaiser 1949, Ponder 1988). Ponder speculates that they may have gotten to Europe in fresh drinking water carried by ships. Bondesen and Kaiser (1949) provide a detailed account of the species' discovery in Great Britain and Western Europe. Initial reports of the snail in Europe attribute it to an entirely distinct species, *Potamopyrgus jenkinsi*, which was thought to be a European native species closely related to *P. antipodarum.* It was not until the later part of the twentieth century that morphometric and molecular analysis confirmed that *P. jenkinsi* was in fact *P. antipodarum* (Winterbourn 1970b, Winterbourn 1972). Three distinct clones EU – A, EU – B and EU – C have been genetically identified in Europe (Figure 7) (Dybdahl, pers. comm.).

Potamopyrgus antipodarum was reported from Tasmania in 1872 and Victoria, Australia, in 1895. Initially, as was the case in Europe, the species was described as a synonymous species, *P. niger,* by early workers (Ponder 1988). The snail spread throughout the state of Victoria, streams around Sydney in New South Wales, and Tasmania (Ponder 1988). Populations in Australia are genetically polymorphic (comprised of numerous clones), but DNA sequence data suggests all these populations originated from New Zealand's North Island (Dybdahl, pers. comm.).

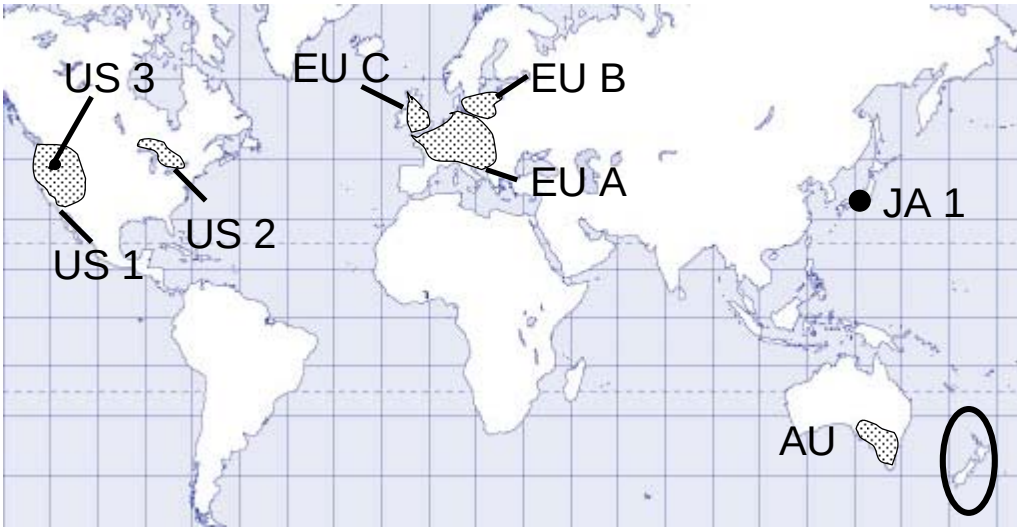

Figure 7. The approximate distribution of introduced clones. Native range is New Zealand. Introduced populations in Australia (AU) are clonally diverse. Introduced populations are comprised of 3 distinct clones (EU A, B, and C) in Europe, of three distinct clones in the U.S. (US 1, 2 and 3), and of one clone in Japan (JA 1). (Figure from Mark Dybdahl)

A single population is also known from Japan and genetic markers suggest that it represents an independently founded population. The genetic markers are consistent with the same origins as US 1 (Dybdahl, pers. comm.).

The origins of clone US 1 might be either New Zealand or Australia, based on genetic markers. US 1 is identical to a widespread clone in Australia (Dybdahl, pers. comm.). On the other hand, US 2 is a single clone that is identical to one of the clones in Europe, EU A (Mark Dybdahl, pers. comm.). Hence, US 1 and US 2 populations were introduced by different mechanisms from different origins. The origin of US 3 found in 2005 in the Middle Snake River located by Bliss, ID is unknown (David Richards, Dan Gustafson, personal observation) (Figure 7).

In western North America, NZ mudsnails were first documented in 1987 from the Middle Snake River in Idaho (Bowler 1991). The exact time of arrival and source of the snails are unknown but it has been speculated that they arrived from the commercial movement of aquaculture products such as trout eggs or live fish (Bowler 1991; Bowler and Fresh 1992). No other populations were discovered until 1993 when NZ mudsnails were found in the Columbia River estuary near Astoria, Oregon, and 1994 during survey work in Montana and Wyoming in the Upper Madison River (Missouri River drainage, F. Pickett, PPL Montana). Figure 8 shows the range expansion during the second decade of the invasion. All western states, except New Mexico, now have known established populations. There are 59 infected drainages, classified as cataloging units in the Hydrologic Unit Code (HUC) system. Some of the HUCs have multiple, discrete populations. These maps were generated by the centralized database at http://www.esg.montana.edu/aim/mollusca/NZ mudsnail/, which can also provide more detailed maps by area or time-frame. Additional collection records can also be entered on-line at this site.

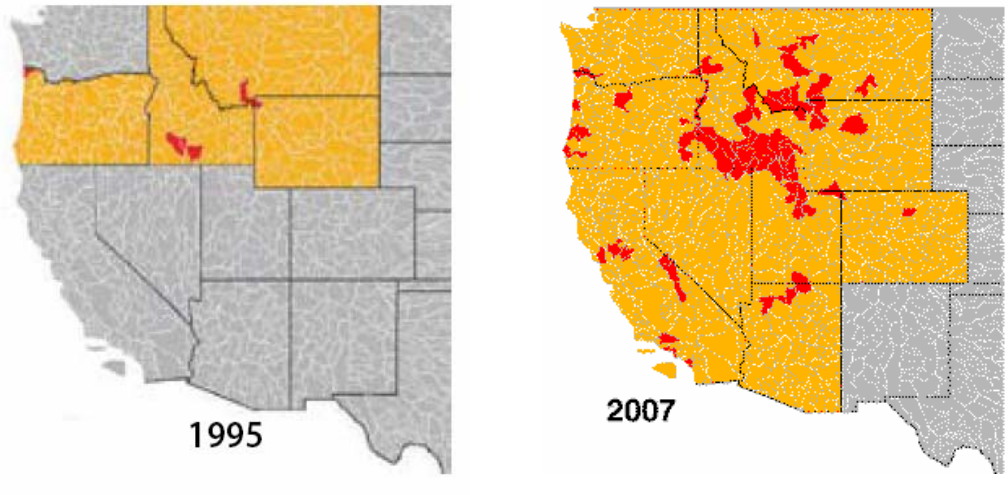

Figure 8. The spread of NZ mudsnails in the western USA during the second decade of its invasion. The small cells on the map represent the USGS cataloging units (8 digit HUCs). Positive HUCs are in red and positive states are in yellow (Maps by Dan Gustafson, 1995 map modified by Oregon Sea Grant).

In 1993, a US 1 clone population of NZ mudsnail was discovered in the lower Columbia River estuary in locations subject to salinities that fluctuate between 0 and 30 parts per thousand where introduction pathways could have included recreational boats/trailers and contaminated hatchery transplants. The Lower Columbia River Aquatic Nonindigenous Species Survey published in October 2004 found that this species now occupies the entire estuary from Clatsop Spit to Calthlamet Bay (River Mile 30), and NZ mudsnails have been found at the mouth of the Kalama River. Other recent discoveries of NZ mudsnails in the Pacific Northwest include Garrison Lake on the southern Oregon Coast (2002); Devil's Lake in Lincoln City, Oregon (2003); Surf Side Estates Lake near Ocean Park Washington (2003); the New River on the southern Oregon Coast (2003); and Bully Creek in southeastern Oregon (2004).

NZ mudsnails were first collected in Utah in 2001 in the Green River downstream from Flaming Gorge Dam. Between September 2001 and May 2004, the snails were found at 28 locations within 16 stream basins. They can currently be found in many of Utah's high quality trout waters including the Green, Bear, Provo, Weber, Ogden, and Logan River Basins.

The NZ mudsnail was discovered in the Colorado River in Arizona in early 1996, having been mis-identified for several years as *Fossaria sp.* In addition, NZ mudsnails were discovered in Boulder Creek in Colorado near and in a private fish aquaculture facility in 2004. In 2005, a second Colorado population was identified in the South Platte River in Elevenmile Canyon.

In California, NZ mudsnails were first discovered in 2001 in the Owens River. A review of past samples shows that low densities were found in 1999 but the snails had been misidentified (Herbst, pers. comm.). Since then, NZ mudsnails have been detected in Upper Owens River, Lower Owens, Bartlett Springs on the Owens dry lake, and Hot Creek within the Owens Basin. Populations were later discovered west of the Sierra Nevada, at

Putah Creek, Lower Calaveras River and Mormon Slough, Lower Mokelumne River, Lower Napa River and Piru Creek. Another disjunct population was discovered in the Mono Basin, at Rush Creek. In 2005, Malibu Creek watershed was found to be positive. In 2006, an intense survey revealed NZ mudsnails were present in Medea Creek, Malibou Lake, numerous sites on Malibu Creek, and in Las Virgenes Creek.

In the Great Lakes region, NZ mudsnails (US 2) were first found in Northeast and Southwest Lake Ontario and in the St. Lawrence River near Prescott, Ontario (Figure 9). The snail is usually found in substrates of silty sand (Zaranko et al 1997). Zaranko et al. (1997) failed to find NZ mudsnails in any other locations in Lake Ontario or in Lakes Erie and Huron in their extensive survey. Since Zaranko et al.'s (1997) study, the snail has been found in Lake Ontario near Rochester, NY in 2003 (Levri, unpublished data) and near Toronto (Zaranko, pers. comm.). The snail has also been found in Lake Superior near Thunder Bay (Grigorovich et al. 2003) and in Lake Erie near Erie, PA in 2005 (Levri and Kelly, in prep.). In the Great Lakes Region, NZ mudsnails have been found at depths ranging from 4 to 45 meters (Zaranko et al. 1997; Levri et al. in prep.). At Wilson, NY, NZ mudsnails have not been found in waters shallower than 15 meters and at depths deeper than 45 meters. Densities peak between 20 and 25 meters in depth. Efforts to find the snail in streams or rivers emptying into Lake Ontario have failed. This distribution, especially in shallow waters is perplexing as this clone is found in shallower waters in Europe. Some factor must be important in keeping the snail out of shallow water in the Great Lakes. This factor may be wave action, but wave action should not be important at 10 meters in depth, and the snails are not found there. Additionally, if wave action is important, the snails should be found in sheltered bays and inlets, but they have yet to be found in these areas.

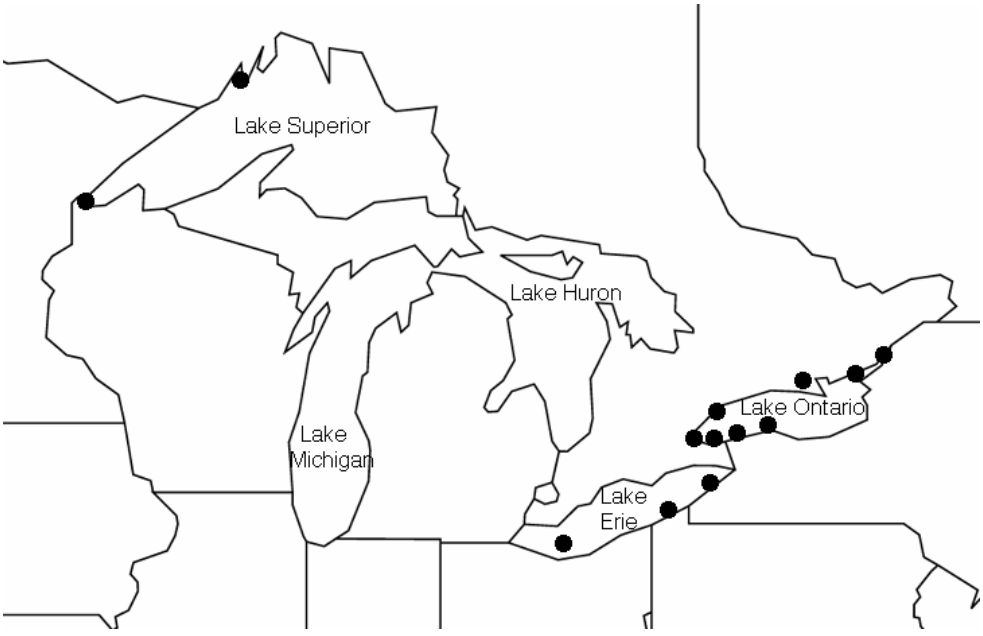

Figure 9. NZ mudsnail populations (US 2) in the Great Lakes (map by Ed Levri).

Unlike many gastropods, NZ mudsnails produce fully formed "crawl-away" juveniles and lack larval forms specialized for dispersal. Because they are fully aquatic, they cannot disperse unaided among isolated watershed and drainages. Nevertheless, their capacity for invasive spread is exemplified by the invasion of Europe, which spanned 2,500 km in less than 140 years (Zaranko et al. 1997). The rate of spread may well be faster in North America. In about 20 years, NZ mudsnail populations have spread to 9 western states and three of the Great Lakes.

Numerous adaptations of the NZ mudsnail would seem likely to aid its spread within watersheds. Adults can pass alive through the digestive systems of several fish species (Bondesen and Kaiser 1949, Haynes et al. 1985) and float on masses of algae (Ribi and Arter 1986). Juveniles can float freely on the water surface without a substrate (Vareille-Morel 1983). The NZ mudsnail is positively rheotactic (movement in the opposite direction of the water flow) (Haynes et al. 1985). It can crawl at speeds exceeding 1 m/hour (Richards 2002) and can move as much as 60 m upstream in 3 months (Adam 1942). However, despite its potential for explosive population growth wherever it is found, rates of growth are highly variable. Downstream dispersal of populations in some western U.S. watershed might be limited by lakes and reservoirs where populations are either at low densities or are absent. (Dybdahl, pers. comm.).

2. Vectors and Pathways of Spread

The broad physiological and ecological tolerances of the NZ mudsnail may render it suited for dispersal by a wide range of vectors. Incomplete information regarding the timing and source of initial introductions within the United States obscures identification or ranking of individual vectors. As used here, a vector is the mechanism by which ANS are spread while pathways are the routes over which vectors pass. Vectors and pathways of spread and transport believed to contribute to the distribution of NZ mudsnails include:

a. **Fish hatcheries and associated stocking operations**: The current infestations by NZ mudsnails at the Hagerman National Fish Hatchery and a number of state hatcheries in Idaho indicate the vulnerability of government and private aquaculture facilities. Contamination of water supplies and the ability of NZ mudsnails to pass live through fish digestive systems (Haynes et. al., 1986) may provide a vector for introductions to occur during fish stocking operations. Transfer of live organisms, their eggs or larvae, and associated water and packing materials between aquaculture facilities provides another vector for spread.

b. **Recreational watercraft and trailers**: When directly exposed to NZ mudsnails boats, canoes, kayaks and associated gear and trailers may become fouled, providing a contamination source when moved to uninfested waters. In addition, NZ mudsnails often attach themselves to aquatic macrophytes and clumps of algae. These plant materials and associated snails can then be moved by boaters or trailers between water bodies. NZ mudsnails can also be

transported within the livewells of boats or entrained into water lines (particularly for jet skis and other jet-drive systems).

c. **Recreational water users**: Particularly when embedded in mud or attached to plant debris, NZ mudsnails may be transported on fishing gear, on waders and boots, swimsuits and swimming toys and even by hunting dogs and horses. Hikers, backpackers, horseback riders, and bicyclists may inadvertently transfer the snail when encountering multiple stream crossings during their outings. The snail's small size allows it to be carried in small crevices that might escape detection. NZ mudsnails inadvertently distributed via live bait sales or use can be transported to new sites if bait containers or their contents are discarded in or near the water. Given their ability to survive in the digestive tract of fish, movement of live or dead fish between watersheds by anglers can also be a vector.

d. **Natural resource management activities**: Personnel involved in monitoring projects, restoration activities, and other natural resource activities that cross watershed boundaries may transport NZ mudsnails to new waterbodies via their gear, vehicles, or clothing. Without pre-planning, field staff may not have access to facilities or equipment that allows decontamination between work sites. Mudsnails can live in moist environments near the edges of streams, and therefore can be picked up and moved by people who are not wading in the water. Citizen and classroom monitoring groups are another potential vector for spread of ANS.

e. **Commercial shipping**: Most ballast water introductions are species with planktonic larval dispersal, which NZ mudsnails lack, making this vector unlikely, but possible. Discharge of ballast water has been associated with many introductions of ANS, including snails, and could be a vector for NZ mudsnail introductions. Ballast water from foreign ports can serve as a continuing inoculation source of new NZ mudsnail clones, while ballast discharge from coastal shipping may spread snails already found in the U.S. (e.g., transport of Columbia River mudsnails to other West Coast estuarine ports). Zaranko et al. (1997) suggested that this mechanism may have been responsible for the presence of NZ mudsnails in Lake Ontario. Ships can also transport NZ mudsnails that have attached to or are embedded in mud on anchors and other surfaces.

f. **Sand/gravel mining, extraction, and dredging**: Any waterway operations that remove and transport mud, sand, and other bottom materials from areas with NZ mudsnails can serve as a vector for new introductions. Dredges that move frequently between rivers and estuaries are particularly vulnerable sources of regional spread. Maintenance of canals and ditches by landowners, ranchers, water and power agencies, and flood control personnel also has the ability to spread ANS.

g. **Aquatic plant trade and collections**: Similar to aquarium contamination, it is unknown if NZ mudsnails have ever been distributed as a contaminant by wholesale or retail aquatic plant suppliers. A study conducted in Minnesota by the Department of Agriculture found that 40 orders placed to 34 aquatic plant vendors across the U.S. yielded 31 orders with live animal species. Sixty five percent of these species belonged to Gastropoda, Diptera and Hirudinea (Montz, 2002, Maki and Galatowitsch, 2003). Therefore, potentially NZ mudsnail can be spread by this vector. Contaminated home water gardens could then serve as a source for new introductions due to flooding, wildlife, or other vectors of secondary spread. Several authors have offered the hypothesis that initial introductions to Europe and Australia were a result of the transport of aquatic plants between Australia and botanical collections in Europe (Winterbourn 1972, Ponder 1988).

h. **Transport by fish, wildlife and livestock**: It is already known that NZ mudsnails can survive passage through the digestive system of trout (Haynes et. al, 1986). Fish could therefore serve as a more localized source of spread, particularly for species that may migrate or stray into other tributaries or watersheds. It has been suggested that waterfowl and other birds could also spread NZ mudsnails between waterbodies via feet or feathers (Bycott 1936, Talling 1951, Lassen 1975). In addition, NZ mudsnails might be spread through consumption by waterfowl, but it is unlikely a snail could pass unharmed through the gizzard (Gangloff et al. 1998). Other wildlife (particularly aquatic and semi-aquatic species like frogs, raccoons, and otters) may serve as vectors via passive transport on a variety of geographic scales. They could also be spread on the feet or fur of domestic livestock which walk through streams, such as goats, sheep, cattle or horses or wildlife such as bison, deer and elk. Since mudsnails can live in moist areas along stream banks, they may be spread by animals that are walking along the riparian areas as well.

i. **Firefighting**: NZ mudsnails could be spread by firefighting machinery or equipment that is moved from one place to another across streams and rivers to fight backcountry or forest fires. Transporting large helicopter-deployed water buckets between water bodies is a particular concern. Spread could also occur through human and pack animal activity.

j. **Transport by water flow**: Water flow can spread NZ mudsnails downstream within a watershed (where they then may come in contact with new vectors that would transport them outside the basin). This vector typically would vary seasonally based on flood events or periodic management of water levels in ponds and reservoirs. In lakes and ponds, snails have been reported to raft on floating algae mats and other vegetation (Vareille-Morel 1983 and Ribi and Arter 1986 as cited in Ribi 1986, Dorgelo 1987). Additionally, NZ mudsnails can simply float at the water's surface or cling to the underside of the surface film (Gangloff et. al, 1998). Both floating and rafting behaviors are

commonly observed in other snails, including anywhere that dead and uprooted vegetation accumulates in ponds. In addition to rafting and floating, gastropods have been reported to undergo "drifting" behavior in flowing water systems. Marsh (1980) found that *Physa gyrina* drifted at rates exceeding 500,000 individuals m^3 sec^{-1} under "normal" flow conditions. It is not known to what extent NZ mudsnails exhibit drift behavior.

k. **Transport by volitional movement**: As noted earlier, NZ mudsnails are capable of moving at speeds exceeding 1 m/hour (Richards 2002). Although unlikely to be a vector between river basins, volitional movement can obviously spread NZ mudsnails within a watershed (to sites where they then may come in contact with new vectors that could transport them outside the basin).

3. Information and Data Management

With funding from the U.S. Fish and Wildlife Service, the New Zealand Mudsnail in the Western USA web site (http://www.esg.montana.edu/aim/mollusca/NZ mudsnail/) is being hosted by the Department of Ecology, Montana State University in Bozeman, Montana. The purpose of the site is to be the most comprehensive and current database for information concerning the ecology of NZ mudsnails.

A basic foundation for the management of NZ mudsnail populations is to document and map its known locations; therefore, it is critical to timely map invasion patterns and abundance throughout the U.S. The sophisticated mapping capability of the New Zealand Mudsnail in the Western USA web site allows for easy input of new sightings and creation of accurate location maps. In addition, each location point has a description of the site and estimates of abundance. This web site and database needs to be expanded nationally to include Great Lakes populations and any others that may show up.

The NZ mudsnail web site also provides conference minutes, recent findings, news, downloadable files, links, people involved with management and research, and a comprehensible bibliography.

4. Objective 1 Implementation

a. Identify additional pathways.

b. Develop guidance or criteria for prevention, management, risk and impact assessments.

c. Develop risk assessment of different pathways.

d. Prioritize pathways for outreach efforts.

e. Support and expand the New Zealand Mudsnail in the Western USA database, maps and web site to include eastern U.S. populations.

f. Facilitate coordination with USGS Nonindigenous Species database in Gainesville, Florida.

Priority pathways for targeting funds include fish hatcheries, recreational watercraft and trailers, anglers and hunters, and natural resource management activities.

5. Objective 1 Research Needs

The following questions need to be addressed to improve the capacity for measuring and managing risk of NZ mudsnail introductions:

a. Risk Assessment

1) Are there specific habitat types and/or environmental conditions that completely preclude establishment of NZ mudsnails? Are there environmental/habitat parameters that make an area more vulnerable to invasion?

2) How can relative risk of introduction and establishment of NZ mudsnails in uninfested waters be quantified?

b. Pathways

1) Using genetic markers, determine the pathway for NZ mudsnail spread in the U.S.

2) How important are the different human-mediated recreational vectors to the spread among watersheds: boat transport, angler movements, swimmers, etc?

3) How important are different vectors associated with economic activities: fish aquaculture, fish hatcheries, water use and transport?

4) Which suspected pathways have had the most prominent role in actual introductions of NZ mudsnails in the U.S.?
   a) between watersheds
   b) within watersheds

5) Are NZ mudsnails being distributed by biological supply houses? Could NZ mudsnails be unintentionally distributed with other species? Similarly, could NZ mudsnails be distributed through the Internet trade?

6) For those fish species used in aquaculture which will consume live snails, what is the maximum time period that NZ mudsnails can live within the digestive tract and still pass through as viable organisms?

7) What are the ranges of natural dispersal rates/distances that have been documented for NZ mudsnails upstream and downstream from initial infestations, and what physical factors affect those rates and distances?

8) Once NZ mudsnails occur in one tributary of a watershed, what is the likelihood that they will eventually occupy all reaches of the entire watershed that support their habitat needs? Under what circumstances might such spread not occur?

B. Objective 2: Develop Methods of Detecting New Populations

1. NZ Mudsnail Sampling Methods and Procedures

NZ mudsnails occur in a wide variety of water bodies and on a wide variety of substrates including sand, leaf litter, organic detritus, silt, algae, aquatic macrophytes, gravel, cobbles, and boulders, as well as any other type of stable substrate (natural or artificial). With such a wide variety of habitats capable of being invaded, no single sampling method can be developed that is applicable in all situations. Numerous benthic invertebrate sampling methods have been developed and are widely used for different purposes and habitats including: Surber and Hess samplers, kick-nets, Ponar grabs, snorkeling, SCUBA, hand picking, suction dredges and colonization samplers or traps (Merritt and Cummins 1996). Once an invertebrate sample has been collected many techniques are used to detect and count NZ mudsnails in the sample including: preservation in alcohol, inspection of collection container for floating snail shells, examination under a microscope, and visually inspecting contents of the live or dry samples.

Any of these methods can be used to detect NZ mudsnails depending upon different conditions, time and budget constraints, or individual preferences. No sampling method can ever guarantee 100% effective detection of NZ mudsnails and therefore not detecting NZ mudsnails at a site does not equate to its absence, or a negative location**.** NZ mudsnails are simply too small and can inhabit too many locations. Samples are not equivalent to a census, and one unobserved 1.0 mm NZ mudsnail can relatively quickly produce a whole population.

Formal sampling to detect NZ mudsnails is time consuming, expensive, requires some statistical sampling knowledge, and probably will not often be conducted. Therefore, prioritization of sampling sites should be established. For example, sites close to existing infestations but in different bodies of water or sites with special management needs such as sites containing endangered or threatened species might be high priority. For specific information for estimating the detectability of NZ mudsnails using power analysis, see Appendix D. A more practical and efficient method with less statistical validity for detecting NZ mudsnails in wadeable waters is the use of a standard heavy-duty D-shaped kick net with mesh size $\leq$ 1 mm. The kick net is vigorously pushed through all available habitats, including vegetation, and also placed downstream of the biologist who vigorously kicks and agitates the substrate (cobbles, gravels, etc.) to collect what is kicked up with the net. Contents of the net are then placed in a large bucket of water; vegetation is washed in the bucket to remove snails and then safely discarded. Snails and other invertebrates are then poured into a < 1.0 mm mesh small, aquaria hand-net or suitable container. All that should remain in the bucket is heavier sand, gravel, or cobbles, which can be discarded. Contents of the small aquarium net are stored in 70-95% ethanol with collection labels written in pencil or alcohol-proof pen placed both in the container and attached on the outside of container. Samples are then visually searched for NZ mudsnails under a dissection scope. Preservation in 70% ethanol is also preferred for genetic analysis but soft body parts will become shriveled. If NZ mudsnails are abundant in the sample and easily visible during collection, some individuals should be removed and dried without alcohol.

A good snail collection consists of three parts: 1) preserved, relaxed soft parts, 2) DNA samples and 3) dried shells. The animals must be relaxed before fixing by keeping them alive in a clean container using the water of origin and adding a few crystals of menthol. After 24+ hours, when the animals do not respond to touching, most of the water is carefully removed and replaced with 10% buffered formalin, Kahle's or Bouin's fluid. The latter 2 solutions provide better fixation, but they are acidic and they will damage or destroy the shells in time. They can be replaced with 5% buffered formalin after only several minutes to preserve the shells. An antacid tablet can be used to buffer formalin. After 2 hours of preservation, wash the snails with running water and store in 70% ethanol.

DNA samples are easily obtained by placing a few snails directly into 90-100% ethanol. Chemically spiked ethanol should not be used for DNA samples. The fluid should be changed if it is much diluted. Different clones are identified by allozyme analysis (Dybdahl and Lively 1995). Samples for this analysis should be either alive or snap frozen, preferably in liquid nitrogen.

Excess shells are best preserved dry as they are easier to maintain and the color is preserved better. These should be placed into in 5-10% buffered formalin. Alcohol can also be used, but the shells will retain an unpleasant odor. In the lab the shells are cleaned with soap or ammonia water and rinsed with clean water before drying. NZ mudsnails can take a long time to completely dry out. The soft parts of large snails can be removed after killing the animal by suffocation or by freezing and then thawing. The operculum, if removed from the animal should be placed within the shell and held in place with a cotton plug.

Proper labeling is important. A standard collection label should include at least the following:

Collector’s name
Water body
Date
County, State
Short description of site
GPS coordinates if available

An estimate of the area covered by the kick net during sampling should also be recorded. Another method would be to calculate the number of man-hours for which the sampling occurred. Either method would allow for estimation of a rough detection probability. Because these are simple qualitative methods, they will always yield rough estimates.

Casual sampling for NZ mudsnails by hand picking cobbles, vegetation, woody debris etc. along shorelines can also be used, if no other methods are available or if time is limited. This method will work reasonably well if the snails are abundant. If NZ mudsnails are detected using this method, proper preservation and documentation methods should be followed. If no NZ mudsnails are discovered by casual observation it should be

documented (including details about the search method and intensity), but the data will be of limited use.

Both NZ mudsnail density and habitat location can vary dramatically seasonally. Typically NZ mudsnail densities are highest in late summer and early autumn, but can vary with location (Richards et. al. 2001, Richards 2004, Kerans et al. 2005). NZ mudsnail habitat location can also vary seasonally depending on food availability and other physical and chemical factors. A locality with NZ mudsnails in one season may not have them in another season. NZ mudsnails in winter often remain on the bottom of cobbles (Richards personal observation), or can possibly burrow into gravels to avoid cold temperatures. Therefore, sampling efforts should not be rigidly defined but adjusted accordingly.

Another sampling method that has not been used widely to detect NZ mudsnails is the databases of county, state, tribal, and federal water quality agencies. These agencies have collected thousands of benthic invertebrate samples from thousands of miles of streams and rivers. If NZ mudsnails were present, there is a likely chance that they would have been collected during these widespread and ongoing sampling efforts. Most agencies typically use D-net or Hess samplers and samples are often collected from riffle habitats. All NZ mudsnail positive sites in these databases likely can be considered valid (if they were not misidentified), but the absence of NZ mudsnails needs to be evaluated. NZ mudsnails may have been misidentified as other species because they are not included in keys used for North American benthic macroinvertebrates. In addition, a probability of detection estimate needs to be established comparing the relationship of NZ mudsnails found in cobble-riffle habitat samples to other non-sampled habitats.

Biologists who collect snails that fit the description of NZ mudsnails but are unsure of the specific identification should contact the ANS coordinator at their State fish and wildlife agency or U.S. Fish and Wildlife Service regional office.

2. Objective 2 Education and Outreach

Although methodology exists for sampling and identifying NZ mudsnails, there are untapped opportunities to involve additional audiences in implementing these methods. Therefore, one goal of the outreach and education efforts must be to reach out to both professionals working in the field and concerned citizens who may assist in monitoring. While the desired result of increased monitoring and detection is the same for each group, the outreach approach needs to be tailored differently to reach these audiences.

A general campaign of public outreach will serve as an excellent first step in encouraging additional public monitoring. Existing community-based programs (e.g., stream watch teams) that include water quality or biological monitoring will need to be identified and contacted with sampling information. These groups have already demonstrated their commitment to the resource and should be easily recruited into the effort. As outreach increases, individual anglers and other recreationists will join the effort and it can be expected that there will be a number of individuals that will routinely inspect the waters they visit.

Fishery workers and other resource professionals that frequently visit aquatic areas need to be recruited into the effort of identifying introductions. Outreach strategies need to be implemented that will educate these professionals about the need to sample. However, a more effective outreach strategy may be to target higher level resource management professionals who have the authority to direct their staff to conduct surveys. These individuals need to be identified and targeted to insure that entire agencies adopt sampling protocols and not just individual employees.

In order to ensure that increased field monitoring is promptly translated to reportable results, it will be important to:

a. Develop a list of local and national sources of taxonomic expertise for identifying potential NZ mudsnail populations and distribute information to a broad national audience.

b. Develop a key of aquatic snails with an emphasis on identifying nonnative snails such as NZ mudsnails, Chinese mystery snails, etc.

c. Outreach to watershed groups and government agencies collecting benthic invertebrate samples regarding identification and data gathering.

d. Promote the Stop Aquatic Hitchhikers Campaign, Habitattitude and the use of HACCP-NRM plans for all groups utilizing aquatic habitats.

3. Objective 2 Implementation

a. Create a national web-base database for easy retrieval on NZ mudsnail sampling efforts (methods, frequency, detection limits, etc.) including those where NZ mudsnails have <u>not</u> been detected. (Note: Objective 3.8.i.)

b. Review of existing local, state, federal databases and/or collections of macroinvertebrate samples (focused on watersheds where intentional NZ mudsnail sampling has not occurred).

c. Increase coordination with agencies and other biologists to obtain more timely input on new populations.

d. Establish priority areas for monitoring, such as sites close to existing infestations or sites with special management needs or sites containing endangered or threatened species. (Note: Objective 3.8.i.)

e. Conduct further outreach to watershed groups and government agencies collecting benthic invertebrate samples regarding identification and data gathering.

4. Objective 2 Research Needs.

a. Develop estimates of detection probabilities for a variety of substrates, ecoregions, sampling methods and sampling designs.

b. Develop standardized techniques for detecting low abundance NZ mudsnail populations and for estimating densities in established populations.

c. Monitor existing populations on a precise schedule to understand population trends and their response to environmental factors.

d. Evaluate different detection methods in different habitat types to provide a way for establishing confidence in data that people submit.

e. Establish baseline data in States which currently have NZ mudsnails populations.

C. Objective 3: Develop Strategies and Methods of Prevention, Control and Management

1. Risk of Future Introductions

The most effective way to avoid NZ mudsnail impacts is to prevent or slow new introductions by the pathways described under Section II.A. Assuming resources and other factors set limits on the scope of a prevention program, it becomes desirable to evaluate the likelihood that a particular action will lead to an introduction. Despite advancements in modeling and analysis of past invasions, predicting if, where, and when nonnative species will become established in a new site is still unreliable. The probability of transport by potential pathways is difficult to calculate and highly variable spatially and temporally. Physiological thresholds that may be evident from current distribution and laboratory experiments do not provide certainty that those thresholds will hold true in locations where the species does not yet occur. This is particularly true for species as apparently phenotypically plastic as NZ mudsnails. Further complicating predictions of future NZ mudsnail introductions are a long list of information gaps regarding physical tolerances, habitat suitability, pathway probabilities, and the complex relationships between these factors (see Research section below). That said, it still may be feasible to assign relative risk rankings in order to prioritize pathway and water body management efforts. For example, introductions to new locations across broad geographic spans are highly likely to stem from human mechanisms rather than volitional movement or dispersal by fish, wildlife, and water flow.

However, unless the risk of an activity introducing NZ mudsnails to a particular location can be confidently determined to be zero, decisions regarding preventative measures not only must consider what is the actual level of risk (both probability and magnitude of impact), but what is the acceptable level of risk. If, for example, a fish hatchery detects one live NZ mudsnail in a sample of fish that will be stocked into an uninfested tributary, should that release be halted or do the benefits outweigh the anticipated level of risk? If there are snails found in another tributary of the same watershed, will other vectors (including volitional movement) minimize the impact of the hatchery's decision regarding distribution in the basin? How should the inherent uncertainty noted above regarding habitat use by invasive species be incorporated? For particular pathways, criteria need to be developed that guide these risk management decisions based on available data for the relevant vectors and watersheds of concern. A precautionary approach needs to be considered given the likely irreversibility of introductions that do occur. Appendix D provides an example of corresponding risk assessment and risk management criteria developed for stocking decisions at the Hagerman National Fish Hatchery in Idaho.

2. Management Options for pathways

Recognizing the challenges in setting prevention thresholds and prioritizing pathways of concern, there are many methods that may contribute greatly to reducing the spread of NZ

mudsnails. Some prevention methods are applicable to a variety of pathways and invasive species. For example, where NZ mudsnail spread is associated with inadvertent contamination of equipment, clothing, or other materials, educational materials and programs aimed at the associated audiences can promote decontamination activities. A strong strategic public outreach campaign targeting various audiences can protect lakes, rivers and streams from the spread of invasive species. Education on the harmful effects that NZ mudsnails have in conjunction with information on how to prevent the spread is essential in the control and management. Prevention of introduction is always the best tool to manage invasive species.

Hazard Analysis and Critical Control Point – Natural Resource Management (HACCP-NRM) planning is another general tool for managing invasive species pathways. HACCP-NRM plans identify potential pathways of introduction of invasive species and identify how the pathways can be broken to prevent the introduction. Development and implementation of HACCP-NRM plans for activities likely to transport NZ mudsnails can significantly reduce spread.

Pathway-specific management actions may include:

a. **Fish hatcheries and other aquaculture operations**: For facilities where no known NZ mudsnail contamination occurs, close visual inspection of water systems, raceways, stocking equipment, as well as regular gut content analysis can detect the arrival of snails before they can be spread. For facilities with contaminated water supplies, well water or other alternative uncontaminated sources should be used for any situation where there is exposure to fish. Similarly, equipment (e.g., nets) used within contaminated water should not be used in areas of the facility that are on clean water. Gear used in the field should not be used in the hatchery, and all gear should be stored in walk-in freezers, if available, treated with decontamination methods or thoroughly dried. When there is evidence or even likely risk that fish are consuming live NZ mudsnails, releasing those fish only at sites already contaminated by mudsnails can avoid further spread. Current research on the effects of fish feeding, snail size, snail meal size, and fish size on snail transit and survival through the gastrointestinal tract of rainbow trout/steelhead will help develop a depuration strategy that infected hatcheries can implement before stocking fish. Preliminary investigations also suggest that copper, carbon dioxide under pressure, and hydrocyclonic separators may prove useful in both decontaminating fish hatchery water supplies and preventing spread into uncontaminated areas of a hatchery. Ozone has not been shown to be effective in killing NZ mudsnails in a hatchery environment (Moffitt, pers. comm.).

b. **Recreational watercraft and trailers**: Given their small size, it may not be practical in many situations to completely eliminate NZ mudsnails that have contaminated recreational boats or trailers. However, providing information and resources (and/or regulatory requirements) to promote thorough inspections, cleaning and removal of organisms from watercraft before it exits

a contaminated site will certainly reduce risk. Promoting thorough drying of boats and trailers before launching at new locations will also prevent introductions. There are a number of guidelines developed under the 100th Meridian Initiative focused on reducing boater transport of zebra mussels that are applicable to NZ mudsnails. Similarly, boater movement surveys developed for the 100th Meridian Initiative that focus on NZ mudsnail infested waters can help target prevention activities toward potential "hot spots" for new introductions. For more information, see http://100thmeridian.org.

c. **Anglers, Hunters, and Natural Resource Management personnel**: In addition to outreach and HACCP-NRM measures noted above, providing resources to facilitate field decontamination of gear will aid in reducing risk. Associated with this approach is having a variety of decontamination methods that are known to be effective via scientific testing and are also practical for field use (see section 6 below). Anglers who catch fish in NZ mudsnail infested waters could be required to clean fish before moving between watersheds. As a more extreme measure, contaminated areas can be closed to public access (although this will only be effective if accompanied by adequate enforcement resources).

d. **Aquarium and aquatic plant trade and collections**: Stronger inspection and quarantine requirements for shipments of aquarium and water garden organisms, accompanied by effective requirements for disinfection or disposal of contaminated shipments, can eliminate this pathway. The Lacey Act can facilitate federal enforcement of NZ mudsnail importations between states that prohibit possession of NZ mudsnails. Please see Section C and Appendix B for individual state regulations.

e. **Commercial shipping**: There are significant programs in place at the state, federal, and international level to reduce the discharge of contaminated ballast water. It is beyond the scope of this plan to analyze those programs. Where applied, it is likely that the current practice of open ocean ballast water exchange, as well as many onboard ballast water treatment methods currently under development, would be sufficient to eliminate the discharge of live NZ mudsnails. However, there are gaps in mandatory ballast treatment requirements that still leave some U.S. waters vulnerable to NZ mudsnail introductions via this pathway. In addition, NZ mudsnail attachment to the surface of the ship could be another pathway.

f. **Sand/gravel mining, extraction, and dredging**: There does not appear to be regulatory requirements governing inspection or cleaning of associated equipment for these activities. Voluntary or mandatory decontamination guidelines may help reduce risk. As a more extreme measure, contaminated areas can be closed to extraction or dredging activity. Individual state agencies may incorporate decontamination requirements when issuing permits for these

activities, but often do not have personnel available to enforce the requirements.

g. **Transport by fish, wildlife and livestock**: Although impractical to limit in natural settings, netting and other predator avoidance methods typically used in fish hatcheries can reduce the ability for birds and other wildlife to spread NZ mudsnails from contaminated facilities. In some cases, trapping and moving animals from the area may be necessary. Livestock should be kept away from invaded streams and riparian areas. Where this induces undue hardship, livestock should be contained to the same drainage.

h. **Transport by volitional movement**: It has been suggested that barriers such as copper stripping or electrical weirs may limit volitional movement of NZ mudsnails, particularly as a means of protecting high risk sites like fish hatchery water systems. Some investigations are underway but there is no applicable tool available yet.

i. **Transport by anchors:** Since the eastern clone is found in relatively deep water, the most likely method of dispersal is by movement of deep sediment by dredging or by movement of anchors. Inspection and cleaning of anchors may be necessary if they are used in invaded areas from 15-45 meters deep. Anchors used in western U.S. rivers by recreational boaters might also become fouled with aquatic vegetation and mud, and require inspection and cleaning.

3. Rapid Response

The following is a stepwise process for the rapid response to the report of a population of New Zealand mudsnails:

1. Confirm reports of new NZ mudsnail invasions.
2. Document the distribution of the NZ mudsnail population.
3. Convene Technical Advisory Body which has been designated to determine the feasibility of eradication and/or control methods.
4. Determine whether to attempt eradication or whether to implement control actions, and make recommendations to appropriate state agencies.
5. Develop a ‘boilerplate’ environmental review document approved during the Programmatic Environmental Assessment /Programmatic Environmental Impact Statement (PEA/PEIS). Allow a maximum of two weeks for completion of the document and availability to the public.
6. Public participation process.
7. Incorporate public comment.
8. State ANS Coordinator and Technical Advisory Panel to make a decision on whether to proceed with the proposed action.
9. If decision is to proceed, implement the proposed action.
10. Monitor the results of the action and determine success.

Eradication and control of newly discovered populations of NZ mudsnails will require quick action on the part of the appropriate agencies. States with ANS Management Plans should have a Rapid Response section which lays out specific actions based on state and federal government agency coordination. States with an invasive species or aquatic invasive species council may designate that body to determine what eradication or control steps need to be taken. States without ANS Management Plans or invasive species councils need to have a clear authority for making this decision. For example, in Colorado, which does not have an ANS Management Plan, the authority for determining actions in response to aquatic invasive species starts with the State Health Board which makes a recommendation to the Colorado Wildlife Commission. This process takes several weeks because it depends on when the two boards have regularly scheduled meetings.

For agencies to have the ability to implement actions in a rapid fashion, one suggestion is that each state conducts a PEA or PEIS for the control of mudsnails and other aquatic nuisance species. After completion of a PEA/PEIS, a systematic process could be implemented to address confirmation and action of mudsnail populations and to implement rapid response measures.

4. Eradication

If prevention efforts fail to stop the spread of NZ mudsnails into a new water body, new populations should be eradicated where it is feasible and practical. It must be determined: 1) if total kill is likely, recognizing the survival of even one NZ mudsnail can negate an eradication attempt; 2) if environmental damage will be caused and if so estimated recovery costs, and 3) if there will be impacts to non-targeted and threatened and endangered species. Development of geographic-specific early detection and rapid response plans will facilitate quick action. These plans can include documents such as intended response actions.

Many times the newly discovered population of NZ mudsnails may be in a river or lake where chemical eradication will not be feasible and physical eradication difficult. This would be the case with large rivers or lakes where it is impossible to isolate the invader and treatment would be difficult to contain. In other situations the invader may occupy too large an area or other ecological or political restraints may rule. However, it must be recognized that there will be some opportunities where either of the methods would be applicable and effective.

Areas where eradication may be possible include small lakes and ponds, waterbodies that can be temporarily hydrologically separated (e.g., curtain, wall), irrigation canals, and fish hatcheries. Many small lakes and ponds are isolated or may easily be isolated from the drainage making it easier to apply chemicals without downstream damage. In other cases draining and allowing the substrate to heat and dry in the summer or freeze in the winter would be equally effective. Irrigation canals are routinely shut down or sections are isolated and treated for eradication of unwanted plants, a method which could be used for snail control also. Fish hatcheries are another example of a situation where the snails could be completely eradicated although in some situations it may be difficult. Water flowing

through most fish rearing facilities can be controlled and many have protocols used to remove bacteria or virus pathogens by chemical disinfection.

Chemical methods used to eradicate NZ mudsnails include: Bayer 73 (Francis-Floyd et al. 1997), copper sulfate, and 4-nitro-3-trifluoromethylphenol sodium salt (TFM). The only molluscicide known to have been tested against NZ mudsnails is Bayluscide (a.i. niclosamide). This test, conducted by Montana Fish, Wildlife, and Parks (FWP), was to determine the feasibility of eradicating NZ mudsnails from a small spring creek along the lower Madison River. One hundred percent mortality occurred after 48 exposure units of Bayluscide. An exposure unit is 1 ppm for 1 hour (Don Skarr, Montana FWP, pers. comm.). Preliminary investigations also suggest that copper and carbon dioxide under pressure may prove useful in both decontaminating fish hatchery water supplies and preventing spread into uncontaminated areas of a hatchery. Ozone has not been shown to be effective in killing NZ mudsnails in a hatchery environment (Moffitt, pers. comm.)

Physical treatments include the use of temperature, humidity or desiccation to kill the target species. This includes draining the infested areas. NZ mudsnails can survive for long periods in a cool damp environment; however, draining the areas where they are congregated and exposing them to sunlight during the summer months may be sufficient for eradication. Using a flame thrower in a hatchery situation against the walls of raceways will kill any mudsnails attached. Mudsnails cannot withstand warm temperatures (Dwyer et al. 2003; Richards et al. 2004) or low humidity situations (Dwyer and Kerans, unpublished; Richards et al. 2004). Alternately, if an infested area could be drained in the winter and the substrate is frozen to a depth containing the mudsnails, then total eradication will occur. There is preliminary evidence that hydrocyclonic separators may also be a useful tool to decontaminate fish hatchery water supplies and prevent the spread of NZ mudsnails within a hatchery (Moffitt, pers. comm.).

5. Control and Containment

When complete eradication is deemed infeasible, the NZ mudsnail population should be isolated to prevent further spread by closing pathways and eliminating vectors. Posting of educational signs about mudsnails and ways to prevent their spread will increase awareness and target the behavior of audiences and their role in the containment of NZ mudsnails. Some jurisdictions may choose to close the invaded area to fishing, hunting or other water sports.

Other techniques which may control the populations of NZ mudsnails without eradication are:

1. Periodic molluscicide or biocide application,
2. Periodic desiccation of waterbody, and/or
3. Periodic introduction of biological control agent.
4. Mechanical methods

III. Objectives

A laboratory study was done in 2005 by Sean Garretson at Portland State University's Center for Lakes and Reservoirs using GreenClean® PRO to control NZ mudsnails. GreenClean® is a non-copper-based algaecide that eliminates a broad spectrum of algae on contact. It is designed for lakes, ponds, and other large bodies of water, as well as for unpainted surfaces, such as beaches, docks, and walkways. Its active ingredient, sodium carbonate peroxyhydrate, creates a powerful oxidation reaction that destroys algal cell membranes and chlorophyll, providing immediate control of algae. The producer, BioSafe Systems, claims that the algaecide is fish, animal and plant safe (see www.biosafesystems.com).

Garretson's study had several objectives: 1) to investigate the effects of GreenClean® PRO on NZ mudsnail mortality, 2) determine the minimum algaecide concentration and exposure times that results in 100% mudsnail mortality, and 3) expose mudsnails to a range of concentrations including those that exceeded the maximum application rate recommended.

Application of GreenClean® PRO is an effective way to hinder if not eliminate NZ mudsnails in the lab. Mortality was 100% within 72 hours of exposure to a 0.5% concentration for 2 and 4 minutes, 1% concentration for 30 seconds, and minimum of 0.33% concentration for 8 minutes. Mortality was also 100% 48 hours after exposure to a 4% concentration for 2 minutes and 0.55% concentration for 8 minutes. Results demonstrate the detrimental effects that GreenClean® PRO has on NZ mudsnails under very specific lab conditions. Garretson concluded that uncertainty remains as to the effectiveness of application by field personnel.

Copper and carbon dioxide under pressure are also being investigated as biocidal compounds that hinder NZ mudsnail movement and spread (Moffitt, pers. comm.).

Parasites of NZ mudsnails from New Zealand may also become useful to control population size by inhibiting reproduction. Studies of the efficacy and specificity of a trematode parasite from the native range of NZ mudsnails as a biological control agent have shown positive results so far (Dybdahl et al. 2005, Emblidge and Dybdahl *in prep*.). The parasite *Microphallus* sp. appears to be highly specific in the native range, infecting the most common genotypes (Dybdahl and Lively 1998, Lively and Dybdahl 2000). Experimental infections have shown that populations of the parasite originating from the US 1 clone source are very effective at infecting the US 1 clone genotype in the western U.S. Experimental infections and molecular genetic studies have also shown that these effective lineages of *Microphallus* sp. are highly specific.

However, biological control entails the introduction of another non-native species, and the costs of this have to be weighed against the costs of ecological damage caused by the NZ mudsnails. In addition, substantial research on specificity and effects on vertebrates is still required before this can be conducted on any scale to ensure that further harm to the environment does not result.

The most prospective mechanical method for removing NZ mudsnails from hatchery source water is the hydrocyclonic separator (Moffitt, pers. comm.). Hydrocyclonic separator technology uses centrifugal force produced in a chamber to separate solids from liquids. One unit can handle 20 to over 1000 gallons per minute of water (see www.polytech-filtration.com/Hydrocyclones.htm).

6. Preventing the Spread on Wading and Other Gear

Given the significance of fishing gear and equipment as a pathway for NZ mudsnail transport, emphasis has been placed on researching effective control methods. At this time, consensus has not been reached on one universal method that consistently eliminates all NZ mudsnails from gear without causing gear damage (see Objective 3 Research Needs below). Recommended practices include:

- Cleaning all mud and debris that might harbor NZ mudsnails from boot, waders and gear with a stiff brush.

- Putting fishing gear in a freezer for 6-8 hours will kill all attached NZ mudsnails (Medhurst 2003, Richards 2004).

- Putting fishing gear in water maintained at 120°F for a few minutes will eliminate NZ mudsnails (Medhurst 2003). The mudsnails can survive at 110°F so the water temperature needs to be accurate.

- Dry fishing gear at 84-86°F for at least 24 hours or at 104°F for at least two hours (Richards et al. 2004). Gear should be thoroughly brushed with a stiff bristled brush prior to drying.

Freezing, hot water and drying at high temperatures may be difficult or impossible for many anglers or researchers who are moving from one water body to another in a short period of time. Two recent tests described below have revealed alternative methods of killing NZ mudsnails that are more adaptable to these conditions.

Researchers at California Department of Fish and Game exposed NZ mudsnails in laboratory tests to solutions of benzethonium chloride, chlorine bleach, Commercial Solutions Formula 409® Cleaner Degreaser Disinfectant, Pine-Sol®, ammonia, grapefruit seed extract, isopropyl alcohol, potassium permanganate, and copper sulfate. With the exception of grapefruit seed extract, potassium permanganate and isopropyl alcohol, these materials all killed mudsnails within five minutes (Hosea and Finlayson 2005). However bleach and Pine-Sol®, at concentrations efficacious in killing snails, did structural damage to wading gear. See Appendix E for more information.

The most effective solutions for killing NZ mudsnails which can be used in the field, according to this research are copper sulfate (252 mg/L Cu), benzethonium chloride (1,940 mg/L) and 50% Commercial Solutions Formula 409® Cleaner Degreaser Disinfectant. Appendix E has a thorough description of the procedure for cleaning wading or fishing

gear using these solutions in the field. Wading gear cleaned using any one of the three methods was, in general, free of live NZ mudsnails that could be transported to another water body. Exposure to these materials causes NZ mudsnails to release from the substrate they're in contact with, which facilitates their removal. Anglers or waders using these methods would need to insure that the cleaning solutions do not enter surface water or other sensitive habitats.

Researchers at the Colorado Division of Wildlife (CDOW) found that Sparquat brand quaternary ammonium disinfectant (benzalkonium chloride) outperformed Commercial Solutions Formula 409® Cleaner Degreaser Disinfectant. Sparquat is the disinfectant routinely used by CDOW fishery biologists for inactivating Whirling Disease (*Myxobolus cerebralis)* spores from field gear. They found that two dilutions of Sparquat at 4 oz/gal and 6 oz/gal outperformed 50% Formula 409 at 5 and 10 minute exposures. The researchers are recommending that field personnel use 6 oz. Sparquat per gallon of water for at least 10 minutes exposure, preferably longer (Colorado Division of Wildlife Aquatic Section, August 2005).

Oregon State University has completed initial research on using dry ice as a disinfection method. Preliminary results indicate 100% mortality can be achieved in some situations with minimal damage to gear (Chan 2005).

7. Objective 3 Education and Outreach

a. Develop corps of volunteer anglers who can provide one-on-one technical assistance to other anglers regarding prevention methods and effective control techniques. (Note Objective 5.C.3)

b. Provide outreach and educational material to watercraft inspectors to educate anglers and recreationists.

8. Objective 3 Implementation

a. Write a model provision for States to adopt that requires HACCP-NRM plans for aquaculture seeking permits.

b. Develop a model/rule that will help State agencies and private organizations do HACCP-NRM plans.

c. Develop a Hatchery certification to indicate whether NZ mudsnails are not detected or present at or near the facility and perform facility evaluations for invasive species prevention strategies.

d. Develop State rapid response plans. (Note: The Western Regional Panel has a model rapid response plan which can be used by States).

e. Develop a rapid response protocol or action plan for responding to new occurrences of NZ mudsnails.

f. Develop watershed-specific rapid response plans.

g. Develop a model interagency/interstate agreement for monitoring and efficacy of control/containment efforts on existing occurrences of NZ mudsnails in waters that cross state lines.

h. Provide tools such as wash kits or wash stations (instead of providing information only) as a means for action.

i. Create a national web-based database on NZ mudsnail monitoring and control efforts for easy retrieval for researchers and managers.

j. Increase coordination with agencies and other biologists to get more timely input on new populations of NZ mudsnails.

9. Objective 3 Research Needs

The following list gives research topics that will increase our understanding of management and control strategies for NZ mudsnails.

a. Biological Control

1) What is the specificity of parasite biocontrol agents against native non-target alternative?

2) What population demographic models can be developed to show under what parameters a parasite biocontrol agent would control NZ mudsnail populations and to what degree?

3) What is the effect of trematode parasite biocontrol agents on other hosts in the life cycle?

4) Is the risk of biocontrol worth the benefits of NZ mudsnail control?

5) Develop and apply control strategies for application and evaluation as HACCP-NRM control actions.

b. Chemical/Physical Control

1) Will chemical and or physical control techniques have acceptable ecological impacts?

2) Is the risk of chemical, physical, or biological control worth the benefits of NZ mudsnail control?

3) Can we develop effective and ecologically-sound control methods in the mechanical, physical, chemical and biological control arenas?

4) Continue to test efficacy of current treatments of gear and determine whether a treatment must *kill* all mudsnails or simply get them to release from the gear.

5) Develop and test prevention methods for wader and gear enabled transport for a variety of ANS including whirling disease, didymo, etc. so that anglers and field crews don't have to use multiple disinfectants or choose to prevent the transmission of one ANS over another. Once a consistently effective method is identified, seek consensus to make it the recommended protocol used consistently by all agencies and organizations.

D. Objective 4: Develop Further Understanding of Ecological and Economic Impacts

1. Ecological Impacts

The extent of the ecological impact of NZ mudsnails to ecosystems in areas where it has invaded in western North America is not yet known, but studies to date indicate the likelihood of wide-ranging consequences. The full ecological impact of NZ mudsnails is likely to include effects on aquatic resources, such as competitive interactions with native aquatic invertebrates, and the associated changes in community structure and ecosystem function. Extremely high invasive snail densities (>500,000 m^2, Hall et al. 2003) contribute to the assumption of negative ecological impacts. Furthermore, the evidence that grazing herbivores like NZ mudsnails are extremely effective primary consumers in aquatic systems is well documented in the literature (e.g. Hawkins and Furnish 1987, Feminella and Hawkins 1995).

NZ mudsnails directly affect native biota by 1) consuming large quantities of the primary production, especially periphyton (Riley 2003, Hall et al. 2003); 2) competing with native gastropods, some threatened and endangered (Richards 2004, Riley 2003, Riley et al., *in review*); 3) competing with other grazing and detritivorous invertebrates that are the foundation of aquatic food webs (Cada 2004, Kerans et al. 2005, Cada and Kerans, *in review*); and 4) negatively impacting both invertebrates and vertebrates at higher trophic levels in aquatic food webs that depend on the aquatic invertebrate food base (Cada 2004, Hall et al. 2006, Vinson, pers. comm.).

NZ mudsnails may displace native biota in aquatic food webs; hence their invasion has caused an alteration in the energy flow pathways among trophic levels thus damaging aquatic ecosystems. In three streams in the Yellowstone region, mudsnail production constitutes the vast majority of total secondary production (Hall et al. 2006). In the Gibbon River and Polecat Creek, NZ mudsnails constitute 88-93% of total secondary production and their rate of production in Polecat Creek is one of the highest ever measured in a river. Community structure is dominated by mudsnails, and this degree of dominance by a single species is comparable to highly degraded communities. The infestation in parts of Lake Superior are localized and relatively new, therefore, it is likely that impacts have not yet been manifested.

Invading NZ mudsnails do not serve as an equivalent substitute energy source for predators. Mudsnails apparently pass through fish intestinal tracts undigested, and have low energetic value for these secondary consumers (Ryan 1982, McCarter 1986). A recent lab experiment in Utah showed that when trout were fed a diet of mudsnails, over 80% of the mudsnails were undigested. These trout lost weight, while trout fed native invertebrates gained weight. This appeared to result from the fish's inability to obtain enough energy to grow (Mark Vinson, pers. comm.). Hence, community structure might be directly and indirectly affected at higher trophic levels in the food web (e.g. predatory invertebrates, fish, and other vertebrates).

The extent to which these changes in community structure and energy flow can affect fish populations is poorly understood. However, in a "worst case scenario," if NZ mudsnails replace higher valued food resources, fish reproduction, condition factor and population densities could be affected. Terrestrial animals such as birds may also be affected since some interact with aquatic food webs as well. Consequently, this invader could have effects that cascade through both aquatic and terrestrial food webs (e.g., Carpenter et al. 1985).

Because NZ mudsnail densities and biomass can be so high, they alter ecosystem processes such as nutrient cycling in rivers. Excretion of ammonium by mud snails supplies about 2/3 of the whole-stream demand by algae and bacteria for this limiting nutrient in Polecat Creek, WY, suggesting that mudsnails dominate the nitrogen cycle when biomass is high (Hall et al. 2003). It is also possible that they make rivers large sources of CO_2 by precipitating calcium bicarbonate to calcium carbonate to make their shells (Chavaud et al. 2003). By changing ecosystem functions such as C and N cycling, NZ mudsnails can indirectly alter the community structure and population dynamics of native organisms, as the snails have changed fundamental attributes of the ecosystem.

In conclusion, based on the current literature, direct effects of mudsnail invaders on stream communities potentially include 1) decreased densities of native herbivorous and generalist invertebrates, 2) decreased densities of attached filter-feeding organisms, and 3) decreased densities of invertebrate and vertebrate predators of native species displaced by NZ mudsnails. Species replacement is one of the most important contributors to the loss of biodiversity in freshwater communities.

Densities of the eastern population in Lake Ontario vary with time of year, peaking in the late summer and early fall and crashing during the winter (Zaranko et al. 1997). In Lake Ontario, densities also vary substantially from year to year ranging from 15 to over 5500 per square meter in several locations (Zaranko et al. 1997; Levri et al. in prep.). In both Lake Superior and Lake Erie, very few individuals have been found (two in each location).

Assessing the ecological effects of the eastern clone is difficult. The areas inhabited by NZ mudsnails have already been substantially impacted by zebra mussel (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*). Thus it is difficult to attribute damage to NZ mudsnails.

Studies of terrestrial communities suggest that changes in community structure associated with nonnative species and biodiversity loss can alter ecosystem function and disturbance regimes, but these effects have been less studied in aquatic systems. The stability of ecosystem functions in terrestrial systems such as nutrient cycling and productivity is reduced at lower levels of plant species richness (Tilman et al. 1996). In a marine system, grazer diversity was positively correlated with ecosystem properties (Duffy et al. 2003). Furthermore, nonnative species in communities can increase the frequency of disturbance and lead to further changes in community structure (Mack and D'Antonio 1998), although these effects have not been studied in aquatic systems.

2. Economic Impacts

Economic impacts associated with the introduction of NZ mudsnails may derive from both direct and indirect effects and may vary regionally. Biofouling is a typical direct economic impact of invasive mollusks, as exemplified by the zebra mussel, *Dreissena polymorpha* (Mills et al. 1993). Zebra mussels may reach high densities, clog intake structures, and foul maritime equipment (Locke et al. 1993, Mills et al. 1993). It is estimated that the damage and control costs of the zebra mussel in intake pipes, water filtration systems, and electric generating plants is $100 million per year (Pimentel et al. 2000). The biofouling potential of NZ mudsnails is probably lower than that of the zebra mussel. However, NZ mudsnails have been documented to pass through water pipes and to emerge from domestic taps (Ponder 1988) and can block water pipes and meters (Cotton 1942 in Zaranko et al. 1997). With population densities as high as 800,000 individuals m^2 (Dorgelo 1987), there is a potential for biofouling, particularly in irrigation systems in arid regions. More study is needed to quantify potential impacts of NZ mudsnails on municipal, industrial, and irrigation/water delivery systems.

Extremely high densities of invasive species can also have ecological consequences that result in indirect economic impacts. Indirect economic effects that occur because of changes in ecology are difficult to measure, but clearly occur. For example, in the Greater Yellowstone Ecosystem, the effects of NZ mudsnails on natural communities and food webs may threaten the economically important recreational fishing industry in the region (Keiter 1991) by decreasing the habitat for the various aquatic organisms, altering the food base and ultimately leading to population declines. Revenue generated by tourists, fishing licenses and outfitters could be significantly decreased.

A second cost is related to the vulnerability of threatened or endangered native fauna to mudsnail invasion. For example, zebra mussels' tendency to settle on hard surfaces has lead to the fouling of native mussel valves, leading to high mortality rates (Schloesser et al. 1996). Many of these native mussels are either endangered or threatened, leading to costly study and salvage operations (Schloesser 1996). Mudsnails overlap with threatened and endangered species in the Snake River, ID, resulting in costs associated with protection of these species. Other associated costs include: research and development expenses incurred by agency and university personnel to prevent further spread; monitoring the distribution and spread of the snail to determine whether sensitive native species are being placed at risk; extra monitoring that must occur for threatened and endangered species within the range of the NZ mudsnail invasion; extra steps taken by agency personnel to ensure that facilities such as hatcheries do not act as vectors; extra requirements placed in permits for activities such as dredging, canal maintenance, etc., extra steps and materials used by agency personnel, researchers, citizen monitors, and consultants to decontaminate gear; and extra costs incurred for materials, transport and time of public outreach and information dissemination. Finally, contamination of private hatcheries and subsequent regulation and prohibition of their operations results in direct economic impact to these operators.

Virtually nothing is known of either impacts or potential impacts of the Great Lakes clone. Since there seem to be significant differences, e.g., depth, density, and potential pathways for spread, from western clones, there is a need for research to determine whether Great Lakes populations pose an ecological risk.

3. Objective 4 Implementation

a. Develop a protocol for pre/post-impact studies (ecological and economic) that can be applied consistently to new locations when incipient NZ mudsnail invasions are found.

b. Develop sources of funding for research, including contacts with foundations and coordination among agencies.

4. Objective 4 Research Needs

Ecological research aimed at predicting the invasiveness and ecological impact of this species in North American waters is needed. Research on economic impacts of these snails as biofoulers on manmade systems and as disruptors of natural systems is also needed.

a. Investigate the effects of NZ mudsnails on community structure, food webs (both vertebrates and invertebrates), and ecosystem function. This should include both field surveys and experimental approaches aimed at understanding the interactions between NZ mudsnails and native species.

b. Investigate the effects of NZ mudsnails on vertebrates at higher trophic levels, including trout and waterfowl. Conduct research that can effectively answer the question “how much do native fish populations decline in specific watersheds after NZ mudsnails become abundant?”

c. Determine whether the costs of control and management of NZ mudsnails are significant. This should compared with a no control with anticipated effects on recreational opportunities, loss of revenue to local economies, construction, enforcement, signage, as well as ecological impacts.

d. Investigate the basic ecology and ecological risk of the Great Lakes Clone.

E. Objective 5: Increase Understanding of the Need to Deal with NZ Mudsnails and Gain Support for Implementing National Plan Objectives

1. Education and Outreach Needs

A successful effort to contain and control NZ mudsnails will only be possible if an effective outreach and education campaign is developed and implemented. Many of the goals and objectives outlined in this plan are dependant upon the support and adoption of a large segment of the public. This cooperation and support must be earned through effective outreach.

Any successful communication effort begins with a plan that establishes who the target audiences are, what the desired outcome is from these audiences, where these audiences will be reached and how to reach them. Four primary audiences have been identified to be targeted with NZ mudsnail outreach efforts. These groups are: resource allocators, agency administrators, natural resource management implementers, and the public. Each of these groups has shared unique message needs. Each group needs to receive a message tailored to them to insure the greatest possibility of success. See Table 1 for list of audiences and messages.

Resource allocators are those key individuals that are capable of providing the financial and human resources needed to advance this effort (e.g., legislators). They are usually not scientists and do not want to receive detailed specifics about the organism. Rather, they are concerned about how NZ mudsnails fit into a larger picture. They want to know what the threat is, what the consequences are of various actions, what economic impact they might have, how they will affect the public and why they should receive a priority. These individuals will often be more receptive to messages that address human related impacts. To effectively reach this audience, materials need to directly address their concerns and be delivered in a fairly brief fashion. The most effective way to deliver messages about NZ mudsnails to these individuals will be through personal contact. A brief, well prepared, presentation is the most effective tool to use. The presentation will be crafted to be equally effective and compelling whether it is delivered as a PowerPoint presentation, a formal briefing or a short conversation.

Agency administrators are another important group to be targeted for outreach. These administrators are often resource allocators but also have the role of establishing priorities and policies for their agencies. These individuals need to be fully informed about NZ mudsnails and make policy decisions that result in a commitment to the control effort. Agency administrators will often need a higher level of detailed information than resource allocators. They need to be provided with concise and accurate information about management options which, when implemented will lead to greater success. The message to them will begin by establishing the need (this is essentially the same message provided to allocators) followed by suggestions for management options and strategies. Finally, they will be encouraged to secure the full cooperation of their implementation staffs. The

message delivery to agency administrators will be most effective when conducted on an individual basis. However, presentations to groups of administrators can also be very effective. Effective tools for communicating with these individuals will be PowerPoint presentations, briefing papers, personal appeals (especially from people with established credibility) and peer communications.

Most natural resource management implementers are those individuals that are charged with the actual field level implementation of actions. They might be biologists, researchers, wardens or others that undertake the actions identified as part of the strategy. The outreach goal for this group is twofold. The first goal is to insure that they understand and adopt any protocols that are established. The second goal is to enlist them as additional communicators to the public. No matter how good the planning and research are, the entire control effort will fail if the implementers do not follow through. They must make field observations to identify range and spread. They must implement cleaning and transport protocols. They must actively work to eliminate pathways of introduction and every other management recommendation developed. All of these require new actions on top of already overwhelming work loads. Outreach strategies should be carefully crafted to ensure their enthusiastic support. First, this audience must be educated as to the threat to the resource. They must clearly understand all aspects of identification, spread, control and prevention. Finally, they must be prepared to share their knowledge with others. The most effective way to reach implementers is to both reach them with the message directly and to have their administrator also ensure that they will adopt the appropriate actions.

Most implementers need more information than any of the target audiences. They need to understand the impacts and threats, the vectors of spread, the life history of the organism, the methods of control, any cleaning or disinfection protocols, how the public can help or hurt the effort, how to present to the public and much more. They will need fact sheets, clearly defined protocols, research results, information on support resources and outreach materials for public distribution. Implementers will best be reached through conferences and meetings, publication in professional journals, professional society meetings and publications, agency meetings and briefings and the like. It is not realistic to envision reaching many implementers through personal contact. PowerPoint presentations with support materials will likely be an effective tool for reaching these individuals. Web-based information and field identification guides/manuals are other important tools. Combining these efforts with communication directly from administrators will increase their impact.

Ultimately, the “public” needs to be completely integrated into the effort to control spread. However, the public is a broad group of people that must be broken into subgroups with targeted information. Although each segment of the public will receive the message through different venues, the basic objective is the same for all. Any reduction in spread will require cleaning, disinfecting or some other action on the part of certain segments of public. Modifying peoples’ behavior is a difficult task due to being asked to take action as opposed to just understanding the problem. This requires adoption of social marketing techniques in addition to education or outreach.

Social marketing efforts are those that are specifically designed to change behavior by identifying the points of resistance and addressing these "costs" to the individual. Successful social marking convinces the target audience to take some sort of action that will "cost" them time or convenience; this happens because they perceive that the benefits gained are more valuable than the cost associated. This requires clear definition of the threat of NZ mudsnails, both current and potential. In addition, it must be demonstrated that the effort they invest will have a beneficial outcome. Key to this effort will be the ability to recommend practical actions. Establishing "pride of accomplishment" in those who participate can achieve a great breakthrough in this effort. Once this pride is established, an incentive is created for non-participants to join the "better" group. Organizations such as the Federation of Fly Fishers, American Rivers, Trout Unlimited and others can be enlisted to help disseminate pertinent information to their members.

Another key to the social marketing effort will be to develop consumer materials that are specifically designed to elicit the desired outcome. There is a large body of work that defines the attributes of successful efforts and this will be used to craft effective tools and materials. Finally, the use of "celebrity" spokespersons will help to establish that it is "cool" to be a part of the solution.

An effective tool to be used in educating the public is to integrate invasive species education into school programs. Although teachers are provided with a host of age appropriate materials and programs, there are significant opportunities to provide them with useful educational products. Any success in educating students will be very effective in a broader sense as they, in turn, educate their parents and other adults.

Many public and private organizations are already reaching out to the public with regular education messages about NZ mudsnails and aquatic invasive species in general. Any further education and outreach efforts on NZ mudsnails requires partnering with these existing efforts wherever possible. Success in this effort requires the broadest possible outreach mechanisms. Additionally, repeated messages often are much more effective than a single exposure so a saturated effort will produce far better results.

NZ mudsnails are a serious threat and must be addressed as such. However, outreach and education efforts must be handled with care to avoid confusing target audiences. There is a constantly growing list of aquatic invasive species that are invading North American waters. It is very difficult if not impossible for the average citizen to be aware of each individual species and in some situations people may feel overwhelmed by multiple messages each focused on single species. Therefore, it is important that the NZ mudsnail efforts be integrated into larger campaigns whenever possible. For example, "Stop Aquatic Hitchhikers"™ is a national branding campaign organized by the U.S. FWS to address all aquatic invasives and in particular pathways associated with outdoor recreation. It is important that all NZ mudsnail efforts focus on those pathways with this existing campaign both to utilize the awareness that already exists and to support the branded concept.

Education and outreach efforts also must recognize that unlike invasive species with long and well-defined histories of major economic and ecological impacts (e.g., zebra mussels),

NZ mudsnail impacts may “underwhelm” many audiences. Species impacts will vary between location, habitat, water conditions, temperature and other co-existing organisms. Furthermore, many current concerns about economic impacts from NZ mudsnails are more related to management actions to prevent spread (e.g., hatchery closures) rather than direct effects from the snails (i.e., those particular impacts would not exist if efforts to control the snails ceased). These factors may result in challenging “so what?” questions from target audiences, and in particular for those that seek answers in simple “sound bites.” Education and outreach messages will need to find a balance between providing sufficient information to explain why concern still exists (e.g. explanation of lag times, food web connections, etc.) versus losing audiences with too much information; and a balance between over-exaggeration of risks versus failure to convince audiences that they should be concerned.

Table 1. List of target audiences, outcomes, and possible vehicles for achieving those outcomes.

Audience	Outcome	Message and Means
Resource allocators (politicians) (may want to know about the priority relative to other invasive species)	• Allocate resources (personnel, funds, statements of intent, etc.)	**Message**: The NZ mudsnail is a real and serious threat to resources and the economy. (We don't know the threats, but we need to contain the threats, so we have to find out what they are.) **Means**: executive summary of the plan, PP presentations directly to resource allocators and key staffers, celebrity spokespeople
Agency administrators (some of whom might be resource allocators and set agendas for agencies)	• Make informed management decisions or direct the resources appropriately	**Message**: (Message should be similar to that above but with added case histories and suggestions such as creating a state ANS plan and rapid-response plan.) **Means**: executive summary, full plan, briefings to key staffers, international organizations, celebrity spokespeople, PP presentations with case histories (maybe about other invasive species)
Implementers (managers, biologists)	• Make wise daily operational decisions (prevention, detection) • Become additional communicators to the public	**Message**: The NZ mudsnail is a real and serious threat. You are an important part of the discoveries we make. You can make a difference (detection, monitoring, communicating to the public, raising awareness of ANS in general). **Means**: reports, presentations at professional meetings (such as American Fisheries Society), agency administrators, interagency meetings, division meetings, state ANS coordinators
Public with the following subgroups: • **Anglers** (or "keenly interested" stakeholders to include conservation groups) • **Other water resource users** (recreationists, equipment operators, etc.) • **General** (such as those on the Lewis & Clark trail) • **Educators** • **Youth**	• Take personal responsibility for reducing spread of NZ mudsnail • Initiate change on a higher level	**Message**: Invasive species are bad. You could be part of the problem. You can take action. (The action part may differ by subgroup: clean your gear and don't haul bait; if it doesn't look familiar, let someone know; etc. The action needs to be short and simple) **Means**: editorial coverage in magazines appropriate for each subgroup, celebrity spokespeople, manufacturing companies (stickers on boats, reels, and waders; John Deere and Caterpillar), insurance companies, trade organizations, radio spots (For youth) state REA coordinator, stations at watershed festivals and kids days, coloring or comic book, video game

Media, including Internet	• Broad dissemination of messages	**Message:** The New Zealand mudsnail is a real and serious threat. You are an important part of the discoveries we make. You can make a difference (detection, monitoring, communicating to the public, and raising awareness of ANS in general). **Means:** Press kits, suggested storylines, field trips with specific writers or broadcasters, website messages, blogs

2. Examples of Outreach Efforts

Several partnerships have evolved in the last five years to develop outreach materials targeting recreational users about the possibilities of moving NZ mudsnails to new uninfested waters. Listed below are examples:

a. Watch cards, brochures, posters, and signs

U.S. Fish and Wildlife Service developed watch cards that provide an alert about NZ mudsnails, explain how to identify them and collect samples, and instruct who to contact for new discoveries. Several versions of these cards have been distributed to a variety of audiences, including biologists and other professionals, recreational users, and participants in Lewis and Clark Bicentennial Commemoration events.

Idaho Department of Fish and Game and Idacorp, the power company, with support from the U.S. Bureau of Reclamation, the U.S. Fish and Wildlife Service, and Idaho State Parks and Recreation developed laminated signs with a cartoon NZ mudsnail, titled "Stop the Mudsnail!" The signs were distributed to various state and federal land agencies in the west.

Alaska Department of Fish and Game, Federation of Fly Fishers and the U.S. Fish and Wildlife Service created a "Most Unwanted" flyer titled "Alert! Dangerous Invader." The flyers were distributed to all the fishing guide services in Alaska to warn them of possible invasion and give them precautions to take with their clients.

b. Web sites

Montana State University maintains a website on NZ mudsnails with funding from the U.S. Fish and Wildlife Service. There are searchable maps of known and reported locations in the western U.S, information about biology and ecology, minutes from national meetings, pictures and a comprehensive bibliography. The website can be found at: http://www.esg.montana.edu/aim/mollusca/NZ mudsnail/

Several state wildlife agencies also have NZ mudsnail alerts or pages on their websites.

c. Workshops and conferences

There have been four New Zealand Mudsnails in the Western USA conferences, held in 2001, 2002, 2003 and 2005 at Montana State University in Bozeman. The minutes for these conferences which have information on current research, are available on the website above.

The Colorado Division of Wildlife and the U.S. Fish and Wildlife Service held a one-day workshop in April 2005 for over 100 biologists from federal, state and local agencies as well as private organizations and companies, on

identification, biology and ecology of NZ mudsnails. The purpose was to provide information for field biologists to look for, identify and report NZ mudsnail sightings in the state.

d. Wash stations
Montana Department of Fish, Wildlife and Parks has purchased one permanent wash station and one mobile wash station for use at strategic locations in Montana where NZ mudsnails occur.

e. General ANS materials
There are many other publications and outreach efforts that feature NZ mudsnails along with other aquatic invasive species, giving guidance on how to avoid spread. Some examples include the "Threats to the West" brochure by the Western Regional Panel and "Aquatic Hitchhikers" by the Utah Division of Wildlife Resources.

3. Objective 5 Implementation

a. Fund staff in every State to do field outreach regarding NZ mudsnails and other invasives.

b. Raise awareness to audiences associated with identified pathways.

c. Develop corps of volunteer anglers who can provide one-on-one technical assistance to other anglers regarding prevention methods and effective control techniques.

d. Coordinate with other programs such as the 100th Meridian Initiative and zebra mussel efforts.

e. Develop press kits and outreach materials.

f. Create sound bites that are understandable to the public.

g. Develop a canned template (in Adobe Illustrator or other usable application) so that information can be easily adapted.

h. Fund a website that is "public friendly." Provide dedicated support and have an outreach person to coordinate information and efforts.

i. Develop a list server discussion group for those interested in sharing information on NZ mudsnails.

j. Develop list of local and national sources of taxonomic expertise for identifying potential NZ mudsnail populations and publicize widely.

k. Develop educational materials that address the reality that NZ mudsnail invasions have not been linked to conspicuous declines in native fish populations but also provide concrete evidence of negative impacts along with analogous stories of other invasions where such measurable impacts took years to hit (at a point where many management options were not longer available).

l. Develop a plan for public awareness of control measures, including biological, chemical and physical, and the potential benefit of their application.

m. Develop a key of North American aquatic snails with emphasis on identifying nonnative snails such as NZ mudsnails, Chinese mystery snail, etc.

4. Objective 5 Research Needs

a. How effective are existing NZ mudsnail education and outreach methods in changing behavior of target audiences?

IV. Implementation Plan

Section III of the NZ Mudsnail NMP contains chapters on each of the five objectives which include ideas for implementing education, management and research actions. In Section IV, all of the suggested action items are brought together and prioritized below.

A. Priorities for Implementation

Tables 2, 3, and 4 give primary, secondary and tertiary priorities for suggested actions. Page numbers for location of the action item in the text are in parentheses.

Table 2. Primary Priorities for Implementation

Objective	Action Item
Objective 1. Pathways and Vectors	**Implementation** 1.1) Develop risk assessment of different pathways. (16) 1.2) Develop guidance or criteria for risk and impact assessments. (16) 1.3) Support and expand New Zealand Mudsnail in the Western USA database, maps and web site to include eastern U.S. populations. (17) 1.4) Facilitate coordination with USGS Nonindigenous Species database in Gainesville, FL. (17) **Research** 1.R1) Are there specific habitat types and/or environmental conditions that completely preclude establishment of NZ mudsnails? Are there environmental/habitat parameters that make an area more vulnerable to invasion? (17) 1.R2) How can relative risk of introduction and establishment of NZ mudsnails in uninfested waters be quantified? (17)
Objective 2. Detecting Populations	**Implementation** 2.1) Create a national web-based database for easy retrieval on NZ mudsnail sampling efforts (methods, frequency, detection limits) including those where NZ mudsnails have not been detected. (22) 2.2) Increase coordination with agencies and other biologists to get more timely input on new populations. (22) 2.3)Establish priority areas for monitoring, such as sites close to existing infestations or sites with special management needs or sites containing endangered or threatened species. (Note: Objective 3.8.i.) (22) **Research** 2.R1) Evaluate different detection methods in different habitat types to provide a way for establishing confidence in data that people submit. (23) 2.R2) Monitor existing NZ mudsnail populations on a precise schedule to understand populations trends and their response to environmental factors. (23) 2.R3) Establish baseline data in States which currently have NZ mudsnail populations. (23)

Objective 3. Prevention, Control and Management	**Implementation** 3.1) Develop a Hatchery certification to indicate whether NZ mudsnails are not detected or present at or near the facility and perform facility evaluations for invasive species prevention strategies. (32) 3.2) Write a model provision for States that requires HACCP-NRM plans for aquaculture seeking permits. (32) 3.3) Create a national web-based database on NZ mudsnail monitoring and control efforts for easy retrieval for researchers and managers. (32) 3.4) Increase coordination with agencies and other biologists to get more timely input on new populations of NZ mudsnails. (32) **Research** 3.R1) Develop effective and ecologically-sound control methods in the mechanical, physical, chemical and biological control arenas. (33) 3.R2) Continue to test efficacy of current treatments of gear and determine whether a treatment must kill all mudsnails or simply get them to release from the gear. (33) 3.R3) Develop and test prevention methods for water and gear enabled transport for a variety of ANS so that anglers and field crews don't have to use multiple disinfectants or choose to prevent the transmission of one ANS over another. (33)
Objective 4. Ecological and Economic Impacts	**Implementation** 4.1) Develop sources of funding for research, including contacts with foundations and coordination among agencies. (37) **Research** 4.R1)Investigate the effects of NZ mudsnails on community structure, food webs (both vertebrates and invertebrates), and ecosystem function. This should include both field surveys and experimental approaches aimed at understanding the interactions between NZ mudsnails and native species. (37) 4.R2)Investigate the effects of NZ mudsnails on vertebrates at higher trophic levels, including trout and waterfowl. Conduct research that can effectively answer the question "how much do native fish populations decline in specific watersheds after NZ mudsnails become abundant?" (37)
Objective 5. Outreach and Education	**Implementation** 5.1) Raise awareness to audiences associated with identified pathways. (45) 5.2) Develop corps of volunteer anglers who can provide one-on-one technical assistance to other anglers regarding prevention methods and effective control techniques. (45) 5.3) Develop a list of local and national sources of taxonomic expertise for identifying potential NZ mudsnail populations and distribute information to a broad national audience. (45) **Research** 5.R1) How effective are existing NZ mudsnail education and outreach methods in changing behavior of target audiences? (46)

Table 3. Secondary Priorities for Implementation

Objective	Action Item
Objective 1. Pathways and Vectors	**Implementation** 1.5) Identify additional pathways. (16) 1.6) Prioritize pathways for outreach efforts. (16) **Research** 1.R3) Using genetic markers, determine pathways of NZ mudsnail spread in the U.S. (17) 1.R4) How important are different human-mediated recreational vectors to the spread among watersheds: boat transport, angler movements, swimmers, etc.? (17) 1.R5) How important are different vectors associated with economics activities: Fish aquaculture, fish hatcheries, water use and transport? (17) 1.R6) Which suspected pathways have had the most prominent role in actual introductions of NZ mudsnails in the U.S? a) between watersheds, b) within watersheds. (17) 1.R7) For those fish species used in aquaculture and that will consume live snails, what is the maximum time period that NZ mudsnails can live within the digestive tract and still pass through as viable organisms? (18) 1.R8) What are the ranges of natural dispersal rates/distances that have been documented for NZ mudsnails upstream and downstream from initial infestations, and what physical factors affect those rates and distances? (18) 1.R9) Once NZ mudsnails occur in one tributary of a watershed, what is the likelihood that they will eventually occupy all reaches of the entire watershed that support their habitat needs. Under what circumstances might such spread not occur? (18)
Objective 2. Detecting Populations	**Implementation** 2.4) Review existing local, state, federal databases and/or collections of macroinvertebrate samples (focused on watersheds where intentional NZ mudsnail sampling has not occurred). (22) 2.5) Conduct further outreach to watershed groups and government agencies collecting benthic invertebrate samples regarding identification and data gathering. (22) **Research** 2.R4) Develop estimates of detection probabilities for a variety of substrates, ecoregions, and sampling methods and sampling designs. (23) 2.R5) Develop standardized techniques for detecting low abundance NZ mudsnail populations and for estimating densities in established populations. (23)
Objective 3. Prevention, Control and Management	**Implementation** 3.5) Develop a model/rule that will help State agencies and private organizations do HACCP-NRM plans. (32) 3.6) Develop State rapid response plans. (32) 3.7) Develop watershed-specific rapid response plans. (32) 3.8) Develop a protocol for responding to new occurrences of NZ mudsnails. (32) 3.9) Provide tools such as wash kits or wash stations instead of providing information only. (32) **Research** 3.R4) What is the specificity of parasite biocontrol agents against native alternative hosts? (32) 3.R5) What population demographic models can be developed to show under what parameters a parasite biocontrol agent would control NZ mudsnail populations and to what degree? (32)

	3.R6) What is the effect of trematode parasite biocontrol agents on other hosts in the life cycle? (33) 3.R7) Is the risk of chemical, physical, or biological control worth the benefits of NZ mudsnail control? (33) 3.R8) Will chemical or physical control techniques have acceptable ecological impacts? (33)
Objective 4. Ecological and Economic Impacts	**Implementation** 4.2) Develop a protocol for pre/post-impact studies (ecological and economic) that can be applied consistently to new locations when incipient NZ mudsnail invasions are found. (37) **Research** 4.R3) Determine whether the costs of control and management of NZ mudsnails are significant? This should be compared with a no control option with anticipated effects on recreational opportunities, loss of revenue to local economies, construction, enforcement, signage as well as ecological impacts. (37) 4.R4) Investigate the basic ecology and ecological risk of the Great Lakes Clone. (37)
Objective 5. Outreach and Education	**Implementation** 5.4) Develop a list server discussion group for those interested in sharing information on NZ mudsnails. (45) 5.5) Develop a plan for public awareness of control measures, including biological, chemical and physical, and the potential benefit of their application. (46) 5.6) Develop educational materials that address the reality that NZ mudsnails invasions have not been linked to conspicuous declines in native fish populations but also provide concrete evidence of negative impacts along with analogous stories of other invasions where such measurable impacts took years to hit (at a point where many management options were no longer available). (46) 5.7) Develop press kits and outreach materials. (45) 5.8) Create sound bites that are understandable to the public. (45) 5.9) Coordinate with other programs such as the 100th Meridian Initiative and zebra mussel efforts. (45) 5.10) Fund staff in every State to do field outreach regarding NZ mudsnails and other invasives. (45) **Research**

Table 4. Tertiary Priorities for Implementation

Objective	Action Item
Objective 1. Pathways and Vectors	**Implementation** **Research** 1.R10) Are NZ mudsnails being distributed by biological supply houses? Could NZ mudsnails be unintentionally distributed with other species? Similarly, could NZ mudsnails be distributed through the Internet trade? (17)
Objective 2. Detecting Populations	**Implementation** **Research**
Objective 3. Prevention, Control and Management	**Implementation** 3.10) Develop a model interagency/interstate agreement for monitoring and efficacy of control/containment efforts on existing occurrences of NZ mudsnails in waters that cross state lines. (32) **Research**
Objective 4. Ecological and Economic Impacts	**Implementation** **Research**
Objective 5. Outreach and Education	**Implementation** 5.11) Develop a key to North American aquatic snails with an emphasis on identifying nonnative snails such as NZ mudsnails, Chinese mystery snails, etc. (46) 5.12) Develop a canned template (in Adobe Illustrator or other usable application) so that information can be easily adapted. (45) 5.13) Fund a website that is "public friendly: Provide dedicated support and have an outreach person to coordinate information and efforts. (45) **Research**

Table 5. Action Item Implementation Table:

Prioritized action items that are either in progress or slated for implementation during federal fiscal years 2005 and 2006 are summarized in tabular form, and correspond to numbered action items listed in the preceding three priority tables (in parentheses).

Actions Funded in Thousands of Dollars					
Related Objective	Action	Funded By	Implemented By	FY2005	FY2006
Objective 1: Identify Foci, Pathways and Vectors	NZ mudsnail web site management (1.3)	USFWS - R6	Montana State University	$6	
Objective 1: Identify Foci, Pathways and Vectors	Development of NZ mudsnail risk assessment in Madison River (1.1)	USFWS – R6	Montana State University	$18.5	
Objective 1: Identify Foci, Pathways and	Identifying sources and dispersal pathways of NZ mudsnail spread (1.5)	USFWS – R6	Washington State University	$28	
Objective 2: Develop Methods of Detecting New Populations	Early detection surveys for NZ mudsnails in western Washington and Oregon (2.R3)	USFWS – R1	USFWS	$15	
Objective 2: Develop Methods of Detecting New Populations	Green River survey, Utah (2.R3)	USFWS – R6	Utah State University	$12	
Objective 3. Prevention, Control and Management	Research and development of control strategies for NZ mudsnails at fish hatcheries (3.R1)	USFWS – R1; USGS	University of Idaho/Idaho CFWRU	$25	$25
Objective 3. Prevention, Control and Management	Development of an angler-based outreach program for preventing the spread of a newly established NZ mudsnail population in the Deschutes River watershed, Oregon (5.2)	USFWS – R1	Portland State University		$10
Objective 3. Prevention, Control and Management	Preventing upstream invasions of NZ mudsnails through manmade structures (3.R1)	USFWS – R6	Colorado State University	$20	
Objective 5. Education and Outreach	NZ mudsnail training for biologists in Colorado (5.1)	USFWS – R6;	CO Division of Wildlife	$1.88	

Appendix A

Biology and Ecology

Life History and Tolerances

Life History and Reproductive Biology

The reproductive biology of NZ mudsnails suggests that it has the potential for rapid colonization. This species is dioecious (separate male and female sexes) and ovoviviparous (Winterbourn 1970a, b, Wallace 1978). Ova develop within the female's brood pouch and are born into the environment as fully functional animals. In New Zealand, female snails may be either sexual or asexual. Asexual female snails undergo a reproductive process known as parthenogenesis whereby eggs are produced that are competent to develop without fertilization. Parthenogenetically derived offspring are genetically identical to the female (i.e., clones). Clonal populations are polyploid and almost entirely female (Wallace 1992, Dybdahl and Lively 1995a). Clonal reproduction in NZ mudsnails increases the probability of success of introductions because populations can be established by only one female. Although NZ mudsnails reproduce both sexually and asexually in New Zealand, exotic populations are entirely clonal (Zaranko et al 1997, M. Dybdahl unpublished data). Only a few males have been documented from populations in Europe (Wallace 1978, 1979, 1992), and North America (Cada, unpublished data, M. Dybdahl, unpublished data), but males may comprise up to ten percent of populations in Australia (Wallace 1978).

Life history traits are a direct function of environmental conditions, as is the case for most poikilothermic organisms. Females reach maturity in about 3 to 6 months (J. Jokela, pers. comm., Dybdahl and Kane *in prep*). Year-round reproduction and recruitment is possible where environmental conditions are moderate (Winterbourn 1970a, Schreiber et al. 1998). A study of one lake population in Australia showed that individual females brooded a maximum of 42 embryos, and the population brooded up to 81,000 embryos per m^{-2} (Schreiber et al. 1998). Snails reproducing in less productive, cooler, and more saline waters have variously been shown to produce fewer offspring, undergo longer gestation periods, and grow more slowly (Winterbourn 1970a, Harman 1974, Lassen 1979, Dorgelo 1991, Jacobson and Forbes 1997, Dybdahl 1997). In U.S. populations, Snake River individuals matured at larger sizes and carried bigger broods in river sites compared to spring sites, and brood sizes reached a maximum at 78 embryos (Dybdahl 1997).

Predicting invasiveness of NZ mudsnail populations is difficult because their performance varies widely among different clones and with environmental conditions. Individual clones in New Zealand differed significantly in size at maturity, brood size and susceptibility to parasites (Jokela et al. 1997a, Dybdahl and Lively 1998). These same traits may exhibit plastic variation under different environmental conditions, and these clone-specific traits remain distinct (Negovetic and Jokela 2001, Dybdahl and Krist 2004). A thorough understanding of the complex interplay between physiological tolerances and

resulting life history traits of individual mud snail clones in invading populations is important for managing and predicting the spread of this species in North America.

Invasive populations of NZ mudsnails tend to be comprised of a single clonal genotype, but populations in the native range and Australia are comprised of a diverse array of clones (Dybdahl and Lively 1995, Dybdahl and Emblidge, *in prep*). In New Zealand, the diversity of clones allows NZ mudsnails to occupy a range of habitats. Genetic analyses indicate that European populations are comprised of three clones that occupy different parts of the range (Hauser et al. 1992, Jacobson et al. 1996, Dybdahl 1997). "Euro A" is found in freshwaters across broad areas of continental Europe, "Euro B" is found in estuaries in the Baltic Sea, and "Euro C" is found in Great Britain. In Lake Ontario, New York, a single clone identical to Euro A has been identified (Dybdahl and Emblidge *in prep*). This clone has also been found in Lake Erie and Lake Superior. In the western U.S., a single clonal genotype identical to a clone found in Australia has been identified (Dybdahl and Emblidge *in prep*). A new clone has been identified from a short section of the Snake River, Idaho (Dan Gustafson, pers. comm.). Each clone is likely to possess unique characteristics that affect invasiveness.

Environmental Tolerance

The environmental tolerances of NZ mudsnails as a species are very broad and increase the risk that this species might be capable of wide-spread colonization. This species is found in a wide range of aquatic habitat types, including diverse temperature, osmotic, flow, and disturbance regimes. However, ecological genetic studies of this species suggest that individual clonal lineages may have either narrow or broad ecological preferences or tolerances. Consequently, although clonal reproduction provides reproductive assurance for small numbers of colonizing individuals, clonal reproduction could limit the invasiveness of these snails depending on the environmental tolerances of clones in invading populations.

Most clones seem to have narrow environmental tolerances, but one European clone has invaded over a wide geographic range. In New Zealand, clonally reproducing females are derived from sexual populations in the same lakes (Dybdahl and Lively 1995a), and are endemic to specific lakes and habitats (Dybdahl and Lively 1995a, Fox et al. 1996, Jokela et al. 1999, reviewed in Jokela et al 2003). Clones seem to fix a narrow range of preference and tolerance for different habitat conditions. In New Zealand narrow preferences often result in distinctive habitat utilization among clones within a single system so that, for example clones found in the littoral zone of a lake may be distinct from the clones found in deep-water macrophyte beds in the same system (Fox et al. 1996, Jokela et al. 1999). Thus, asexual populations in New Zealand seem to be able to occupy a range of habitats because of a high diversity of clones, each of which is specialized on specific habitats. On the other hand, genetic analyses indicate that European populations are composed of only three clones (Hauser et al. 1992, Jacobson et al. 1996, Dybdahl 1997), but the colonization of freshwaters in continental Europe has been by a single broadly tolerant clonal lineage (Jacobson and Forbes 1997). Thus, the invasiveness and success of this species is likely to be a function of the clone present and local environmental conditions.

Although individual clonal lineages have been shown to have distinct habitat preferences, temperature, salinity, and flow tolerances for the species in general appear to be broad. It has been documented from nearly every freshwater habitat in New Zealand, including lakes, rivers, streams and springs. In lentic systems, the snails utilize a variety of microhabitats including; littoral shore lines (Quinn et al. 1996, Schreiber et al. 1998), submerged weed beds (Dorgelo 1987, Talbot and Ward 1987, Cogerino et al. 1995, Cunha and Moreira 1995, Quinn et al. 1996, van den Berg et al. 1997), deep bentho-pelagic regions (Zaranko et al. 1997), and floating vegetation masses (Vareille-Morel 1983, Ribi 1986, Ribi and Arter 1986). Snail habitat usage in lotic systems is similarly broad with no real trend towards specific preferences although several authors found that NZ mudsnail densities were highest in areas with an abundance of fine substrate (Tomkins and Scott 1986, Cunha and Moreira 1995), aquatic macrophytes (Lucas 1959, Dorgelo 1987, Cogerino et al. 1995, Cunha and Moreira 1995, Savage 1996), and low velocities (Jowett et al. 1991). However, densities were high in cobble habitat in Yellowstone National Park (Kerans et al. 2005). In New Zealand streams, NZ mudsnails are not common in streams prone to periods of sediment-moving flood flows (Winterbourn 1997, Holomuzki and Biggs 1999).

NZ mudsnails also seem to have wide temperature tolerances. Upper thermal tolerance (expressed as LD50) as determined in experimental analysis was found to be 32 C for snails acclimated at 15 C (Quinn et al. 1994). The lower lethal thermal tolerances of NZ mudsnails are less clear. In Norway, NZ mudsnails were restricted to estuaries in southern Scandinavia, leading to the conclusion that winter temperature may limit colonizing success (Okland 1979, Okland 1983). However, the species' ability to survive in the intermountain west of North America and in continental fresh waters of northern Europe, where mean temperatures at or below freezing persist for three to four months, suggest that it is capable of acclimating to temperatures below those encountered in its native range. In laboratory experiments, Hylleberg and Siegismund (1987) reported that NZ mudsnails were less tolerant than European *Hydrobia* species to temperatures less than 0 C in fresh water, but that nearly 100% survival was observed at 0 C for salinities between 5-30% for up to 7 days. I need to get full document to clarify)Analysis of life-history traits suggests that the suitable temperature range for successful invasion of the western U.S. clone is much narrower than indicated by these survival tolerance studies (Dybdahl and Kane *in review*). Lower temperatures caused slower rates of development and lower fecundity than higher temperatures, but had a weaker effect on size at maturity. Hence, overall fitness showed a peak at 18^{o} C and declined at cooler and warmer temperatures.

Hylleberg and Siegismund's (1987) field surveys found winter mortalities of NZ mudsnails approaching 100% followed by rapid recolonization in northern European estuaries. Similar, seasonal density fluctuations have been documented from other European populations (e.g. Dussart 1976, van den Berg et al. 1997), and in rivers in Yellowstone National Park where temperatures fluctuate seasonally but winter temperatures are moderated by the influence of geothermal inputs (Kerans et al. *in press*). However, it should be noted that several other researchers found that changes in density did not correspond to seasonal temperature extremes (Dorgelo 1987, Cunha and Moreira 1995).

Additionally, population fluctuations have been observed in regions of Australia where climates are more stable (Quinn et al. 1996, Schreiber et al. 1998).

Observations of drastic fluctuations in naturalized temperate-zone populations (with population low points correlated with winter months) would seem to implicate winter water temperature as a direct limiting factor (temperatures below a certain threshold are lethal). It may be that population fluctuations associated with seasonal changes in temperature are an indirect effect of temperature on some aspect of snail biology. The reproductive rate of NZ mudsnails is directly related to temperature (Winterbourn 1970a, Dybdahl and Kane *in review*). In lab experiments, the western U.S. population of NZ mudsnails survived and reproduced at a constant temperature of 12^0C, but optimal temperatures for reproduction and population growth were about 18^0C (Dybdahl and Kane *in review*). However, in field experiments performed during winter in Yellowstone National Park, NZ mudsnails reproduced when temperatures averaged approximately 7^0C (M. Dybdahl, unpublished data). Populations with low reproductive rate are more vulnerable to the effects of disturbances, so that perceived seasonal die-offs may be simply a temperature-dependant reproductive lag following an undetected disturbance. In addition, mathematical models developed for NZ mudsnails suggest that such drastic fluctuations may result from factors intrinsic to the population dynamics of the snail and not necessarily be related to extrinsic environmental drivers (Kerans 2003).

NZ mudsnails are euryhaline organisms. Populations are known from both brackish and fresh water habitats in New Zealand and Europe (Winterbourn 1970a, b, Lassen 1979, Okland 1983, Hylleberg and Siegismund 1987), and the recently discovered population in the Columbia River estuary near Astoria, OR. Winterbourn (1970a) reported a maximum acute salinity tolerance of 21 parts per thousand (ppt) (seawater being 32 ppt) in laboratory trials. However, he collected NZ mudsnails from the field at salinities approaching 27 ppt (Winterbourn 1970a). Jacobson and Forbes (1997) found that two clones collected from Europe had broad salinity tolerances and were able to feed, grow, and reproduce at salinities ranging from 0 to 15 ppt but that the salinity optima for these functions occurred at 5 ppt. The clone found in the Columbia River estuary experiences salinities that vary daily from 0 to 32 (Dybdahl and Kane *in review*).

NZ mudsnail individuals can tolerate the high frequency of disturbance (scouring events) characteristic of many South Island, New Zealand, river systems (Winterbourn 1981), but population sizes are strongly affected by flow regime. The animals' tough shells, small size, and hydrodynamic shape make them likely to survive scouring flows. In an experimental flume, Holomuzuki and Biggs (2000) found that only 8% of NZ mudsnails were dislodged because they behaviorally shifted to deeper, more stable sediments as flows increased. Mortality rates associated with the effects of dislodgement and downstream displacement were very low. Scouring flows merely serve to redistribute snails rather than kill them outright (Holomuzuki and Briggs 1998). Nevertheless, local densities of NZ mudsnails were inversely correlated with disturbance frequency in New Zealand streams (Holomuzuki and Briggs 1998).

NZ mudsnails seem tolerant of most anthropogenic impacts. Several authors have noted that it does well in moderately eutrophic systems (Dorgelo 1987, Scott et al. 1994). In stable habitats with high nutrient loads and abundant macrophytes, mud snails dominated (relative abundance of 90%) invertebrate communities in New Zealand streams (Duggan et al. 2002). In Australia, the success of NZ mudsnail introductions seems to be associated with agricultural runoff and nutrient inputs (Scheiber et al. 2003). However, in some instances, elevated nutrient levels were shown to adversely affect population densities and individual survival (Tomkins and Scott 1986, Hickey and Vickers 1994).

Similar to most gastropods, NZ mudsnails are sensitive to dissolved metals and a range of lethal and sub-lethal effects have been documented (Harman 1974, Moller et al. 1994, Dorgelo et al. 1995, Golding et al. 1997). Golding et al. (1997) demonstrated that snails undergo avoidance and immobility behavior in response to elevated levels of dissolved arsenic. However, the levels of arsenic used in the Golding experiments were one to two orders of magnitude greater than those found in geothermal heated waters with naturally occurring high levels of arsenic (Savka 1993). Dorgelo et al. (1995) found that elevated levels of cadmium and copper resulted in a decrease in growth rate of approximately 50 % for NZ mudsnails. These findings suggest that NZ mudsnails have metal tolerances similar to those observed for other gastropods (Harman 1974).

It is apparent from the literature that the NZ mudsnail is a species that has unusually broad habitat tolerances. Furthermore, the US 1 clone found in western U.S. rivers has spread across a range of habitats and environmental conditions from Oregon to Montana to Arizona and California. Habitats include a variety of rivers and streams, although two lakes (Hebgen in Montana and Crawley in California) in heavily colonized river systems have not been colonized (M. Dybdahl, unpublished data, D. Becker pers. comm.). Hence, it is difficult to envision abiotic environmental conditions that would pose an obstacle to the further spread of this species in the middle latitudes of North America.

Population and Community Ecology

Abundance

NZ mudsnails have been found to be the numerically dominant organism or gastropod in lakes and streams in New Zealand (Hopkins 1976, Towns 1981a, b, Talbot and Ward 1987, Scott et al. 1994), Europe (Cogerino et al. 1995, Cunha and Moreira 1995, Savage 1996), and Australia (Schreiber et al. 1998). However, the species is not numerically dominant in all New Zealand systems, especially in small streams and rivers. For example, Winterbourn (1978) found that the snail was absent from small forested streams on the South Island. Rounick and Winterbourn (1982) found it present in only eight of 43 low-order streams surveyed throughout New Zealand, and Scrimgeour and Winterbourn (1989) found that it comprised less than 0.1% of organisms collected from the Ashley River, New Zealand.

As discussed earlier, densities of NZ mudsnails can fluctuate widely. In Australia, densities ranged between seasonal highs of 50,000 m^{-2} during summer and lows of 1800 m

$^{-2}$ during the winter (Ponder 1988, Schreiber et al. 1998). Similarly, densities often undergo broad fluctuations in Europe (Siegismund and Hylleberg 1987, Dorgelo 1987, van den Berg 1997, Savage 1996) where water bodies freeze in winter and are re-colonized the following spring. However, Quinn et al. (1996) and Schreiber et al. (1998) found that NZ mudsnail densities fluctuated between 1800 and 50,000 individuals m^{-2} in Lake Purrumbete (Australia) where seasonal temperature fluctuations are considerably less extreme. Similar patterns have been observed in New Zealand (Talbot and Ward 1987, Scott et al. 1994). Patterns of density fluctuations observed over such a range of climates may indicate that factors other than environmental parameters contribute to mudsnail population demography. Fluctuations could result from factors intrinsic to snail biology (Kerans 2003) and/or snail lifespan.

Reports of NZ mudsnails reaching densities in excess of 100,000 individuals m^{-2} exist in the literature, the most spectacular of which is a report of 800,000 individuals m^{-2} (Lucas, 1959 in Dorgelo, 1987). Investigations of the snail's distribution in the Greater Yellowstone Ecosystem have shown that it is capable of reaching densities approaching 300,000 individuals m^{-2} at some locations (Kerans et al *in press*), and over 500,000 individuals m^{-2} in one geothermally-influenced stream (Hall et al 2003). Population densities fluctuate seasonally in Yellowstone rivers, reaching highest levels in July or September, and very low levels in March (Kerans et al *in press*).

Feeding Habits

The feeding habits of NZ mudsnails have been subject to a number of experimental investigations. Generally, they are thought to be grazers (herbivores of attached periphyton) and/or detritivores (consumers of decaying plant and animal material). Hanlon (1981) found that NZ mudsnails feeding on decaying deciduous leaf material grew faster and fed more rapidly on soft-cuticle leaves such as willow and aspen than on tougher beech and oak leaves. However, it was not known if the snails in this experiment were feeding on the decaying plant material itself or on associated bacteria. Similarly, Haynes and Taylor (1984), while conducting a food-preference experiment, found that NZ mudsnails were attracted to stones soaked in crushed amphipods, indicating that they feed on decaying animal material. Snails were also attracted to stones colonized by algae. NZ mudsnail spent more time on patches with periphyton than those without periphyton in a field experiment using slate tiles, (Kerans et al. in prep.). Both these results indicate the importance of herbivory in this species.

Herbivory in NZ mudsnail have been documented in field experiments (Towns 1981a, Rounick and Winterbourn 1983, Winterbourn and Fegley 1989). Rounick and Winterbourn (1983) found that NZ mudsnails grazing rates were considerably less than those observed for mayflies, stoneflies, and caddisflies in a New Zealand stream, but that its assimilation efficiency (amount of food material converted to animal biomass) was higher than that observed for these taxa. Additionally, Winterbourn and Fegley (1989) reported that NZ mudsnail affected periphyton biomass on their experimental tiles.

The feeding habits of NZ mudsnails are, like its life history traits and environmental tolerances, potentially quite variable in nature. Indeed, the above discussion makes it clear that everything from diatoms to detritus is fair game. Given that NZ mudsnail are capable of exploiting a wide array of resources, this species will potentially compete with a wide array of organisms that fill different trophic niches in North American aquatic systems.

Interspecific interactions—competition and facilitation

The effect of NZ mudsnails on the invertebrate fauna of New Zealand, Europe and Australia is largely unknown. However, in North America, a few studies demonstrate complex and variable interactions between NZ mudsnails and other gastropod and macroinvertebrate species, including both negative (competition) and/or positive (facilitation) interactions. The mechanisms of competition may include both interference (direct agonistic encounters, e.g. for space) and exploitation (e.g. for resources).

NZ mudsnails may compete with other gastropods, and potentially reduce gastropod biodiversity. Bowler (1991) and Bowler and Frest (1992) speculated that NZ mudsnails could have an impact on the diversity of Snake River gastropods. In the Snake River (Idaho), NZ mudsnail have invaded areas occupied by five threatened or endangered species of native aquatic snails (Federal Register 1992, Richards et al. 2001). Competition between mudsnails and native gastropods could be for resources or for moisture refugia (undersides of rocks) during water fluctuations in this highly regulated system (Bowler 1991). Consistent with this speculation, the distributions of the threatened Bliss Rapids Snail (*Taylorconcha serpenticola*) and NZ mudsnails did not overlap in field studies (Richards et al. 2001). The densities of NZ mudsnails and a narrowly endemic snail in a Yellowstone stream (*Pyrgulopsis robusta*) were positively correlated among sites, but preliminary evidence suggested that they were negatively correlated in another stream (Riley et al. *in review*).

NZ mudsnails may also affect other grazing macroinvertebrates. NZ mudsnails negatively affected the survivorship but not the growth of mayfly species in experiments conducted in a tributary of the Madison River in Montana (Cada 2004). However, a survey of NZ mudsnails and native benthic macroinvertebrate densities across four Yellowstone rivers revealed few negative correlations as expected if interactions were negative (Kerans et al. 2005 *in press*).

Interference competition is common in studies of gastropods and other benthic animals. Brown et al. (1994) observed that at high densities, agonistic interactions between snails (in the form of shell-shaking activity) increased significantly in an experimental population of *Physella*. Similarly, Cuker (1983) found that at high gastropod density, the densities of attached invertebrates such as Chironomidae were lower. It was thought that high snail densities resulted in the dislodgement of these organisms and their fixed benthic feeding retreats. Interference competition, for space as an example, may result from extremely high densities known from invasive NZ mudsnail populations. Densities of 100,000 to 800,000 individuals m^{-2} are known from Europe (Lucas 1959 in Dorgelo 1987), and measured

densities over 500,000 individuals m^{-2} in the Greater Yellowstone Ecosystem (Hall et al. 2003). At such high densities, NZ mudsnails may simply physically exclude other grazing organisms by occupying attachment space.

Experimental studies demonstrate that NZ mudsnails interfere with other benthic macroinvertebrates. The numbers of mayflies foraging for periphyton on the tops of tiles declined in a short-term (2 h) field experiment when NZ mudsnails were present (Kerans et al. in prep.). In addition, in a longer (2 mo) experiment in the Madison River in Yellowstone National Park, densities of macroinvertebrates from many different functional feeding groups were lower on tiles with high abundances of NZ mudsnails (Kerans et al. 2005). Both these results suggest that interference was the mode of competition.

Exploitative competition for periphyton also may occur between NZ mudsnails and other benthic invertebrate grazers. Studies have shown that snails are capable of changing both algal density and community composition in stream systems (review in Hawkins and Furnish 1987; see also Winterbourn and Fegley 1989, Attwood 1996, Kjeldsen 1996). Kjeldsen's work in lowland streams of Denmark demonstrated that gastropod grazing was an important factor in regulating periphyton biomass. In New Zealand, Winterbourn and Fegley (1989) remarked that NZ mudsnails were capable of influencing their studies of periphyton, necessitating control measures. Death (1991) showed that NZ mudsnails depressed periphyton biomass in experiments in several New Zealand streams. In the Yellowstone area, studies of competition between NZ mudsnails and a narrowly endemic snail (*Pyrgulopsis robusta*) showed that both species reduce algal food resource levels, and that NZ mudsnails have a negative effect on the growth of *Pyrgulopsis robusta* (Riley et al. *in review*). Laboratory and field experiments between the threatened Bliss Rapids Snail (*Taylorconcha serpenticola*) and NZ mudsnails suggest they compete (Richards and Kerans, in prep). Finally, Cada and Kerans (in review) showed that periphyton biomass was lower in reaches of a Madison River tributary where NZ mudsnail abundance was higher. Reduction of periphyton biomass may negatively affect other invertebrates and have wide-ranging affects on ecosystem processes in streams dominated by bottom-up interactions (Carpenter et al. 1985).

Not all interactions among NZ mudsnails and other species are negative. Schreiber et al. (2002) showed that NZ mudsnails facilitated the colonization of macroinvertebrates in an experiment in an Australian stream. In a Yellowstone stream, periphyton biomass increased with NZ mudsnail density, suggesting self-facilitation (Riley et al. *in review*). Further study in this system showed that a likely mechanism is fertilization, which suggests that the negative effects of resource exploitation by the invasive snails may be negated (Riley et al. *in preparation*).

The role of species interactions in the success and impact of NZ mudsnails is far from clear. Mathematical models show that both positive and negative interspecific interactions between NZ mudsnails and other species may add even greater complexity to already complex interactions (Kerans, 2003). Low densities of NZ mudsnails may attract some macroinvertebrates (Schreiber et al 2002), but high abundance might inhibit colonization of other species, as shown in the Madison River (Kerans et al. 2005). Low and

intermediate densities may stimulate algal growth and ameliorate the impact of invasion, but high densities can have negative effects on resources shared with native species. More studies are needed to determine the effect that invasion will have on native community structure. Specific studies of competitive interactions in North American populations are also needed**.**

Interspecific interactions—Predators and Parasite

Predators and parasites of NZ mudsnails occur in both native and introduced populations, but their effect in regulating population size is not well known. In New Zealand and Australia short-finned and long-finned eels (*Anguilla australis* and *A. dieffenbachii*), brown trout (*Salmo trutta*), and bullies (*Gobiocloneus spp.*) have been reported to consume NZ mudsnails, but it is unclear if these accounts represent actual targeted feeding behavior or if individuals found in stomach samples were accidentally ingested with other prey (Burnet 1969, Cadwallader 1975, McDowall 1991, Levri 1998). There is no strong evidence that predators control populations in New Zealand (Nyström and McIntosh 2003)

It has been suggested that North American Ostariophysine fish (Catostomidae and Cyprinidae), which posses pharyngeal teeth, may be capable of consuming and crushing the shell of this species. However, in many streams where NZ mudsnails have invaded in North America, fish lack these specialized adaptations to feed on snails. In a tributary of the Madison River, Montana where NZ mudsnail densities were moderate (20,000 individuals/m^2), only one NZ mudsnail was found in the stomachs of 29 brown trout and 17 sculpin (*Cottus bairdi*) (Cada 2004) when most stomachs contained several food items. On the other hand in the upper Madison River right outside the boundaries of Yellowstone National Park, stomachs of mountain whitefish (*Prosopium williamsoni*) contained many NZ mudsnails (W. Dwyer, United States Fish and Wildlife Service, personal observation). These results suggest that some fish species may avoid NZ mudsnails, whereas others eat them readily

Even if some trout and other species eat NZ mudsnails they may gain little energy because studies have shown that NZ mudsnails are capable of passing through the digestive canal of trout alive and intact (Bondesen and Kaiser 1949, Haynes et al. 1985). Additionally, it has been shown that NZ mudsnails offer little or no energy compared to other common food items to those fish successful in crushing its shell (Ryan 1982). Thus, there may be consequences to fish that eat NZ mudsnails over other food sources. In an experiment done in a tributary of the Madison River where areas exist where NZ mudsnails have low and high abundances, the sculpin lost more weight when caged in areas where NZ mudsnails were abundant than where NZ mudsnails were rare (Cada 2004). On the other hand, no difference in weights was recorded for brown trout. More experimentation and field studies are needed to determine how NZ mudsnails influence fish communities.

Mudsnails are infected by up to 14 species of trematode parasites in New Zealand (Winterbourn 1974, Jokela and Lively 1995a and b, Dybdahl and Lively 1998), and because these parasites castrate or sterilize their hosts, they could have important population regulatory effects. These trematode parasites have a two-host life cycle; they alternate between the snail and the digestive tract of a vertebrate host. In the snail, the

parasites undergo asexual proliferation in the gonad, thereby eliminating reproduction in infected individuals. None of these parasites have been found in introduced populations in Europe or North America. One of these parasites (*Microphallus* sp.) is known to occur in the Australian introduced range (Schreiber et al. 1998, Emblidge and Dybdahl *in preparation*). In Europe, the colonization in very low frequencies of NZ mudsnails by a European castrating trematode species has been reported (Gerad et al. 2003). Preliminary studies of parasite populations in streams of the Greater Yellowstone Ecosystem showed that a digenetic trematode of fish might use NZ mudsnails as an intermediate host (Beck et al. 2004).

One particularly well-studied trematode of mudsnails, *Microphallus sp*., seems to have strong population regulatory effects on its snail host populations. *Microphallus sp*. uses a variety of water birds as a vertebrate host to complete its life cycle. For example, dabbling waterfowl such as the native grey duck and the introduced mallard become infected after consuming snails found on the surfaces of aquatic macrophytes (Winterbourn 1974). The parasites reproduce sexually in the vertebrate host, and eggs pass into aquatic habitats where they may be ingested by snails. Whether a parasite egg leads to infection of a particular snail is genetically determined. For example, parasites are locally adapted to infect snails from the same lake or habitat (Lively 1989, Lively and Jokela 1996, Lively and Dybdahl 2000). Furthermore, parasite populations differ in their infectivity to different snail clones (Dybdahl and Lively 1995b, Jokela et al. 1997b, Dybdahl and Lively 1998, Dybdahl and Krist 2004). These specific interactions, along with the castration of members of different clones, lead to large fluctuations in population density in specific clones over time (Dybdahl and Lively 1998).

Appendix B

State and Federal Regulations and State ANS Plans

Note: As with all State and Federal regulations, they are current at a specific time. These are currently in effect at the time of approval of this management plan.

Alaska: While NZ mudsnails are not specifically classified as prohibited under Alaska law, AS 16.05.241 gives the Board of Fisheries the authority to prohibit and regulate the live capture, possession, transport and release of native and exotic "fish" (which is defined to include aquatic invertebrates) or their eggs. With that authority, 5 AAC 41.070 - which prohibits the import of "fish" for the purpose of stocking or rearing in the waters of the state - was developed. Another statute, AS 16.05.920(a), states that unless permitted by regulation adopted under AS 16.05, a person may not take, possess, transport, or purchase "fish" or any "fish" part (again with fish defined to include aquatic invertebrates). Alaska does have an approved state ANS management plan which identifies NZ mudsnails as one of the highest potential threats. Key elements of that plan include development of a NZ mudsnail education and outreach plan, and NZ mudsnail monitoring and detection. The Alaska Department of Fish and Game has partnered with the USFWS to increase sport fish industry awareness in particular and the public in general with posters, ID cards, preserved samples and presentations at outreach events.

Arizona: A pending proposed rule change to R12-4-401 of the Arizona Administrative Code would add NZ mudsnails as a restricted wildlife species, making them illegal to possess, transport, or import without special license. Currently, Arizona law requires granting of an exemption or special license to possess "aquatic wildlife" (which includes mollusks) unless the specimens are intended for use in the aquarium trade or for restaurants or markets licensed to sell food. Arizona does not have an approved state ANS management plan at this time.

California: Title 14, Section 671 (c)(9) of the California Code of Regulations classifies NZ mudsnails as "restricted." Therefore, it is unlawful to import, transport, or possess live NZ mudsnails in the state except under permit issued by the California Department of Fish and Game. California does not have an approved state ANS management plan at this time.

Colorado: The Colorado Wildlife Commission listed the NZ mudsnail as a prohibited species in 2003 in the Colorado Wildlife Regulations, Chapter 0, Article 012 B.1. The regulation prohibits the release, importation, transportation, stocking, sale, acquisition or possession for release without authorization in writing by the Colorado Division of Wildlife. Colorado DOW has a statewide NZ mudsnail management plan in place. Because NZ mudsnails were identified in Boulder Creek and in a private aquaculture facility on the Creek, the Colorado DOW closed the area to fishing for 90 days and worked to create a Best Management Practices document to keep the snails from being transported through fish stocking. Colorado does not have a state ANS management plan.

Hawai'i: NZ mudsnails are not specifically classified as "prohibited", "restricted," or "conditionally-approved" under Chapter 4-71 of the Hawaii Administrative Rules. As a result, live snails can not be imported or possessed in the state without a permit until classified by the Board of Agriculture. The Hawai'i state ANS management plan defines four management classes for species already in the state. NZ mudsnails are included under a separate section listing species not yet established in Hawai'i (note that the plan includes NZ mudsnails as a potential marine aquatic invasive species but does not include them in the list of potential inland water aquatic invasive species). The Hawai'i plan does not include any action items specific to NZ mudsnails, but many of its general action items regarding prevention, detection, and control are applicable.

Idaho: NZ mudsnails are not specifically regulated by the state of Idaho. However, under Idaho Administrative Code 13.01.10.100, "no person shall import, export, transport into or cause to be transported within, release or sell within the state of Idaho any living wildlife including wildlife eggs" without first obtaining a permit from the Idaho Department of Fish and Game. Further, the Director of IDFG is prohibited from issuing permits for species that pose a threat to wildlife in Idaho either via threat of disease, genetic contamination, or displacement of/competition with existing species. The exceptions to these provisions do not apply to NZ mudsnails. Idaho does not have an approved state ANS management plan at this time. However, Idaho has formed a state invasive species council and is drafting a state invasive species management plan. Idaho is also addressing NZ mudsnails by Hazard Analysis and Critical Control Point (HACCP) plans for state fish hatcheries and field crews as well as improving public awareness with signage and other outreach materials.

Kansas: New Zealand mudsnails are specifically prohibited from being possessed, released, or imported under Kansas Administrative Rules 115-18-10. The Kansas ANS Management Plan, completed in 2005, lists NZ mudsnails as a priority species of special concern.

Minnesota: Minnesota has proposed that the NZ mudsnail be a prohibited invasive species, which will prohibit import, possession, transport and introduction into the wild.

Montana: New Zealand mudsnails are listed as prohibited in the Administrative Rules of Montana (ARM 12.6.2201-2230). NZ mudsnails may not be possessed, sold, purchased, exchanged, or transported in Montana, except as provided in Montana Code Annotated 87-5-709. Permits for the possession of NZ mudsnails can be issued to colleges, universities or government agencies if they are being used for scientific research. In the Montana ANS Management Plan, completed in 2002, NZ mudsnails are listed in Priority Class 2: species that are present and established in Montana and have the potential to spread in Montana. There are limited or no known management strategies for these species. These species can be managed through actions that involve mitigation of impact, control of population size, and prevention of dispersal to other water bodies.

Nevada: The list of prohibited species in Nevada Administrative Code (NAC) 503.110 does not include NZ mudsnails. Nevertheless, state statute NRS 503.597 prohibits any person to receive, bring or have brought or shipped into the state, or remove from one stream or body of water in the state to any other, or from one portion of the state to any other, or to any other state, any aquatic life or their spawn, eggs, or young, except with written consent and approval by the Nevada Department of Wildlife. NAC 503.140 lists a number of taxonomic groups that are exempt from this general statutory restriction, although even exempt species cannot be released to the wild without written NDOW authorization. Although this list of exempted species does not exempt NZ mudsnails specifically, it does exempt "saltwater fish, crustaceans, or mollusks." It is unclear whether that category would be applied to NZ mudsnails given their estuarine range. Nevada does not have an approved state ANS management plan at this time.

New York: The state of New York has no specific laws or regulations governing the control or prohibition of NZ mudsnails within the state. However, Environmental Conservation Law (ECL) does have a general provision that would cover NZ mudsnail as "wildlife." ECL 11-0507 (3) states: "No person shall willfully liberate within the state any wildlife except under permit from the department. The department may issue such permit in its discretion, fix the terms thereof and revoke it at pleasure. These provisions do not apply to migratory game birds, importation of which is governed by regulation of the department."

Oregon: NZ mudsnails are not specifically classified as "prohibited", "controlled", or "non-controlled" under Oregon Administrative Rule 635-056. As a result, live snails are prohibited from being possessed; imported; purchased; sold; exchanged; or offered for sale, purchase or exchange without a state permit until they are classified. The Oregon ANS Management Plan does not include any action items specific to NZ mudsnails, but many of its general action items regarding prevention, detection, and control are applicable. The first version of the Plan completed in 2001 classified NZ mudsnails under Management Class 1, which are species not known to be present in Oregon but with a high potential to invade, or reported in Oregon with limited populations. The Plan assigns prevention of introduction and eradication of pioneering populations as appropriate management activities for this class. However, in 2003 the Oregon Invasive Species Council removed NZ mudsnail from its list of "100 Most Dangerous Invaders Threatening Oregon" because the snails' expansion within the state no longer met the list's criteria of absence and/or range restricted to a small area. As a result, revisions currently under development for the Oregon ANS Management Plan would shift NZ mudsnails to Management Class 3, which are species that are established throughout Oregon with impacts but no available or appropriate management techniques. "These species warrant further evaluation and research to ascertain potential control and to prevent establishment in new waterbodies."

Pennsylvania: In Pennsylvania, though no regulations pertain specifically to NZ mudsnail, one general regulation would cover this species. While the language used is for fish, the term "fish" actually applies to any animal placed into Pennsylvania waters. Chapter 73.1, Title 58 of the Pennsylvania Code, Part II – Fish and Boat Commission,

states, “Species of fish may not be transported into this Commonwealth from another state, province or country and liberated in a watershed of this Commonwealth without previous written permission from the Commission, nor may a species of fish be transferred from waters in this Commonwealth into another drainage of this Commonwealth where this particular species is not always present without prior written consent from the Commission. Inspection for species composition or presence of disease, or both, will be required at the discretion of the Commission on all lots of fish transported into this Commonwealth.” This regulation can be accessed online at: http://www.pacode.com/secure/data/058/chapter73/s73.1.html

Utah: NZ mudsnails are a prohibited species that may not be collected, imported, transported or possessed without procuring a variance to Wildlife Resources Rule R657-3, Collection, Importation, Transportation and Possession of Zoological Animals. Utah does not have a state ANS management plan, however many staff have been trained in HACCP-NRM planning. Utah Division of Wildlife Resources staff chair the Utah ANS Task Force, a partnership of agencies and other interested stakeholders to increase the education and outreach on ANS across the state.

Washington: Washington Administrative Code 220-12-090 classifies NZ mudsnails as “prohibited.” Live specimens of prohibited species can not be possessed, purchased, sold, imported, transported, propagated, or released without a permit. This restriction does not apply to the transportation or release of organisms in ballast water (note that Washington has other statutory and administrative requirements addressing ballast water management). Prohibited aquatic animal species that are captured in state waters and not immediately returned to the water from which they were captured must be killed before removing the prohibited aquatic animal species from within the riparian perimeter of the body of water. State requirements are also established regarding removal of aquatic vegetation and transport of water. The Washington ANS Management Plan classifies NZ mudsnails under Management Class 2, which are species that are present and established in the state. Assigned management activities for this class include mitigating impact, controlling population size, and preventing dispersal to other water bodies. The plan does not include any action items specific to NZ mudsnails, but many of its general action items regarding prevention, detection, and control are applicable.

Wyoming: NZ mudsnails are specifically prohibited from being imported, possessed, confined and/or transported into the state of Wyoming as specified by the Wyoming Game and Fish Commission Chapter 10 – Regulations for Importation, Possession, Confinement, Transportation, Sale and Disposition of Live Wildlife, Section 5, subsection b (i) (C). Wyoming does not have a state ANS management plan. The Wyoming Game and Fish Department is training staff in HACCP-NRM planning and requires that fish imported from out of state come from a facility that has an approved HACCP-NRM plan in place.

Appendix C

Detecting NZ Mudsnails Using Power Analysis

Given that many methods are currently being used in benthic surveys, the most important criterion for use is to define some level of probability of detection. An example of a probability of detection level used for hydrobiid snails was that used by Richards et al. (2005) for *Taylorconcha* sp. in the Snake River, Hells Canyon. This species primarily occupies cobble habitat. They tested a simple 20-cobble count method and estimated that it had a detection probability of > 0.95 for densities $\geq$ 1 *Taylorconcha* sp./m^2 on cobble habitat in the Snake River below Hells Canyon Dam using ten 20-cobble counts. Cobble counts could also work for NZ mudsnails, but estimates of detection probabilities would have to be established based on the number of NZ mudsnails found on cobbles relative to other substrates. Thus detection level densities of NZ mudsnails using cobble counts would have to be quite high and would be most useful after NZ mudsnails had become well established in a system. Ideally it is desired to detect NZ mudsnails when they first become established at low densities and when they are technically a 'rare' species in the community composition. There is a large selection of literature on methods for detecting rare species, including freshwater mollusks (Merritt and Cummins 1996, Green and Young. 1993, Strayer and Smith 2003, and others).

Determining if a water body contains NZ mudsnails when they occur in low densities can be difficult. It would be extremely difficult to state that no NZ mudsnails are present at a site, because that would require sampling every square centimeter of substrate. Therefore, the ability to find the snails depends on sampling design and effort. Informal searches that state for example, "researchers failed to detect NZ mudsnails in a 2 hour search" are of limited value. In a formal sampling design, instead of saying that NZ mudsnails are truly absent from a site, it can be stated that NZ mudsnails were not detected given a certain amount of effort using a certain design, or that a design with 'x'% chance of detecting a NZ mudsnail population with a density of 'y'/m^2 failed to find any (Strayer and Smith 2003). Power analysis easily can provide a means to state the later, given the assumption that NZ mudsnails are distributed in the system that approximates a Poisson distribution (Green and Young 1993). This is usually the case when the probability of collecting an individual in any given sample is low (rare) and/or populations are aggregated (i.e. when we want to detect NZ mudsnails at low densities). All that is needed for power analysis is:

1) An agreed upon probability of detection (power; 1-β),
2) An agreed upon detection level or density (for example 1 individual/10m^2 of substrate), and
3) The appropriate number of quadrats sampled.

In marine invertebrate studies the generally accepted standard power is 1- β = 0.80 (Green and Young 1993), while in freshwater invertebrate studies a power of 0.85 is often used (Merritt and Cummins 1996). Commonly used detection level probabilities (density, # NZ

mudsnails/quadrat) for rare species is more arbitrarily defined, but a value of 0.1 individual /sample unit size has been suggested as a maximum value (Green and Young 1993).

The following graph (figure 3) gives a range of sample sizes needed for four levels of probability of detection (1-β = 0.75, 0.80. 0.85. and 0.90) at a given mean density per quadrat. For example, to state that there was an 85% chance of detecting NZ mudsnails at a density of 0.05 individuals/quadrat (e.g. 1 individual/20 m^2 using a 1.0 m^2 quadrat) a sample size of 38 quadrats would be required. Also, if this protocol was followed and 38 quadrats were sampled and no NZ mudsnails were collected, it would be statistically correct to state that, "following this sampling design, there was an 85% confidence that NZ mudsnail density was < 1 snail/20 m^2."

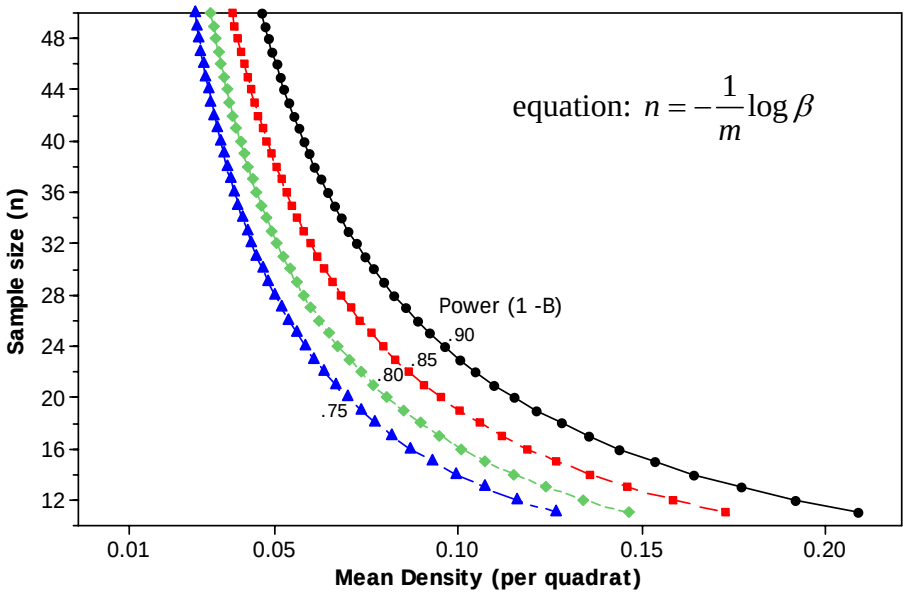

Figure 10. Necessary sample size *n* as a function of mean density (per quadrat) for various degrees of power 1 – β, when sampling the Poisson distribution (modified from Green and Young 1993).

Appendix D

NZ Mudsnail Risk Assessment and Management Criteria For the Hagerman National Fish Hatchery

(*Excerpted from: Burge, H. and P.J. Heimowitz. 2005. Risk Assessment and Risk Management Recommendations for New Zealand mudsnail introduction from Hagerman NFH steelhead releases. USFWS.*)

Introduction

Current policy of the Department of the Interior (Executive Order #13112, Invasive Species) and the U.S. Fish and Wildlife Service requires that programs "*. . . not authorize, fund, or carry out actions that it believes are likely to cause or promote the introduction or spread of invasive species in the United States or elsewhere unless, pursuant to guidelines that it has prescribed, the agency has determined and made public its determination that the benefits of such actions clearly outweigh the potential harm caused by invasive species; and that all feasible and prudent measures to minimize risk of harm will be taken in conjunction with the actions.*"

The New Zealand mudsnail (NZ mudsnail) was first discovered in the Snake River, Idaho in 1987 (Richards 2002c). In 2002, they were discovered in springs at the Hagerman National Fish Hatchery in Idaho, prompting concerns about subsequent spread through hatchery operations. This report evaluates the risk of such spread and associated risk management considerations relative to Executive Order #13112.

In Idaho, NZ mudsnail are widespread in the Hagerman Valley, Snake River, and Snake River reservoirs, but are absent from Brownlee Reservoir (Shinn 2002). Although numerous sites have been surveyed in Northern Idaho, the only recorded finding occurred in 2001 when a single NZ mudsnail was collected in Kalispell Creek. Up to date locations of NZ mudsnail positive sites in Idaho and other western states is available at http://www2.montana.edu/NZ mudsnail/

In the Clearwater Basin the Service sampled 14 sites in the South Fork Clearwater River (Burge 2003a) in addition to more than 50 sites sampled throughout the Clearwater basin by Dr. Gustafson of Montana State University. None of these surveys have found NZ mudsnails in the Clearwater basin. Note that there is no standardized, nationally-accepted sampling protocol for NZ mudsnail surveys; therefore there are no methods for establishing statistical confidence regarding absence determinations. The Nez Perce Tribe did find NZ mudsnails in Sweetwater Creek, a tributary to Lapwai Creek in the Lower Clearwater drainage in July, 2003.

The Service also sampled 34 sites in the Salmon River basin (Burge 2003b) to add to Dr. Gustafson's 55 survey sites. NZ mudsnails were found at 6 locations in the Salmon River basin. The Service found a few NZ mudsnails approximately 50 miles below the Pahsimeroi River in the main Salmon River at Tower Rock Recreational Site. A moderate

to abundant population is known to occupy the mouth of the Pahsimeroi River and Pahsimeroi Hatchery, and the Service found a moderate number of snails approximately 2 miles above the Hatchery in the Pahsimeroi River. Last September moderate numbers of NZ mudsnails were found in the main Salmon River below the Pahsimeroi, however they could not be relocated on a recent trip in April, 2004. Most significantly, an abundant population was discovered approximately 40 miles above the Pahsimeroi in Squaw Pond. Squaw Creek Steelhead Pond is a man-made, earthen pond adjacent to Squaw Creek, approximately 1 km upstream from its confluence with the Salmon River (Osborne and Rhine 2000). It is used by Idaho Department of Fish and Game (IDFG) as an acclimation and release site for steelhead smolts from Magic Valley Hatchery. The pond is also used as a fish-out pond for rainbows stocked from Nampa Hatchery. Both Nampa and Magic Valley hatcheries are infected with NZ mudsnails, to varying degrees. The pond is drained early each fall after steelhead are released, but when full, the pond supports a healthy growth of algae. When surveyed in September, 2003 the pond was already drained although ground water maintained a small pool and outflow channel. NZ mudsnails were observed on the substrate and within the algal mats remaining in the pool. In April, 2004 the pond was recently refilled, the flow in the outflow channel was increased, and pools had been created in the channel to provide a release site for steelhead smolts. Although NZ mudsnails were abundant in the outflow channel prior to refilling the pond (Fred Partridge, pers. comm.) we only observed them in a small side channel below the recently created pools. The increased flow had obviously flushed snails in the main outflow channel downstream.

The potential for NZ mudsnail introduction to the upper Salmon River (Stanley area) from currently occupied areas in the Salmon River (Pahsimeroi area) is greater than in the South Fork Clearwater River. The upper Salmon is typically used by wading anglers (Tom Curet pers. comm.) that are more likely to carry NZ mudsnails in the laces of their wading boots, whereas South Fork Clearwater anglers are mostly bank fishermen that seldom get in the water. Also an angler unknowingly transporting NZ mudsnails from the lower Salmon River would have a shorter travel time to the upper Salmon River than to the Clearwater River. The longer travel to the upper Clearwater River from a NZ mudsnail positive site would provide a longer duration for desiccation, which is one of the preferred methods for control of NZ mudsnails (Richards *et al*. 2004). Additionally because of the recreation aspect of the Stanley basin the upper Salmon River is used more heavily by rafters and floaters than the upper Clearwater basin. Recreationalists also do day float trips downstream from Stanley, but it is unlikely they get far enough downstream and into areas known to have NZ mudsnails, then unknowingly transport them back upstream.

NZ mudsnails have no natural predators in North America, whereas in New Zealand several native fish species frequently eat them (Richards 2002a). They have been found in catchable size rainbow trout at Hagerman State Hatchery, (IDFG data) and in whitefish stomachs (Cada 2003). Dwyer (2001) force fed NZ mudsnails to rainbow trout and observed an 85% survival rate after 2.5 hours in the trout; he also predicted some survival out to about 5 hours. Food passage time for trout is variable ranging from 6 or 8 hours up to 24 hours, and is affected by temperature, fish size, and other factors. So given these factors, a possible scenario could be for a fish to ingest a live snail prior to loading into a

distribution truck and either passing a live snail in the tank during transport or in the stream after release. Either way the snail could be introduced into that water body and potentially start a population via cloning.

Currently Hagerman NFH is releasing steelhead into the Salmon River at several locations above and below the farthest known upstream infestation at Squaw Creek Steelhead Pond. Although NZ mudsnails can move upstream volitionally as noted earlier, any point in the main stem Salmon River downstream of Squaw Creek is particularly susceptible to invasion from that population. When the pond is drained in early fall, algae mats carrying NZ mudsnails are likely flushed downstream. It is interesting to note, however, that no NZ mudsnails were observed in Squaw Creek above the mouth or in the Salmon River directly below Squaw Creek. Lower Squaw Creek appeared to be suitable habitat and supported an abundant population of native *Physa* snails. Current Hagerman NFH stocking sites in the upper Salmon basin upstream of Squaw Creek include the Yankee Fork tributary and Sawtooth Hatchery. They also release steelhead into East Fork Salmon River and the Little Salmon River drainage, a tributary to the lower Salmon River. All of these sites have been used by Hagerman and other IDFG hatcheries as fish release sites for the past 10-15 years.

Potential Establishment

NZ mudsnails were initially found in the Hagerman Valley in 1987 by Dr. Peter Bowler (Richards 2002c). Hagerman NFH has been releasing steelhead into the Salmon River basin since 1978. We do not know exactly when NZ mudsnails colonized the springs at Hagerman, however based on the size of the population we can surmise that it was before they were first discovered in the fall of 2002. Nampa and Magic Valley Hatcheries, which are also infected to varying degrees with NZ mudsnails, also release fish into the Salmon River basin.

Recent releases from Hagerman NFH into the South Fork Clearwater River occurred in Newsome Creek and American River from 2001 to 2003 (Magic Valley Hatchery in 2000). There was a Hagerman NFH release into the Clearwater River in 1989, but the presence of NZ mudsnails at Hagerman NFH at that time is unknown. Hagerman NFH is the only station infected with NZ mudsnails that was programmed to directly release fish into the South Fork Clearwater River. While rainbow trout from Nampa Fish Hatchery (NZ mudsnail positive) are transferred to Clearwater Hatchery then redistributed into the South Fork Clearwater, IDFG is utilizing fish only from the clean part of Nampa Hatchery for this program.

There are several environmental factors that may prevent the colonization or limit the success of NZ mudsnails in the Upper Salmon and Clearwater rivers. Under higher water velocities (>.5 m/s) (Richards 2003; Lysne 2003) the long spiral shell of the NZ mudsnail causes it to wash away easily. While average water temperature of 7^{o}C did not prevent survivorship, growth, or reproduction, optimum growth occurs at 19^{o}C, so colder winter temperatures will slow population growth. Also, Dr. Gustafson (pers. comm.) theorized that ice formation and scouring may limit successful colonization. Recent observations suggest that the clone that has invaded the Western U.S. is a “river” clone and is unlikely

to invade lakes or reservoirs in ecologically disruptive densities (Dybdahl 2002). Concerning the Snake River reservoir populations, Dybdahl (2002) suggested that they are not self-sustaining, but are maintained by immigration from riverine habitats, whereas the absence of NZ mudsnails from Brownlee Reservoir is possibly due to the large fluctuation zone and depths greater than 60 feet (Shinn 2002).

The South Fork Clearwater River has many of the features that would classify it as unsuitable habitat for widespread establishment of NZ mudsnails. However, there is always the possibility for a small population surviving in a pocket of suitable habitat. Given that possibility, a small colony could become the point of invasion, potentially seeding establishment of larger populations of NZ mudsnails in more suitable habitat downstream or a stepping stone to other waters.

While the upper Salmon River may also be unsuitable habitat, if a small colony was established upstream of Squaw Creek Steelhead Pond there is no increased risk of invasion into more suitable habitat downstream, due to the present occurrence of NZ mudsnails. Additionally there are other factors that add support to the theory of potentially unsuitable habitat in the upper Salmon River. The length of time that stocking into an area from infected facilities has been occurring must be considered. In the Salmon River, stocking from hatcheries has been occurring probably as long (greater than 20 yrs) as there have been NZ mudsnails in the facilities, whereas in the South Fork Clearwater River, stocking from Magic Valley Hatchery occurred in 2000 and from Hagerman NFH in 2001 to 2003. Also, the level (number of fish) of stocking in the Salmon River was much greater than in the Clearwater River. Approximately 900,000 steelhead are released annually into the upper Salmon River from Hagerman NFH, compared to 200,000 into the South Fork Clearwater River. While more than 20 years of large releases does not ensure that NZ mudsnails will not become established in the future, it does support the theory of low potential for establishment. Additionally, the lack of a contiguous population downstream of the two locations that currently have well established NZ mudsnail colonization in the Salmon River drainage help support the theory of unsuitable habitat. The Little Salmon River can also be grouped with the upper Salmon River regarding unsuitable habitat and the potential for downstream introduction of NZ mudsnails already present.

Water chemistry played a minor role (5%) in growth and reproductive rates, but may determine distribution (Dybdahl 2003). Hall *et al*. (2002) reported that NZ mudsnails production is highest in vegetated habitats, but cobble can also support high densities.

Schreiber *et al*. (2003) found that NZ mudsnails frequently occurred in sites draining catchments with multiple types of human activities (grazing, agriculture, towns). This is typical pattern for successful invaders (D'Antonio *et al*. 1999 in Schreiber *et al*. 2003). The pattern may not be related to disturbance, but to other factors. In its native habitat the NZ mudsnail occurs in higher densities in agricultural catchments than in forested catchments (Quinn and Hickey 1990 in Schreiber *et al*. 2003). These streams also have higher amounts of algae, which provide increased food resources, possibly leading to higher abundance of NZ mudsnails.

As a final note, adaptation and habitat change need to be considered when contemplating potential distribution of NZ mudsnails. Already endowed with phenotypic plasticity, genetic change in existing NZ mudsnail populations could lead to greater tolerance of habitats in Idaho that currently may not support establishment. Such genetic changes could be disseminated relatively rapidly given the snail's asexual method of reproduction. Similarly, future climate or habitat change as well as other broad-scale environmental changes could potentially transform isolated NZ mudsnail refugia into continuous and wide-ranging populations.

Risk Mitigation

Hagerman NFH has developed a HACCP-NRM Plan for both steelhead and rainbow trout production. These Plans provide a structured method to identify risks and focus procedures on minimizing the unintended spread of species through natural resource pathways. These Plans include visual inspections in all springs, rearing units, and at all phases of the rearing cycle. To date, the presence of NZ mudsnails has been confirmed in all the open springs and spring ponds at Hagerman NFH; however, they are not found in the egg incubation water or the water source used for filling distribution trucks. They have not been observed in the inside rearing tanks or on raceway walls, however since a small number has been found in the head boxes and tailraces they have undoubtedly passed through the raceway (Kurt Schilling, pers. comm.). The raceways are also desiccated annually which contributes to the control of NZ mudsnails at the facility.

Fish are also checked for presence of snails in their stomach at several times during the rearing phase. To date, no live snails have been found in over 1,200 steelhead sampled annually and only recently (March 2004) two empty NZ mudsnail shells were found in steelhead from the upper deck at Hagerman NFH (Kurt Schilling, pers. comm.) Whether the shells were empty when ingested or live snails were digested is unknown; however, the incidence of snail consumption by steelhead is very low.

The HACCP-NRM Plans call for specific measure to be taken to reduce the risk of transporting snails off station. These measures include; using a clean water source to fill the distribution truck, taking fish off feed 48 hours prior to transport, and sweeping raceway floors and walls 24 to 48 hours prior to transport. Hatchery staff utilize large mesh screens on the dewatering tower of the fish pump to allow any NZ mudsnails to fall back into the raceways rather that be loaded into the transport truck. Staff also conduct visual checks of transport trucks and fish pump water and any NZ mudsnails, if seen, would be physically removed (Kurt Schilling, pers. comm.).

Even by instituting all the steps outlined in the Hagerman NFH HACCP-NRM Plans, there is no way to guarantee that NZ mudsnails will not be transported off station during fish stocking. The only way to guarantee no possible introduction from Hagerman NFH would be to curtail stocking. While this would work in the South Fork Clearwater River since Hagerman NFH is the only infected hatchery stocking there, in the upper Salmon River this management action would be pointless unless matched by IDFG for their infected hatcheries.

The HACCP-NRM Plan calls also for surveys of current release sites for the presence/absence of NZ mudsnails. The Clearwater and Salmon rivers were surveyed and plans are in place to establish annual monitoring sites in the Clearwater and upper Salmon rivers to see if NZ mudsnails colonize these areas in future years.

Risk Assessment

A long list of unknowns makes it difficult to quantify the risk of NZ mudsnail spread by Hagerman NFH operations. For example, what are the odds that NZ mudsnails will survive if introduced into new sites like the Clearwater and if they survive, will they cause ecological problems? Eventually, many of these issues will be addressed in the ANS Task Force National NZ Mudsnail Management and Control Plan and assessed in the Hatchery-based NZ Mudsnail Introduction Risk Assessment Model, both of which are currently under development. In the interim, the following criteria have been developed to assess the risk of NZ mudsnail spread by hatchery release operations. A hatchery release will likely cause or promote the spread of NZ mudsnails if**:**

- Evidence of live or dead NZ mudsnails in any quantity has been found associated with water used in rearing or transport of subject fish, inside facilities that indicate availability for consumption by subject fish, or inside subject fish within the last 12 months, and;

- NZ mudsnails have not yet been found in the watershed of the tributary where the hatchery release is to occur.

Risk Management Recommendations

The above risk assessment involves a conclusion about likely risk based on a scientific analysis of available information. The rest of this report addresses the decision of how to manage this risk. This decision considers the science-based conclusions of the risk assessment, but also needs to factor in scientific uncertainty, mitigating circumstances (e.g., additional sources of risk), and other consequences of the decision (ecological, political, socio-economic, etc.).

The following factors were compiled and prioritized to guide decision-making for Hagerman NFH operations that are likely to introduce or spread NZ mudsnails into the South Fork Clearwater River, Upper Salmon River, and Little Salmon River. These factors should be used to determine whether continued hatchery release operations are justifiable despite a risk assessment conclusion that the operation will likely cause or promote the spread of NZ mudsnails. Note that these factors need to be reevaluated to determine if they are appropriate for guiding decision-making for other Pacific Region Fisheries operations, and modified accordingly.

1) **Ongoing stocking by other parties** (i.e. any advantage from not stocking from a Service hatchery is negated by practices in the watershed by other parties)

2) **Potential introduction from other vectors** (i.e. type and level of human recreation, natural waterfowl or fish movement, etc.)

3) **Contamination abundance/history of infected water, facility, and/or fish**

4) **Effectiveness of HACCP-NRM plan or control measure implemented at the infected facility**

5) **Habitat suitability** (i.e. water velocity, mean water temperature, ice formation and scouring, vegetation, substrate, nutrient loading, food availability, natural or man-caused habitat disruption, reservoir water level fluctuation, etc.), recognizing uncertainty due to potential changes in habitat quality or NZ mudsnail tolerance

6) **History of previous stocking for infected hatcheries** (i.e. number of fish and years, this may help support or refute a determination of habitat suitability)

7) **Contiguous NZ mudsnail populations downstream of established colonies** (this may help support or refute a determination of habitat suitability)

8) **Distance of nearest NZ mudsnail population**

9) **Public benefit of continuing the operation relative to the anticipated costs of resulting NZ mudsnail spread**

10) **Potential for development of a new invasion point or stepping stone population** (i.e. possibility of seeding unoccupied habitat downstream or an intermediate step for NZ mudsnails to reach a new water body)

11) **Natural resource or societal benefit of continuing the operation relative to the anticipated risks of resulting NZ mudsnail spread**

12) **Potential for development of a 'significant' population** (i.e. marginal habitat, pockets or fragmented suitable habitat availability, well established native snail or macroinvertebrate populations, etc.) (significant could be defined as one that may impact listed species or reach densities high enough to displace native invertebrates through spatial factors)

13) **Potential for continued stocking from infected Service hatcheries to promote continued stocking from infected facilities by other parties**

14) **Potential for continued operation to compromise other Service invasive species programs even if biological risk is inconsequential**

Literature Cited can be found in Appendix F: Bibliography

Appendix E

Controlling the Spread of New Zealand Mudsnails on Wading Gear

California Department of Fish and Game, Administrative Report 2005-02

The following procedures for cleaning NZ mudsnail infested wading gear can be followed upon exiting infested waters. Wading gear should be cleaned prior to leaving the site. If this is not possible then wading gear should be completely sealed inside a large plastic bag and cleaned before it is used in any other waters. Three different cleaning protocols have been tested and found to be effective using specific cleaning solutions.

1. Immersion Procedure

a. Remove wading gear upon exiting NZ mudsnail infested waters. Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tests or trailers. NZ mudsnails can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.

b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be completely covered by a cleaning solution.

c. Pour sufficient cleaning solution into the container with the infested wading gear to completely cover the gear. It may be necessary to weight down the gear to ensure that it remains immersed in the cleaning solution.

d. Allow the wading gear to remain in the cleaning solution for at least 5 minutes.

e. Remove the wading gear from the cleaning solution one piece at a time and inspect it to make sure that all debris that could harbor NZ mudsnails has been removed from the gear as well as any NZ mudsnails that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.

f. Rinse wading gear in clean water. Do not use water from the mudsnail infected source. This may reintroduce NZ mudsnails to the wading gear.

g. Return cleaned wading gear to its appropriate storage container.

2. Dry Sack Procedure

a. Remove wading gear upon exiting NZ mudsnail infested waters. Avoid allowing infested wading gear to com in contact with interior surfaces of vehicles or camping gear such as tests or trailers. NZ mudsnails can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.

b. Place wader, wading boot and boot insoles into a dry sack (recommended size: 65 liter). Walking sticks will need to be cleaned separately outside of the dry sack to avoid rupturing the sack.

c. Add 8 to 10 liters of cleaning solution to dry sack and seal.

d. Pick up the dry sack and shake it back and forth using a rolling motion to ensure that the contents are thoroughly coated with the cleaning solution. Continue shaking for approximately 30 seconds.

e. Let dry sack sit undisturbed for at least 5 minutes. Then repeat the shaking and mixing for another 30 seconds.

f. Open the dry sack and remove the contents one piece at a time and inspect it to make sure that all the debris that could harbor NZ mudsnails has been removed from the gear as well as any NZ mudsnails that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.

g. Rinse wading gear in clean water. Do not use water from the mudsnail infected source**.** This may reintroduce NZ mudsnails to the wading gear.

h. Return cleaned wading gear to appropriate storage container.

3. Spray Bottle Procedure (Note: This procedure has only been tested using a copper sulfate cleaning solution)

a. Remove wading gear upon exiting NZ mudsnail infested waters. Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tests or trailers. NZ mudsnails can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.

b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be easily accessed.

c. Using a standard one liter squeeze-trigger type spray bottle containing the cleaning solution, spray the wading gear to the point of saturation and runoff with the cleaning solution. Be sure to treat the inside of the wading boots as well as the outside. Use the stream setting to be sure and dislodge any debris from the wading boots. Be sure to treat both top and under side of gravel guards if they are permanently attached to the waders.

d. Allow the wading gear to set for at least 5 minutes with the cleaning solution on it. Remove the wading gear one piece at a time and inspect it to make sure that all debris that could harbor NZ mudsnails has been removed from the gear as well as any NZ mudsnails that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris or mud.

e. Rinse wading gear in clean water. Do not use water from the mudsnail infected source. This may reintroduce NZ mudsnails to the wading gear.

f. Return cleaned wading gear to appropriate storage container.

4. Cleaning Solutions

a. **Copper sulfate:** Dissolve 3.785 grams of copper sulfate pentahydrate crystals (99.1% purity) for each gallon of solution you want to make. This will achieve a concentration of 252 mg/L of copper in the cleaning solution.

b. **Benzethonium chloride:** Dissolve 7.57 grams of benzethonium chloride (97% purity) for each gallon of cleaning solution you want to make. This will achieve a concentration of 1.947 mg/L in the cleaning solution.

c. **Commercial Solutions Formula 409® Cleaner Degreaser Disinfectant:** Dilute the commercially available solution 1:1 with clean water to achieve the needed concentration for the cleaning solution (i.e. one gallon of Formula 409® Disinfectant to one gallon of water).

Appendix F

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Appendix 2

New Zealand Mudsnail Procedures of

LA County Sanitation Districts (2012)

Treatment Methods to Prevent the Spread of Aquatic Invasive Species

Introduction

The purpose of implementing treatment methods to sampling equipment is to prevent the spread of aquatic invasive species, specifically the New Zealand Mudsnail (NZMS). The NZMS is an introduced aquatic species that has invaded lakes, rivers, and streams throughout the United States, and has been found locally in Malibu Creek and Piru Creek in Los Angeles County. The NZMS is a threat to waterways as it out-competes native invertebrates for food and habitat. The NZMS is frequently moved between streams and lakes by attaching to waders, nets, and other sampling gear; therefore, it is important to inspect and treat gear before moving to a new water body. There are many techniques used to prevent the spread of the NZMS including freezing equipment, using hot water, and chemicals. It is important for the SJC-WQL sampling staff to implement these procedures as a precautionary measure to ensure that, if the snail is introduced into a portion of our watershed, it will not be spread throughout the watershed. During river monitoring and bioassessment sampling events, the sampling staff will be required to inspect gear and change waders before moving to another water body. Inspecting gear is important, but it is often hard to see the snails since they are on average one-eighth of an inch long and can be as small as a grain of sand. The waders that come in contact with receiving water will be placed into large plastic bags to separate them from clean waders. These waders will be placed in the walk-in freezer at the SJC-WQL. Freezing will ensure complete mortality of the snails.

Field Procedures (*These procedures only apply if the waders come in contact with the receiving water)*

1. Inspect sampling equipment before leaving each sampling location.
    a. Ensure that there are no invertebrates clinging to gear. This includes any equipment that comes in contact with the receiving water, including field meters, sampling pumps, buckets, stadia rods, etc.
2. Change waders between different water bodies. The same pair of waders can be worn along a river in which receiving water locations are in close proximity. Table 1 indicates which receiving water locations do not require a clean pair of waders when traveling between locations.

Table 1.

River/Water body	**Location**
San Gabriel River (concrete-lined portion)	SJC-R2 LC-R31 LC-R31B LC-R4 LC-R5 LC-R9W
Coyote Creek	LB-RA

	LB-RA1 LB-RA1B
Rio Hondo River	WN-RD WN-RDB WN-RD1
Zone 1 Ditch	WN-RB WN-RBB WN-RC
San Gabriel River	WN-RA WN-RAB
San Jose Creek/San Gabriel River (impounded section)	SJC-C1 SJC-C2 SJC/WN-R11 SJC-R10
San Gabriel River (impounded section)	SJC-R12
San Jose Creek (concrete-lined)	Pom-RA
San Jose Creek (concrete-lined)	Pom-RC Pom-RD
Santa Clara River	SA-RA
Santa Clara River	SA-RB
Santa Clara River	VA-RB01 VA-RC VA-RD
Santa Clara River	VA-RE VA-RF

3. When waders are switched out, the used pair of waders should be placed in a large plastic bag to distinguish between these waders and clean waders. A clean pair of waders must be used between different water bodies. If a trash bag is not used, make sure the clean waders are kept in a separate area from the used waders.
4. Nets used for BMI sampling should follow the same procedures as the waders. Nets must be changed between different water bodies and placed in a plastic bag after use.

Laboratory Procedures

1. Upon arrival at the SJC-WQL, all contaminated waders and sampling nets must be rinsed and then taken to the walk-in freezer and hung on the boot rack.
2. The waders and nets must freeze for at least eight hours. It is best to leave the waders in the freezer overnight.
3. An alternative to freezing, is to leave the contaminated waders out to dry for 48 hours. Snails will not survive if completely dried out.
4. It is important to review Table 1 before leaving on a sampling run to ensure that each field member has enough pairs of waders and/or sampling nets to complete

the run. It is suggested that one field member enters the receiving water when possible to avoid contaminating additional pairs of waders.

Appendix 3

CDFG Controlling the Spread of New Zealand Mudsnails on Wading Gear (2005)

State of California
The Resources Agency
DEPARTMENT OF FISH AND GAME

CONTROLLING THE SPREAD OF NEW ZEALAND MUD SNAILS ON WADING GEAR

OFFICE OF SPILL PREVENTION AND RESPONSE
Administrative Report 2005-02
May 16, 2005

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CONTROLLING THE SPREAD OF NEW ZEALAND MUD SNAILS ON WADING GEAR

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SUMMARY

New Zealand mud snails were first reported in Europe during the 1800s and in North America (Idaho) in 1987. Mud snails quickly colonize habitable waters, and they were first discovered in the Owens River in Eastern California in late 1999 and have since spread to the Mokelumne, Calaveras, and Napa rivers, as well as Rush, Hot and Putah creeks. This invasive species will likely have impacts on native species, fisheries, and aquatic ecosystems of the Sacramento-San Joaquin watershed. Unintentional transport on fishing gear and equipment, notably wading gear, is likely one of the primary vectors spreading mud snails among water bodies. In this study, a phased approach identified several chemicals and cleaning methods that could easily be used in the field, and were efficacious in removing snails from wading gear with minimal corrosiveness to the gear.

New Zealand mud snails were exposed in laboratory tests to solutions of benzethonium chloride, chlorine bleach, Formula 409® Disinfectant, Pine-Sol®, ammonia, grapefruit seed extract, isopropyl alcohol, potassium permanganate, and copper sulfate. With the exception of grapefruit seed extract, potassium permanganate and isopropyl alcohol, these materials all killed mud snails within five minutes. Wading gear was repeatedly exposed to bleach, copper sulfate, Pine-Sol®, benzethonium chloride, and Formula 409® Disinfectant for prolonged periods. Bleach and Pine-Sol®, at concentrations efficacious in killing snails, did structural damage to the wading gear. Solutions of copper sulfate (252 mg/L Cu), 1,940 mg/L benzethonium chloride, and 50% Formula 409® Disinfectant killed New Zealand mud snails within five minutes and had minimal effects on wading gear integrity. Wading gear was completely submersed or put in a dry-sack with the cleaning solutions and shaken in field trials, and copper sulfate solution was sprayed on fishing gear in a separate trial. Copper sulfate (252 mg/L Cu), benzethonium chloride (1,940 mg/L) and Formula 409® Disinfectant (50% dilution) solutions under field conditions can prevent the spread of New Zealand mud snails on wading gear.

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INTRODUCTION

The New Zealand mud snail (NZMS), *Potamopyrgus antipodarum*, is one of many non-native species invading California waters. The NZMS is a cosmopolitan species that was spread to Europe and Australia, during the 19th century (Gangloff 1998). NZMS were first discovered in North America in the Snake River, Idaho in 1987 (Bowler 1990) and have since been reported in all fully western states except New Mexico (Montana State University, New Zealand mud snail website, http://www.esg.montana.edu/aim/mollusca/nzms/). A genetically distinct population of NZMS was also found in the state of New York, in Lake Ontario, in 1991. It is a new invasive species in California waters, only recently discovered in the Owens River in 1999 (Dawne Becker, personal communication). It has since spread by 2005 to the Calaveras, Mokelumne and Napa rivers as well as Hot, Rush and Putah creeks spanning both sides of the Sierra Nevada Mountains.

NZMS is a member of the Gastropod order prosobranchia (lungless or gilled snails). Species in this order have a calcified operculum that fits tightly over the shell opening. The long-range dispersal of NZMS is restricted to transport in water or damp media. The operculum forms a tight seal, and NZMS have been reported to survive out of water for several hours (Gangloff 1998). The survival of NZMS increases if kept in damp media such as a wading boot; Winterbourn (1970) reported 50% survival after 25 days in damp media. It is likely that their spread within California and from Idaho to Montana and Wyoming were the result of unintentionally being transported on damp media such as wading gear.

NZMS can reproduce by parthenogenesis, where generally a female produces offspring without fertilization. The young snails are fully functional versions of the adult, complete with immature larvae developing in their ovaries (Gangloff 1998). The populations in western North America have been found to be predominately females and are believed to be clones originating from one original source population (Gangloff 1998). Several researchers have reported NZMS densities near and in excess of 50,000/m^2 (Hylleberg and Siegismund 1987; Schreiber et al. 1997; Noda 2003); numbers in excess of 100,000/m^2 have been observed in Putah Creek (Ken Davis, personal communication) and rivers in Yellowstone National Park (Riley 2002).

Impacts of NZMS can fall into three categories: (1) out competing native gastropods (Richards 2003); (2) exclusion of other grazing aquatic organisms through high density (Cada 2003); and (3) competing with other macro-invertebrates for periphyton (Gangloff 1998, Cada 2004). It is also possible that very dense snail populations may have a significant adverse impact on available nutrients in streams. Mud snails are capable of passing through the digestive canal of most fish, alive and intact (Bondesen and Kaiser 1949; Haynes et al. 1985). In addition, energetic studies have indicated that NZMS, even when digested is a trophic dead end with fish receiving little, if any nutrition from feeding on them (Vinson 2004, Ryan 1982). This will ultimately have a significant adverse impact on the fish populations through reductions in nutritious benthic invertebrate fauna to the benefit of low-nutritional value mud snails.

Initially in California, NZMS were only found in a portion of the Owens River on the east side of the Sierra Nevada Mountains. Snails have now been found along a significant stretch of the Owens River and some tributaries, as well as in streams and rivers on the west side of the Sierras (Bergendorf 2004). It is believed that the snails were inadvertently transported from one stream location to another by hitchhiking on waders, wading boots and other gear used in infested streams. NZMS are very small, typically < 7 mm in length. The ability of NZMS to survive for days in damp environments, combined with their parthenogenic nature, makes wading boots and equipment that contact infested waters a high risk vector for spreading populations (Chapman 2003). An immediate threat exists of NZMS invasion of suitable habitats within the Sacramento-San Joaquin River Delta.

Little research has been conducted on fishing gear to determine the sensitivity of NZMS to potential cleaning compounds. Studies among various researchers show somewhat inconsistent results, likely due to the lack of a consistent testing method. NZMS in a Petri dish were killed by immersion in a 5% bleach solution for 1 hour (Medhurst and Herbst 2003). Dwyer (2001) however, was unable to kill NZMS with chlorine at any concentration within one minute, but did have some success with copper sulfate. For any cleaning method to gain widespread acceptance for routine use in reducing the spread of NZMS, it should be easy to perform and not damage gear. Medhurst (2003) noted that fishing gear placed in water at a temperature of 54° C for five minutes completely killed NZMS. Medhurst (2003) also noted that freezing fishing gear for four to six hours should also kill NZMS. However, using hot water or freezing to remove NZMS from wading gear in the field is impractical and there is some concern that such methods may damage gear. Little research has been conducted on the efficacy of cleaning compounds to kill NZMS on wading gear or on the potential corrosive effects such treatments would have on gear. This uncertainty limits wide-spread efforts by the public to help prevent the spread of NZMS.

In an effort to prevent the unintentional spread of NZMS to uncontaminated waterways in California, the Department of Fish and Game (DFG) investigated methods of cleaning wading gear between uses. In this study, a phased approach identified several cleaning solutions that were efficacious in killing mud snails, minimally corrosive on wading gear, and could easily and effectively be applied in the field. In the first phase of this study, NZMS were exposed to readily available compounds to determine what concentrations elicited 100% mortality in five minutes. The second phase of the study examined the corrosiveness of the compounds on wading gear under prolonged exposures. Finally, the efficacious compounds that had minimal effects on the integrity of the wading gear were tested under field conditions.

MATERIALS AND METHODS

Toxicity of Potential Cleaning Solutions

Several cleaning solutions were selected for testing on NZMS. A protocol was developed for determining the lowest concentrations of these products/compounds that killed 100% of the NZMS within 5 minutes of exposure (Appendix 1).
Compounds investigated for killing NZMS were:

- Grapefruit seed extract (GSE), (Nutribiotic "The Original GSE®", 33% Citricidal)
- Benzathonium chloride (BZCl), (Alfa Aesar, 97% benzethonium chloride)
- Household bleach, (Clorox®, 6% sodium hypochlorite)
- Formula 409® Disinfectant
- Copper sulfate pentahydrate, (Malinckrodt, 99.1% cupric sulfate granular U.S.P.)
- Potassium permanganate, (J.T. Baker, 0.1N volumetric solution)
- Isopropyl alcohol, (generic rubbing alcohol 70% isopropyl alcohol by volume.)
- Pine-Sol®, (5% pine oil)
- Household non-sudzing, unscented ammonia, (generic ammonia, 4% as NH_3)

NZMS used for toxicity testing were collected from Putah Creek upstream of Lake Solano in Yolo County, California. Toxicity tests were conducted at temperatures of 5°C and 15°C. Large colonies of collected snails were maintained at the Aquatic Toxicology Laboratory (ATL) in Elk Grove in 2,000-ml glass beakers in constant temperature incubators set at temperatures of 5°C or 15°C. NZMS were maintained on a diet of deciduous leaves and algae, *Selenastrum* sp. in excess. NZMS used in the tests had a carapace length > 2 mm.

All toxicity tests employed laboratory controls using well water from ATL. Well water was also used for dilution of the nine compounds. Quality of ATL well water was 68 mg/L $CaCO_3$ hardness, 84 mg/L $CaCO_3$ alkalinity, 8.2 pH, and 255 µmho/cm conductivity. Concurrent toxicity tests were conducted using NZMS with opercula open or closed at two water temperatures (5°C and 15°C). Forty NZMS were exposed per treatment, with four chambers, 100-ml borosilicate glass beakers, per each treatment (10 NZMS per chamber). Test temperatures were maintained at 5 ± 1°C and 15 ± 1°C using constant temperature incubators. Prior to testing, NZMS were confirmed to be alive by observation of movement by the individual snails. For tests with opercula open groups, NZMS were placed in the individual test chambers with 10 ml of laboratory water ensuring the snails were active with their opercula open at the time of exposure. For tests with opercula closed, NZMS were gently disturbed with a blunt probe to close their opercula prior to placement in the test chambers with the test solutions. Test solutions for the opercula open tests were prepared at a concentration 11% higher than needed to account for the dilution of the 10 ml of laboratory water in the test chambers at the beginning of the test. This was not possible for the materials that were tested as original strength or "undiluted".

NZMS were exposed to the test solutions for 5 minutes (Figure 1), after which the test solutions were decanted and NZMS rinsed twice each with 50 ml of ATL well water. The test chambers were then refilled with 100 ml of ATL well water, covered, and returned to the incubators for 48 hours. NZMS were not fed during this period. At the end of 48 hours NZMS were observed under 10X magnification for survival. The criteria for death were

opercula open and no movement for a minimum of 5-minutes; occasionally the body of the snail was observed to be separated from the shell. At the end of the tests all NZMS were killed by immersion in undiluted bleach; NZMS were killed within five to ten minutes.

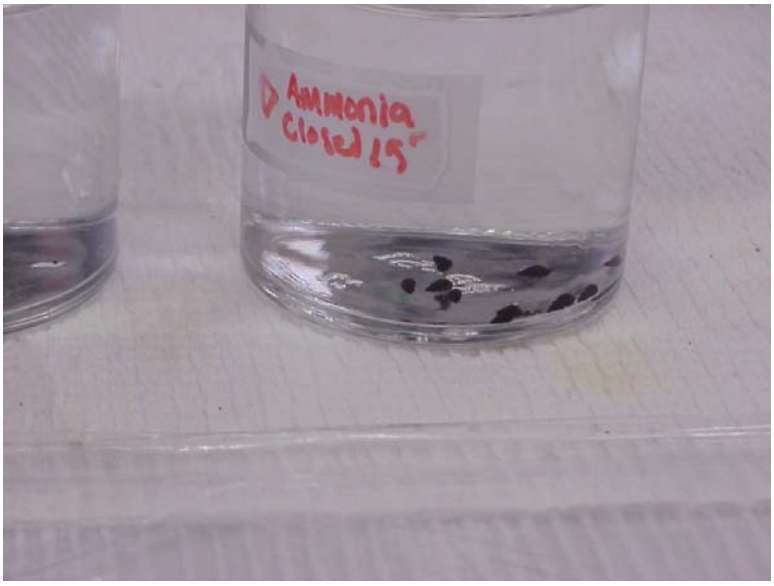

Figure 1. NZMS in test chamber with ammonia test solution

Preliminary results indicated that the solutions were more toxic to NZMS at lower temperatures with their opercula open. To mimic worst-case conditions, dilutions of the test solutions found to be most effective during the initial tests were retested on NZMS at a temperature of 15°C with their opercula closed. In the next phase of the study, solutions that were efficacious at killing NZMS were tested for corrosiveness on wading gear. The solutions that were efficacious were undiluted bleach, 50% Pine-Sol®, 50% Formula 409® Disinfectant, 1,940 mg/L benzethonium chloride, 991 mg/L copper sulfate (252 mg/L Cu), and undiluted non-sudzing household ammonia.

Corrosiveness of Cleaning Solutions

The second phase of the study tested the corrosiveness of the efficacious cleaning solutions on wading gear. The testing was conducted using the protocol in Appendix 1. Waders and wading boots were donated by three manufacturers (Orvis Outfitters, Simms, and Patagonia).

Each cleaning solution was tested on waders and boots from each manufacturer. One mate from each pair of gear was used as a control (ATL well water) and the other mate was exposed to the cleaning solution. Exposure consisted of immersing the gear in a five-gallon plastic container containing a cleaning solution or control water for 30 minutes, followed by drying in direct sunlight at ambient temperatures for a period of not less than one hour or until the gear were dry to touch, which ever was longer. Gear exposed to the cleaning solutions were not rinsed with control water following exposure. Gear were exposed on alternating days for a period of two weeks; a total of seven exposures for each set of gear. Test solutions were not renewed during the course of the testing.

At the end of the seven exposures, the gear were examined and physically tested to identify any adverse effects from exposure to the test compounds. Examination included checking for changes in: fabric flexibility, fabric color, texture of fabric, water repellency, seam integrity (stitching and glue), the condition of neoprene as well as the presence of surface and/or structural cracking on rubber parts, corrosion on metal parts, surface residues, and odors. The efficacious cleaning solutions that did not significantly damage the gear were tested under field conditions for removing NZMS from wading gear. The cleaning solutions that were efficacious against NZMS and were not corrosive on wading gear were 991mg/L copper sulfate (252 mg/L Cu), 1,940 mg/L benzethonium chloride, and 50% Formula 409® Disinfectant.

Field Testing of Cleaning Solutions and Methods

The last phase of the study tested the efficacy of the cleaning solutions under field conditions using the cleaning protocol in Appendix 1. Three cleaning methods used during the field trials were: (1) Full immersion of gear in a solution (approximately 20 to 30 liters of cleaning solution); (2) Shaking gear in a dry sack with a solution (approximately 8 to 10 liters of cleaning solution); and (3) Spraying gear with a solution to the point of saturation/runoff (only done using copper sulfate solution (252 mg/L Cu)). Following the 5-minute cleaning procedure, the gear was rinsed in control water. The cleaning solutions were not renewed during the course of the field trials.

Over fifty volunteers were recruited from local fishing clubs (Figure 2). The volunteers, wearing their own wading gear, followed a marked trail along approximately 1,300 feet of the stream bed in Putah Creek in Yolo County, California. This section of the creek had been previously identified as supporting high densities of NZMS (>100,000 snails/m^2). After wading the trail, gear were removed and randomly assigned to one of seven treatment groups (Table 1). Field trials continued over a period of two days until a minimum of seven sets of gear had been processed using each of the cleaning solution/method combinations.

Table 1. Distribution of wading gear sets by combination of cleaning solution and method.

Treatment	Cleaning Solution	Cleaning Method	Sets of Wading Gear
1	Copper sulfate (252 mg/L Cu)	Immersion	7
2	Benzethonium chloride (1,940 mg/L)	Immersion	7
3	Formula 409® Disinfectant (50% dilution)	Immersion	8
4	Copper sulfate (252 mg/L Cu)	Shaken in dry sack	8
5	Benzethonium chloride (1,940 mg/L)	Shaken in dry sack	7
6	Formula 409® Disinfectant (50% dilution)	Shaken in dry sack	8
7	Copper sulfate (252 mg/L Cu)	Sprayed	7

Figure 2. Volunteers participating in the field trials

All wading gear were examined after cleaning to detect the presence of living or dead NZMS. If substrate material (mud, gravel, etc) that could harbor NZMS was present, the gear was rinsed again in fresh water and examined. If substrate material remained, the wading gear was scrubbed using a stiff, nylon-bristled brush until all foreign material was removed. The cleaning solutions and rinse water were filtered through a 500-µm mesh nylon net following each cleaning (Figure 3). The filter and filtrate for each pair of wading gear were placed into individually labeled, clean containers with fresh water. The containers were placed on wet ice and returned to the laboratory to identify the numbers of living and dead NZMS. Live NZMS were collected from Putah Creek during the field trials and held in clean containers with fresh water served as process controls.

The filtrate samples were examined in the laboratory under 10x magnification. Survival was determined for each sample. Because size may have affected survival, all NZMS were counted and assigned to the following size classes based on the length of the carapace: (1) $\leq$ 1 mm; (2) 2 mm; (3) 3 mm; (4) 4 mm; (5) 5 mm; and (6) $\geq$ 6 mm. Data from the examination of the samples were tabulated to determine the efficacy for each cleaning solution/method combination.

Figure 3. Filtering cleaning solution after removing wading gear.

RESULTS

Toxicity of Cleaning Solutions

Benzethonium chloride (1,940 mg/L), copper sulfate (504 mg/L Cu), undiluted Formula 409® Disinfectant, undiluted household ammonia, and a 50% dilution of Pine-Sol® were effective at killing NZMS in a 5-minute exposure at a temperature of 5°C (Table 2). A 5% dilution of bleach (3,000 mg/L sodium hypochlorite), potassium permanganate (200 mg/L), undiluted isopropanol (700,000 ml/L) and grapefruit seed extract (700 ml/L) were ineffective at killing NZMS at temperatures of either 5°C or 15°C.

The survival rate for NZMS was notably higher for isopropyl alcohol and potassium permanganate at the higher temperature (15°C) (Table 2). Similarly, the survival rate was higher when the NZMS opercula were closed when exposed to isopropanol, potassium permanganate, and bleach. Hence, additional tests were conducted only at a temperature of 15°C and with the NZMS opercula closed.

Additional toxicity tests were performed using dilutions of those materials that were efficacious on NZMS to determine the minimal effective dose. Copper sulfate solution at 252 mg/L Cu and the 50% dilution of Formula 409® Disinfectant continued to exhibit 100% control (Table 2). Dilutions of Pine-Sol® below 50% and of benzethonium chloride below 1,940 mg/L were not effective. Grapefruit Seed Extract (GSE) was retested at a higher concentration (2,000 ml/L) but remained ineffective in killing NZMS (Table 2).

Laboratory toxicity tests demonstrated that copper sulfate pentahydrate (252 mg/L Cu), Formula 409® Disinfectant (50% dilution), Pine-Sol® (50% dilution), benzethonium chloride (1,940 mg/L), and undiluted household ammonia (4% NH_3) were effective at killing NZMS over a range of temperatures (5°C - 15°C). Dilutions of bleach appeared to be effective at controlling NZMS when their opercula were open (Table 2).

Table 2. Survival of New Zealand mud snails at temperatures of 5°C and 15°C following 5-minute exposure to cleaning solutions and a 48-hour recovery in fresh water

Compound	5° C		15° C	
	Opercula Open	Opercula Closed	Opercula Open	Opercula Closed
ATL Well Water (Control)	97.5	99.5	99.5	100
Grapefruit seed extract (700 ml/L)	100	97.5	100	100
Grapefruit seed extract (2,000 ml/L)	-	-	-	62.5
Isopropanol (undiluted, 700,000 ml/L)	47.5	75	85	72.5
Potassium permanganate (200 mg/L)	10	25	22.5	52.5
Bleach (5% dilution; 3,000 mg/L HOCl)	0	30	0	80
Bleach (17% dilution; 10,000 mg/L HOCl)	-	-	-	97.5
Benzethonium chloride (1,940 mg/L)	0	0	0	0
Benzethonium chloride (970 mg/L)	-	-	-	67.5
Benzethonium chloride (485 mg/L)	-	-	-	87.5
Formula 409® Disinfectant (undiluted)	0	0	0	2.5
Formula 409® Disinfectant (50% dilution)	-	-	-	0
Pine-Sol® (undiluted; 50,000 ml/L pine oil)	0	0	0	0
Pine-Sol® (50% dilution)	0	0	0	0
Pine-Sol® (25% dilution)	-	-	-	62.5
Pine-Sol® (10% dilution)	-	-	-	100
Ammonia (undiluted; 40,000 ml/L)	0	0	0	0
Ammonia (50% dilution)	-	-	-	30
Ammonia (25% dilution)	-	-	-	47.5
Copper sulfate (504 mg/L Cu)	0	0	0	0
Copper sulfate (252 mg/L Cu)	-	-	-	0

Corrosiveness of Solutions

Examination revealed the most noticeable changes were to those waders and boots exposed to bleach (undiluted) and Pine-Sol® (50% dilution) (Table 3).

Bleach (undiluted) – Bleach caused a noticeable color change in the fabric of waders and wader boots. Cracking of neoprene, overall loss of flexibility (stiffening) of the fabric, failure of stitching, and tearing of fabric on the boots were all observed (Figure 4 and 5). The waders exposed to bleach leaked through the neoprene booties. However, water continued to bead up on the fabric portion of the Simms® waders exposed to bleach.

Table 3. Effects of NZMS cleaning solutions on wading gear.

Compound and Concentration	Color Change	Seam Integrity	Leaks[A]	Comments
ATL control well water	None	No observed impact	No	No observed changes
Copper sulfate (252 mg/L Cu)	None	No observed impact	No	No observed changes
Benzethonium chloride (1,940 mg/L)	None	No observed impact	No	Rubber of boot toe guard developed small cracks, waders lost surface water repellency
Formula 409® Disinfectant(50% dilution)	None	No observed impact	No	Rubber of boot toe guard developed small cracks, waders lost surface water repellency
Pine-Sol® (50% dilution)	Some dark staining inside and outside	Glue dissolved on neoprene seams	Yes in the seams	Fabric retained odor of Pine-Sol®, developed a greasy feeling, rubber of boot toe guard developed small cracks, waders lost surface water repellency
Bleach (undiluted)	yellowing, fading	Stitching began to dissolve on boots	Yes	Fabric became brittle, neoprene developed cracks, fabric fractured and tore along boot seams

[A] All waders were tested for leaks prior to initiation of Phase 2 testing.

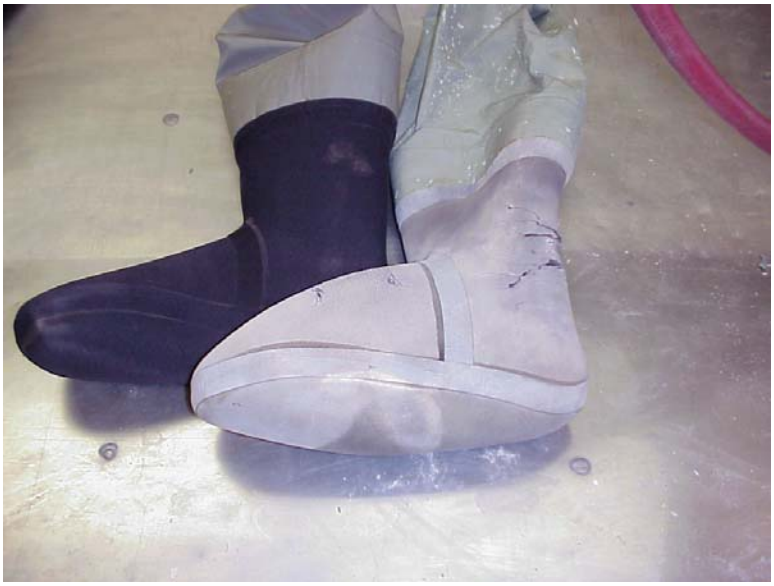

Figure 4. Neoprene wader bootie exposed to bleach (right) compared to bootie exposed to ATL control well water. The fabric of the neoprene bootie exposed to bleach has cracked and lost color.

Figure 5. Wader boot exposed to bleach had changed color of boot liner and failure of material where tongue had been sewn.

Pine-Sol® (50% dilution) – Wading gear exposed to Pine-Sol® (50% dilution) continued to emit an odor characteristically associated with the compound several weeks after the tests ceased. The seams of the neoprene booties were weakened and in one case actually failed. The glue used in the seams appears to have been dissolved (Figure 6). Surface cracking of the rubber toe portion was observed for boots. The inside of the leg of the Simms® brand waders exposed to the Pine-Sol® solution exhibited some staining but did not leak.

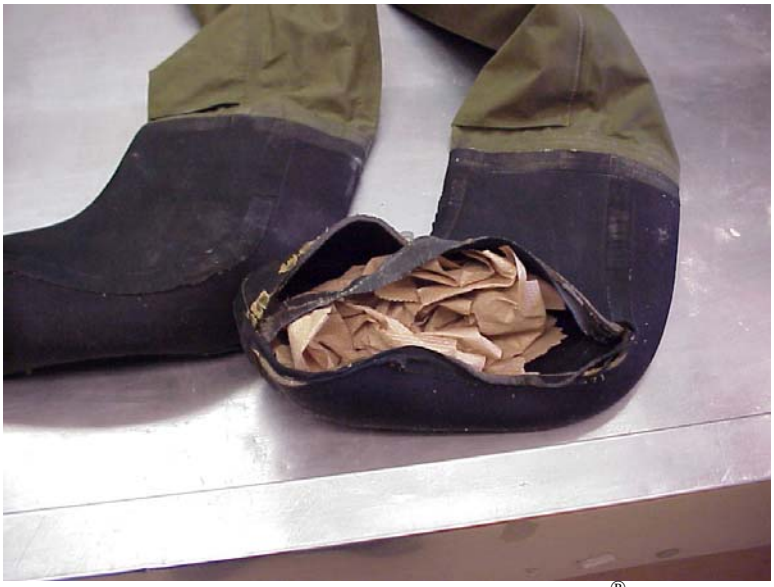

Figure 6. Neoprene bootie exposed to Pine-Sol® had seam failure. The glue appears to have dissolved.

Formula 409® Disinfectant (50% dilution) – Cracking of the rubber toe portion of the boots was observed. The cracking was similar to that observed for the boots exposed to the Pine-Sol® solution. This cracking appeared to be surficial and did not appear to have compromised the integrity of the boots. Water did not continue to bead up on the exterior of the waders. However, they did not develop any leaks.

Benzethonium Chloride (1,940 mg/L) – The only observed changes in the wading gear exposed to benzethonium chloride were minor, surficial cracking of the rubber toe guard of the boots similar to that observed with Formula 409® Disinfectant and Pine-Sol® exposure (Figure 7), and a loss of surface water repellency (beading of water) on the legs of the waders similar to that observed for Formula 409® Disinfectant. Waders exposed to benzethonium chloride did not develop any leaks. The fabric in the waders or the boots did not appear to lose any flexibility.

Figure 7. Wader boot exposed to benzethonium chloride had cracking of rubber toe.

Copper Sulfate (252 mg/L Cu) – There were no observed changes in wading gear exposed to the copper sulfate solution (252 mg/L Cu). The fabric of the waders and the boots did not appear to have become stiff or brittle. Cracks were not observed in any of the rubber on the wader boots. There were no observable stains present on the outside or the inside of the waders. The waders did not develop any detectable leaks. Water continued to bead on the surface of the waders unlike those exposed to the benzethonium chloride, Pine-Sol® or Formula 409® Disinfectant solutions.

ATL Well Water – There were no observed changes to any of the waders or boots exposed to the ATL well water (controls).

Field Testing of Cleaning Solutions and Methods

Fifty-two sets of wading gear were tested during the field trials. At least seven sets of wading gear were tested for each cleaning solution and method. NZMS were separated from each filtrate sample and divided into 1-mm size classes based on length of the carapace (Table 4 and Appendix 2). All NZMS collected as control samples survived confinement in the containers, transportation and storage at the laboratory. All cleaning solutions and methods were 100% efficacious in removing NZMS from wading gear. No NZMS were recovered alive from wading gear, or from the filtrate samples.

Table 4. Numbers of NZMS isolated from individual filtrate samples for each treatment group. Refer to Table 1 for treatment specifics.

Treatment	NZMS Size Class						Total NZMS
	≥6 mm	5 mm	4 mm	3 mm	2 mm	≤1 mm	
1	0	3	25	24	40	116	208
2	5	53	64	27	66	309	524
3	0	17	30	29	51	150	277
4	3	18	47	52	53	185	358
5	0	16	11	17	21	81	146
6	0	2	1	9	18	36	66
7	0	7	15	10	39	80	151

The majority of NZMS recovered were associated with wading boots. NZMS were observed on the tongue area of wading boots, associated with the laces or the area of the tongue that was tucked beneath the lacing eyelets. Large numbers of small NZMS were present inside of the boots, having worked down between the boot and the neoprene bootie of the wader. If the boots contained padded insole inserts, NZMS were also found underneath the inserts, associated with sand grains. NZMS were recovered from every treated set of wading gear. Numbers of NZMS per sample (Figure 8) ranged from 1 to 227 with a mean of 33 (Appendix 2). Over 50% of NZMS recovered were $\leq$ 1 mm in size (Table 4).

Figure 8. NZMS recovered from one set of wading gear cleaned during the field trials.

DISCUSSION

Prior to this study, the primary methods of eliminating NZMS from wading gear were primarily physical in nature, either freezing equipment for over 6 hours at temperatures of -10°C (Bergendorf 2004) or immersing in heated water at a temperature of 45°C for at least 1 minute (Gangloff et al. 1998). Desiccation has also been reported to be effective at removing NZMS from equipment. However, all three physical methods have significant limitations (lack of a freezer or heating device) for making them feasible for cleaning gear in the field. Complete desiccation of NZMS could take several days. Several studies report greater than 50% survival of NZMS for 25 days on "damp media" (Bergendorf 2004). "Damp media" may consist of small void spaces under seams on waders and gravel guards or under insoles in wading boots that could retain small amounts of moisture for days. During this drying period the gear would not be available for use in other waterways.

In reviewing potential threats to California habitat and fisheries posed by NZMS, Becker (2001) noted that control measures for the snails appeared to be more effective at cold temperatures. Our data corroborate these findings as survival of NZMS was notably lower at a temperature of 5°C compared to a temperature of 15°C for bleach, potassium permanganate, and isopropanol. We believe this is related to the increased time for the NZMS to close the opercula at lower temperatures, allowing more cleaning solution inside the shell.

Our results indicate that copper sulfate (252 mg/L Cu), Formula 409® Disinfectant (50% dilution) and benzethonium chloride (1,940 mg/L) are effective at removing NZMS from wading gear in a feasible amount of time. These materials were equally effective if the snails' opercula were open or closed and had no apparent effect on gear integrity. Observed impacts to the gear appeared to be cosmetic rather than structural, and the exposure was far more rigorous than the necessary exposure period indicated by our lab trials (5-minutes). All three solutions were effective at removing NZMS from wading gear under field conditions. We conclude that wading gear properly cleaned using any one of the three methods are free of live NZMS that could be transported to another water body. It also appears that exposure to materials causes NZMS to release from the substrate they're in contact with, which facilitates their removal. The data further support the conclusion that solutions of these materials can remain efficacious for cleaning several sets of wading gear without renewal.

CONCLUSIONS

We believe that the use of copper sulfate, benzethonium chloride or Formula 409® Disinfectant immersion baths or in dry sacks provides an acceptable alternative to the current physical methods of removing NZMS from wading gear. Copper sulfate was also effective when sprayed on the gear. These have the advantage of requiring less than 30 minutes to complete versus freezing (4 to 6 hours) or desiccation (possibly days) and cleaning can be done in the field. However, it may be necessary to carry a container to place the gear in during cleaning. After cleaning, the gear should not be rinsed with site water as this may place NZMS back on the gear. Care must also be taken to ensure that the cleaning solutions not enter surface water. We propose that a possible cleaning protocol based on the results of this study could be distributed through an outreach program to various fishing groups, consultants and researchers that may visit NZMS infested waters (Appendix 3).

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APPENDICES

Appendix 1:

Standard Operating Procedure (SOP) For Testing The Effects Of Cleaning Solutions On New Zealand Mud Snail, *POTAMOPYRGUS ANTIPODARUM* And Wading Gear

STANDARD OPERATING PROCEDURE (SOP) TESTING THE EFFECTS OF CLEANING SOLUTIONS ON NEW ZEALAND MUD SNAIL, *POTAMOPYRGUS ANTIPODARUM* AND WADING GEAR

Prepared by: _____ Date:
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1.0 Scope and Application

1.1 The purpose of this protocol is to screen for short-term lethal effects of select cleaning solutions on the New Zealand mud snail (NZMS) *Potamopyrgus antipodarum* with their opercula open and with their opercula closed. Previous studies have suggested a considerable difference in toxicity between the two opercula states. The compounds tested are being considered for use to clean waders, wading boots and water contact fishing equipment containing NZMS in an attempt to prevent its spread to uncontaminated waters. The compounds currently being considered include (1) Grapefruit seed extract; (2) Benzethonium chloride (a quaternary ammonium compound); (3) Household bleach (6% sodium hypochlorite); (4) Formula 409® Disinfectant brand all purpose cleaner; (5) Copper sulfate; (6) Potassium permanganate; (7) 70% Isopropyl alcohol; (8) Pine Sol®; and (9) Household use non-sudzing ammonia.

2.0 Equipment

2.1 100-ml borosilicate clear glass beaker test chambers (four replicates per test solution and four control replicates).

2.2 NZMS of a size large enough to be seen with the unaided human eye, approximately 2-mm carapace length or larger (ten snails per replicate, including controls).

2.3 1-L chemically clean amber glass bottles for mixing and holding test solutions.

2.4 Temperature control incubator to hold test chambers.

2.5 Disposable glass pipettes.

2.6 Glass graduated cylinders.

3.0 Preparation of Test Equipment

3.1 Tests are conducted at the Aquatic Toxicology Laboratory (ATL) of the Department of Fish and Game (DFG), Elk Grove. Tests are run at two temperatures, 5°C and 15°C (40° and 60° F) using constant temperature incubators.

4.0 Preparation of Test Solutions for Opercula Open Tests[1]

Test materials are diluted using ATL control water (1:1 ATL well water : RO water).

4.1 0.20% (2,000 ppm) grapefruit seed extract (GSE), 33% Citricidal in glycerin:
200 drops (165 ml) of GSE diluted with control water to a total volume of 900 ml.

4.2 0.194% (1,940 ppm) benzethonium chloride (QAC):
2 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 900 ml.

4.3 5% (3,000 ppm) household bleach (6% sodium hypochlorite):
50 ml bleach diluted with control water to a total volume of 900 ml

4.4 100 % Formula 409® Disinfectant (undiluted):

4.5 0.198% (1,980 ppm) copper sulfate pentahydrate (504 ppm CU):
2 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 900 ml

4.6 0.02% (200 ppm) potassium permanganate ($KMnO_4$):
63.7 ml of 3.171 mg/ml potassium permanganate standard solution diluted with control water to 900 ml total volume

4.7 100% household rubbing alcohol (70%, isopropyl alcohol):

4.8 100% Pine Sol® cleaner (5% pine oil):

4.9 100% household non-sudzing ammonia (4% as NH_3):

5.0 Preparation of Test Solutions for Opercula Closed Tests

Test materials are diluted using ATL control water (1:1 ATL well water : RO water).

5.1 0.20% (2,000 ppm) grapefruit seed extract (GSE), 33% Citricidal in glycerin:
200 drops of GSE (165 ml) diluted with control water to a total volume of 1,000 ml.

5.2 0.194% (1,940 ppm) benzethonium chloride (QAC):

[1] The diluted materials are 11% stronger than indicated to account for dilution by 10 ml of control water in the test chambers

2 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

5.3 5% (3,000 ppm) household bleach (6% sodium hypochlorite):
50 ml bleach diluted with control water to a total volume of 1,000 ml

5.4 100% Formula 409® Disinfectant (undiluted):

5.5 0.198% (1,980 ppm) copper sulfate pentahydrate (504 ppm Cu):
2 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 1,000 ml

5.6 0.02% (200 ppm) potassium permanganate ($KMnO_4$):
63.7 ml of 3.171 mg/ml potassium permanganate standard solution diluted with control water to 1,000 ml total volume

5.7 100% household rubbing alcohol (70%, isopropyl alcohol):

5.8 100% Pine Sol® cleaner (5% pine oil):

5.9 100% household non-sudzing ammonia (4% as NH_3):

6.0 Preparation of Dilutions of Efficacious Solutions for Opercula Closed Tests

6.1 (10,000 ppm) Bleach: 167 ml Bleach (60,000 ppm) diluted to 1,000 ml with control water.

6.2 Pine Sol® 25% Solution: 250 ml of Pine Sol® diluted to 1,000 ml with control water.

6.3 Pine Sol® 10% Solution: 100 ml of Pine Sol® diluted to 1,000 ml with control water.

6.4 Household ammonia 50% Solution: 500 ml of ammonia diluted to 1,000 ml with control water.

6.5 Household ammonia 25% Solution: 250 ml of ammonia diluted to 1,000 ml with control water.

6.6 0.097% (970 ppm) benzethonium chloride (QAC):
1 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

6.7 0.049% (485 ppm) benzethonium chloride (QAC):

0.5 g crystalline benzethonium chloride (97% purity) in control water to a total volume of 1,000 ml.

6.8 0.099% (991 ppm) copper sulfate pentahydrate (252 ppm Cu): 1 g copper sulfate pentahydrate (99.1% purity) in control water to a total volume of 1,000 ml.

6.9 Formula 409® Disinfectant 50% Solution: 500 ml of Formula 409® Disinfectant diluted to 1,000 ml with control water.

7.0 Collection of Test Organisms

7.1 A minimum of 440 NZMS (2 mm or larger) are needed for the tests. Each replicate will require ten (10) snails..

7.2 There will be one trial with four replicates each for a control and each of the nine (9)) test solutions. A five minute exposure will be conducted for each material. One set of 40 snails will be retained as a control group. The total is ten (10) test and control groups.

8.0 Preparing the Test Chambers

8.1 Ten snails will be held in each test chamber and covered by ten (10) ml of control water (1:1 ATL well water:RO water) at the appropriate test temperature for the opercula open tests.

8.2 Snails will be held in the 2,000-ml glass beaker with control water at the appropriate test temperature until ready for use in the opercula closed tests.

9.0 Loading the Organisms

9.1 Snails will be maintained in the laboratory in 2,000-ml beakers containing control water at either 5°C or 15°C. The snails will be fed deciduous leaves and algae, *Selenastrum* sp. to excess as a maintenance diet. Snails will be randomly assigned to each test chamber. Snails will not be fed for one day before test solutions are administered.

10.0 Daily Tasks

10.1 Opercula Open Tests – Day 0: Place ten snails (at least 2 mm in size) in each test chamber (four replicates per test solution and control), add 10 ml of control water to each chamber and bring to a temperature of 5°C or 15°C. Allow snails to acclimate for 1 hour prior to exposure. This ensures that the

snails are active with open opercula during exposure. Add ninety (90) ml of test solution to each test chamber to make a final solution of 100 ml. Test solutions take into account the additional 10 ml in the test chamber. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water : RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

10.2 Opercula Closed Tests – Day 0: Place 100 ml of test solutions into 4 chambers and bring temperature to either 5°C or 15°C. Add 100 ml of control water to four test chambers and bring temperature to either 5°C or 15°C. Disturb snails with blunt probe prior to transfer to test chamber to ensure opercula are closed. Transfer the snails, making sure that the opercula are closed prior to immersion into test chambers. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water : RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

10.3 Opercula Closed Tests – Day 0: Place 100 ml of each test solution into each of 4 chambers and bring temperature to 15°C. Add 100 ml of control water to four test chambers and bring temperature to 15°C. Disturb snails with blunt probe prior to transfer to ensure opercula are closed. Transfer snails, making sure that the opercula are closed prior to immersion into test chambers. Exposure times in each test chamber are five minutes. Remove all test solution, and rinse snails in each test chamber with 50 ml of control water (1:1 ATL well water:RO water), twice. Drain second rinse and fill test chamber with clean control water. Replace test chamber in rack. At completion of all tests, randomize placement of test chambers in rack and return test chambers to incubator for 48 hours. Snails will not be fed during the test.

Day 1: Do not disturb snails.

Day 2: At the end of 48 hours remove test chambers from incubator and record numbers of live snails (active snails) in each test chamber. All snails are viewed under magnification to confirm survival or mortality.

11.0 Ending the Test

11.1 Count the number of snails surviving in each of the test chambers.

11.2 Write up test summary.

11.3 At the completion of the tests all contents of test chambers will be disposed of in a concentrated solution of bleach. All test chambers and equipment contacting snail containing water will be rinsed in a concentrated bleach solution to preclude inadvertently introducing snails to any uncontaminated waterways.

12.0 Corrosiveness Testing

12.1 Samples of various waders and wader boots are identified and obtained.

12.2 Specific compounds and dilutions from Phase I toxicity tests on NZMS are identified.

12.3 Samples of each of the waders and wader boots are completely immersed in solutions of the identified Phase I compounds under the following conditions on alternate days for a period of two weeks:

12.3.1 Immersion in the designated concentration of the compound for a period of thirty (30) minutes. The waders and wader boots are then placed in direct sunlight until dry or not less than one hour which ever is greater.

12.4 Following completion of the exposure regimen, all waders and wader boots are examined for evidence of adverse impacts due to exposure to the specified compounds. Adverse impacts include:

12.4.1 Discoloration of waders or wader boots

12.4.2 Cracking or evidence of brittleness of wader material or wader boots.

12.4.3 Separation of layers of materials in waders or wader boots.

12.4.4 Failure of seams in waders.

12.4.5 Loss of water repellency

12.4.6 Presence of odors

12.4.7 Presence of surface residues

12.5 The compounds identified from Phase I to be tested for adverse effects to wader material include:

12.5.1 Formula 409® Disinfectant (50% dilution)

12.5.2 Copper sulfate (252 mg/L Cu)

12.5.3 Benzethonium chloride (1,940 mg/L)

12.5.4 Pine-Sol (50% dilution)

12.5.5 If time and resources permit, ammonia (4% NH_3, undiluted) and bleach (6% sodium hypochlorite, undiluted) will also be tested.

13.0 Field Testing

13.1 Different solutions and methods for decontaminating waders and wader boots (equipment) contaminated with immature and mature NZMS are tested.

13.2 Three specific solutions have been identified (see 13.6) for 100 % control of NZMS (Phase I) and subsequently, no adverse structural changes to equipment (Phase II).

13.2.1 The solutions to be tested during Phase III include:

13.2.1.1 Copper Sulfate (252 mg/L Cu)

13.2.1.2 Benzethonium Chloride (1,940 mg/L)

13.2.1.3 Formula 409® Disinfectant (50% dilution)

13.3 Equipment tested in Phase III is either donated to the program or belongs to fishermen fishing in Putah Creek who are actively solicited by DFG staff to participate in the decontamination trials. The focus of the trials is on those configurations of equipment that are most prone to becoming contaminated with NZMS.

13.4 Equipment is worn in Putah Creek in an area known to support high concentrations of NZMS. The equipment is examined upon exiting Putah Creek to confirm the presence of NZMS. Equipment contaminated with NZMS is subjected to one of three decontamination protocols using one of the three test solutions (seven test groups). Each treatment group will be used for at least 7 sets of waders and wader boots for a total of at least 49 sets of waders and wader boots (Table 1).

Table 1. Combinations of decontamination methods and solutions to be investigated (49 sets of waders and boots)

Solution	Tub	Dry Sack	Spray Bottle
Copper sulfate (1,000 ppm)	7 Sets	7 Sets	7 Sets
Benzethonium chloride (2,000 ppm)	7 Sets	7 Sets	N/A[1]
Formula 409® Disinfectant (50% solution)	7 Sets	7 Sets	N/A

[1] Combination not planned for initial field trials

13.5 The following three protocols are used to investigate the decontamination of waders and wader boots. Sufficient sets of contaminated waders and wader boots are used for each decontamination method/test solution combination to obtain a statistically valid result (minimum 7). The waders and wader boot sets are assigned to a specific combination of test solution and decontamination method by means of a random number table. Each set of waders and wader boots is assigned to one of seven treatment test groups using a table established prior to testing based on a random number table.

13.5.1 The waders and wader boots are placed in a large (approximately 40 liter capacity) plastic tub containing a sufficient quantity of the decontamination solution for complete immersion (25-30 Liters). The waders and wader boots are immersed in the decontamination solution for five minutes.

13.5.1.1 At the end of the five minute exposure period the decontamination solution is poured through a mesh filter into a holding container. All filtered material is placed in a clean Ziploc bag and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.1.2 The waders and wader boots are removed from the container and rinsed with ATL control water. The rinseate is poured through a mesh filter. All filtered material is placed in a clean Ziploc bag and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.1.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.5.2 Waders and wader boots are placed in a 65 liter dry sack and sufficient decontamination solution added to the sack to ensure all surfaces will be exposed to the decontamination solution. The sack is sealed and shaken for 30 seconds. The waders and wader boots then remain in the sack for five minutes. Prior to removing the waders and wader boots they are shaken again for 30 seconds.

13.5.2.1 The liquid solution is poured out of the dry sack through a mesh filter and returned to the tub container. All material filtered from the decontamination solution is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.2.2 The waders and wader boots are rinsed with ATL control water and the rinseate is poured through a mesh

filter. All material filtered from the rinse water is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.2.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.5.3 The waders and wader boots are placed in an empty plastic tub. Using a hand operated kitchen type spray bottle containing the copper sulfate test solution, the waders and wader boots are thoroughly sprayed by hand to the point of runoff of decontamination solution. The waders and boots are manipulated by hand as necessary to ensure all surfaces are sprayed with the decontamination solution. The waders and wader boots are then left to sit for 5 minutes.

13.5.3.1 The waders and boots are removed from the tub and placed into a second tub. Any decontamination solution and any snails that have fallen off of the equipment are rinsed into a mesh filter. All material filtered from the decontamination solution is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.3.2 The waders and wader boots are then rinsed using ATL water. The rinseate is poured through a mesh filter and any filtered material is placed in a clean container and sealed for later examination to determine the presence, sizes and numbers of NZMS, and to determine if any snails are alive.

13.5.3.3 The waders and wader boots are examined using appropriate magnification equipment to determine if NZMS remain and if they are alive or dead. If NZMS

are present and determined to be alive the numbers and estimated sizes of snails are recorded. Locations where the snails are found on the waders and wader boots is recorded and the waders and wader boots are actively scrubbed with a brush and rinsed using ATL control water. They are reexamined and returned to the fishermen once it is determined that all NZMS have been removed.

13.6 The effectiveness of immersion decontamination method is to be investigated and compared to the dry sack method and the spray bottle method to determine if the test solutions are effective using any of the three decontamination methods under field conditions. Following completion of the initial Phase III trials it will be decided if other decontamination techniques are to be investigated further.

Appendix 2:

Distribution Of New Zealand Mud Snails In Filtrate Samples From Field Trials Of Cleaning Solutions And Methods

®Distribution and numbers of NZMS, by size class, for individual sets of wading gear from field trials at Putah Creek, Yolo County California, March 5-6, 2005.

Wader #	Treatment #	Size Classes 6mm	5mm	4mm	3mm	2mm	1mm	Total	Treatment Total	Treatment Mean
3	1	0	0	0	0	0	1	1		
5	1	0	0	1	3	4	10	18		
32	1	0	2	0	2	2	9	15		
38	1	0	0	0	8	11	23	42		
43	1	0	0	24	0	23	48	95		
47	1	0	1	0	11	0	23	35		
49	1	0	0	0	0	0	2	2	208	30
7	2	5	28	49	16	21	98	217		
9	2	0	0	0	0	1	7	8		
13	2	0	0	4	0	6	15	25		
15	2	0	0	3	2	0	10	15		
28	2	0	0	0	0	2	22	24		
39	2	0	25	8	7	36	151	227		
45	2	0	0	0	2	0	6	8	524	75
8	3	0	1	6	4	0	2	13		
12	3	0	1	2	1	1	6	11		
18	3	0	3	7	5	17	45	77		
22	3	0	1	0	0	1	0	2		
30	3	0	0	1	1	3	4	9		
33	3	0	11	14	8	8	48	89		
48	3	0	0	0	8	12	19	39		
51	3	0	0	0	2	9	26	37	277	35
4	4	0	0	2	2	1	6	11		
20	4	3	7	9	6	7	28	60		
26	4	0	7	20	27	30	91	175		
29	4	0	4	2	2	3	6	17		
50	4	0	0	0	3	0	4	7		

41	4	0	0	4	6	7	18	35		
44	4	0	0	10	5	2	28	45		
53	4	0	0	0	1	3	4	8	358	45
1	5	0	1	1	2	0	2	6		
6	5	0	0	1	1	4	37	43		
11	5	0	0	0	10	6	14	30		
19	5	0	0	0	1	1	0	2		
24	5	0	0	0	1	1	4	6		
25	5	0	4	0	2	2	8	16		
27	5	0	11	9	0	7	16	43	146	21
2	6	0	1	1	0	0	2	4		
10	6	0	1	0	0	2	11	14		
17	6	0	0	0	1	2	4	7		
21	6	0	0	0	1	3	6	10		
31	6	0	0	0	1	3	4	8		
35	6	0	0	0	1	0	3	4		
40	6	0	0	0	3	2	2	7		
54	6	0	0	0	2	6	4	12	66	8
14	7	0	0	2	6	3	21	32		
16	7	0	7	6	1	14	16	44		
23	7	0	0	3	2	13	15	33		
34	7	0	0	4	0	2	7	13		
52	7	0	0	0	1	6	10	17		
42	7	0	0	0	0	0	6	6		
46	7	0	0	0	0	1	5	6	151	22
Totals by size class		8	116	193	168	288	957	1730		
Mean # snails by size class		0.1	2	4	3	6	18	33		

Appendix 3.

Proposed Cleaning Procedure for NZMS Infested Wading Gear

The following procedures for cleaning NZMS infested wading gear can be followed upon exiting NZMS infested waters. Wading gear should be cleaned prior to leaving the site. If this is not possible then wading gear should be completely sealed inside of a large plastic bag and cleaned before it is used in any other waters. Three different cleaning protocols have been tested and found to be effective using specific cleaning solutions:

1) Immersion Procedure
    a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
    b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be completely covered by a cleaning solution.
    c. Pour sufficient cleaning solution into the container with the infested wading gear to completely cover the gear. It may be necessary to weight down the gear to ensure that it remains immersed in the cleaning solution.
    d. Allow the wading gear to remain in the cleaning solution for at least 5 minutes.
    e. Remove the wading gear from the cleaning solution one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
    f. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
    g. Return cleaned wading gear to it appropriate storage container.

2) Dry Sack Procedure
    a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
    b. Place waders, wading boots, and boot insoles into a dry sack (recommended size: 65 liter). Walking sticks will need to be cleaned separately outside of the dry sack to avoid rupturing the sack.
    c. Add 8 to 10 liters of cleaning solution to dry sack and the seal dry sack.
    d. Pick up the dry sack and shake it back and forth using a rolling motion to ensure that the contents are thoroughly coated with the cleaning solution. Continue shaking for approximately 30 seconds.

e. Let dry sack sit undisturbed for at least 5 minutes. Then repeat the shaking and mixing for another 30 seconds.
f. Open the dry sack and remove the contents one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
g. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
h. Return cleaned wading gear to it appropriate storage container.

3) Spray Bottle Procedure (**Note:** this procedure has only been tested using a copper sulfate cleaning solution).
    a. Remove wading gear upon exiting NZMS infested waters. **Avoid allowing infested wading gear to come in contact with interior surfaces of vehicles or camping gear such as tents or trailers.** NZMS can be transferred to any surface they come in contact with and they could later be transferred back to cleaned wading gear. Turn waders right side out and remove insoles from wading boots.
    b. Place waders, wading boots, boot insoles and the streambed contact end of a wading stick, if used, in a container of sufficient size to allow the gear to be easily accessed.
    c. Using a standard 1 liter squeeze-trigger type spray bottle containing the cleaning solution, spray the wading gear to the point of saturation and runoff with the cleaning solution. Be sure to treat the inside of the wading boots as well as the outside. Use the stream setting to be sure and dislodge any debris from the wading boots. Be sure to treat both top and under side of gravel guards if they are permanently attached to the waders.
    d. Allow the wading gear to set for at least 5 minutes with the cleaning solution on it. Remove the wading gear one piece at a time and inspect it to make sure that all debris that could harbor NZMS has been removed from the gear as well as any NZMS that could be lodged in cracks or crevices. If necessary, use a stiff plastic bristled brush such as a kitchen brush to remove any remaining debris and mud.
    e. Rinse wading gear in clean water. **DO NOT USE WATER FROM THE NZMS INFESTED SOURCE**. This may reintroduce NZMS to the wading gear.
    f. Return cleaned wading gear to it appropriate storage container.

4) Cleaning Solutions.
    a. Copper sulfate: Dissolve 3.785 grams of copper sulfate pentahydrate crystals (99.1% purity) for each gallon of solution you want to make. This will achieve a concentration of 252 mg/L of copper in the cleaning solution.

b. Benzethonium chloride: Dissolve 7.57 grams of benzethonium chloride (97% purity) for each gallon of cleaning solution you want to make. This will achieve a concentration of 1,947 mg/L in the cleaning solution.
c. Formula 409® Disinfectant: Dilute the commercially available solution 1:1 with clean water to achieve the needed concentration for the cleaning solution (i.e. 1 gallon of Formula 409® Disinfectant to 1 gallon of water).

Appendix 4

CDFG New Zealand Mudsnail Warning Poster

RESOURCES AGENCY
CALIFORNIA
DEPARTMENT
FISH&GAME

Caution!

Don't Spread

New Zealand Mudsnails

Snails range in size from a grain of sand to 1/8 inch in length and are black or brown in color

The Threat

- **Rapid reproduction of this invader can lead to densities of 1 million per square yard. A single snail could result in the production of more than 40 million snails in one year.**
- **They outcompete and replace native invertebrates that are the preferred foods of fish.**
- **They can cause drastic, harmful changes in the native plant and animal food web of streams and lakes.**

What You Can Do

- **If you wade, freeze waders and other gear overnight (at least 6 hours).**
- **Have extra waders and boots that are used only in infested waters. Store them separately.**
- **After leaving the water, inspect waders, boots, float tubes, boats/trailers-any gear used in the water. Remove visible snails with a stiff brush and follow with rinsing. If possible, freeze or completely dry out any wet gear.**
- **Never transport live fish or other aquatic animals or plants from one water to another.**

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Appendix 5

New Zealand Mudsnail Prevention Guide (2010)

Design by Stefania M. Padalino.
Cover photos: top three photos by D. L. Gustafson; bottom two photos by Jane and Michael Liu.

ORESU-G-10-001

IF YOU FIND MUDSNAILS

If you suspect you have found mudsnails, collect 5 to 10 individuals and place them in a plastic bag into which you have sprinkled water. Check against the simple traits above and on this Web page to confirm identification: http://www.esg.montana.edu/aim/mollusca/nzms

Please save the samples and contact the Oregon Invasive Species Council (1-866-INVADER or online at oregoninvasiveshotline.org) and one of these specialists:

Sam Chan and Tania Siemens
Oregon State University
Oregon Sea Grant Extension
samuel.chan@oregonstate.edu

Robyn Draheim
Center for Lakes and Reservoirs
Portland State University
draheim@pdx.edu

Paul Heimowitz
U.S. Fish and Wildlife Service
Paul_Heimowitz@fws.gov

Cynthia Tait
USDA Forest Service
ctait@fs.fed.gov

Sherri L. Johnson
PNW Research Station
USDA Forest Service
johnsons@fsl.orst.edu

To order copies of this brochure, call 541-737-4849 or e-mail Oregon Sea Grant, sea.grant.communications@oregonstate.edu. You can also download a pdf at http://seagrant.oregonstate.edu/sgpubs/onlinepubs.html

New Zealand Mudsnails

How to Prevent the Spread of New Zealand Mudsnails through Field Gear

This brochure is a guide for field detection and for treating field gear to prevent the spread of New Zealand mudsnails. It is intended for researchers, monitoring crews, watershed survey groups, and anyone else who travels frequently between aquatic or riparian locations.

Second Edition
February 2010

Devils Lake, Oregon, is heavily infested with New Zealand mudsnails. Prevent the spread of New Zealand mudsnails by cleaning gear and boats and not moving water from infested waters into new bodies of water. (Photo by Jane and Michael Liu.)

INTRODUCTION

The New Zealand mudsnail (*Potamopyrgus antipodarum*) is an introduced aquatic species that has invaded estuaries, lakes, rivers, and streams in Washington, Oregon, California, and many other states in the western U.S. It was first noted in North America in the late 1980s in the Snake River and has since spread throughout the West.

The small size (< 5 mm), cryptic coloration, and ability to survive out of water for weeks make the New Zealand mudsnail an ideal hitchhiker.

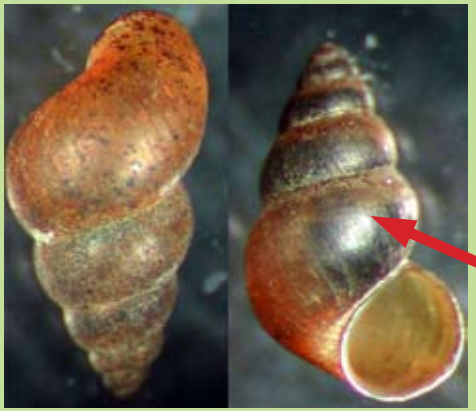
Range expansion of the mudsnail has been unwittingly hastened by anglers, hunters, and field personnel—in other words, people who frequently move between streams and lakes in watersheds, hauling wet waders, nets, and other gear with them. Once the mudsnail is established in a new habitat, it is impossible to eradicate it without damaging other components of the ecosystem. Thus, inspecting, removing, and treating gear before moving to a new water body is the most effective means of preventing the spread of mudsnails.

Snails can be inadvertently transported in bootlaces (center—note different color). (Photo by Jane and Michael Liu.)

The New Zealand mudsnail is often less than 5 mm long. (Photo by Jane and Michael Liu.)

IDENTIFYING THE NEW ZEALAND MUDSNAIL

Size: A mature snail is usually less than 5 mm (.2 in) long. (Photo by Jane and Michael Liu.)



Shape: Shell is elongated and dextral (its whorls or spirals lean toward the right). Snail typically has between 5 to 6 whorls on its shell. (Photo by D. L. Gustafson, http://www.esg.montana.edu/aim/mollusca/nzms.)

Color: Most snails have a light- to dark-brown shell that may appear to be black when wet. (Photo by Jane and Michael Liu.)

Embryos: Upon dissection, mature snails will have brooded embryos. (Photo by D. L. Gustafson, http://www.esg.montana.edu/aim/mollusca/nzms.)

Operculum: The mudsnail operculum (a rounded plate that seals the mouth of the shell when the animal's body is inside) can be seen on live snails but is not easily visible on dead or preserved snails. (Photo by D. L. Gustafson, http://www.esg.montana.edu/aim/mollusca/nzms.)

chloride [DDAC]). Formula 409® Cleaner Degreaser Disinfectant has been proven effective for killing mudsnails at 50% dilution.

- The compounds Quat 128® and Sparquat 256® are commercial disinfectants with an active ingredient (QAC) similar to that of Formula 409® Cleaner Degreaser Disinfectant, which has proven effective for killing mudsnails and other aquatic invasive species (see the table on the foldout page for dilution rates).
- Many household bath and kitchen disinfectants contain quaternary ammonium compounds (check the label for active ingredients containing alkyl dimethyl benzylammonium chloride [ADBAC]; diecyl dimethyl ammonium chloride [DDAC]).

These and other chemical treatments are constantly being evaluated and are updated online at seagrant.oregonstate.edu/themes/invasives/

Fishing docks and boats are potential conduits for spreading the New Zealand mudsnail. (Photo by Jane and Michael Liu.)

This test chamber contains a New Zealand mudsnail with chemical test solution. (Photo by Robert Hosea.)

CAUTION

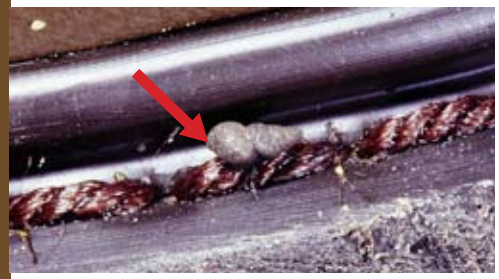
Treating field gear with chemical methods may result in unintended contamination of the environment. In particular, extreme caution must be taken to avoid contamination of waterways and wetlands. DO NOT rinse your treated gear in a water body.

Treating rubber gear or boots with Formula 409® and other disinfectants with QACs may result in surface cracking of the rubber and loss of water repellency. Chemical methods are not always effective in killing mudsnails. Always scrub your gear and consider using physical methods before resorting to chemical methods. For more information on the testing of chemical treatment methodology, see R. C. Hosea, and B. Finlayson, 2005, *Controlling the Spread of New Zealand Mud Snails on Wading Gear,* Administrative Report 2005-02, Rancho Cordova, California: Resources Agency, California Department of Fish and Game.

THE MUDSNAIL PROBLEM

The New Zealand mudsnail is a threat to our waters. By competing with native invertebrates for food and habitat, it has a detrimental impact on fish populations, vegetation, and other native biota.

Mudsnails can tolerate a wide range of habitats, including brackish water, and are found living in high densities (often over 400,000 snails/sq meter) on many different substrates (rock, gravel, sand, mud, vegetation, and even the shells of other organisms).



Mudsnails can attach to the seam of a stream boot. Unintentional transport from one stream location to another by hitchhiking on waders or wading boots is one of the primary vectors for spreading New Zealand mudsnails. (Photo by Jane and Michael Liu.)

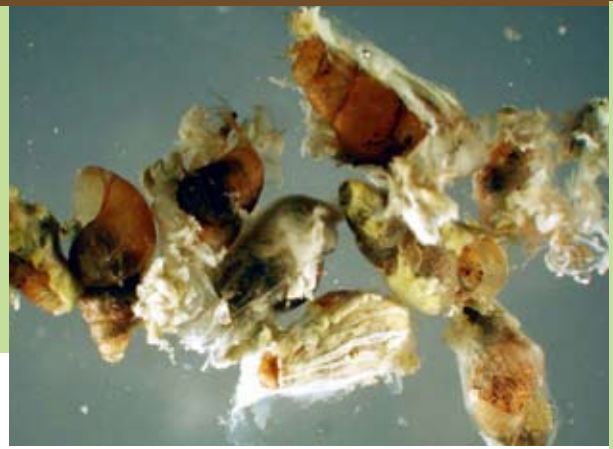
The biology, ecology, and distribution rate of the mudsnail suggest that many habitats are suitable for further expansion.

Mudsnail populations in the West are self-reproducing brooders; they clone themselves and retain the embryos inside their shell until they are large enough to release. Also known as parthenogenesis, this reproductive technique means that a single mudsnail can rapidly colonize a new location.

Mudsnails are easily transported to new habitats by recreationists and field crews because the snails readily attach to or are wedged into the many cracks, crevices, and crannies presented by waders, boot soles, nets, and buckets. New Zealand mudsnails can live for weeks in damp, cool conditions; can easily survive on field gear for long periods of time; and can be transferred to a new environment when that gear is reused.

1995 2001 2009

These maps show the spread of the New Zealand mudsnail from 1995 to 2009 in the western U.S. New Zealand mudsnails have recently been found in parts of the Great Lakes region. (Maps courtesy of Amy Benson, U.S. Geological Survey.)



Mudsnails can pass through the intestinal tract of a fish. Almost half the mudsnails survived this trip. (Photo by M. Vinson, http://www.esg.montana.edu/aim/mollusca/nzms.)

PREVENTION

To prevent the survival of mudsnails on field clothing and equipment, you will need first to clean your field gear and then to treat it, using either the physical or chemical methods listed below. We recommend the following steps:

1. **If possible, keep several changes of field gear for use in different bodies of water.**
2. **Clean all gear before leaving a site, scrubbing with a stiff-bristled scrub brush and rinsing with water, preferably high-pressure. This is often the simplest and most effective for prevention.**
3. **Inspect gear before it is packed for transport. Visible traces of sand, mud, gravel, and plant fragments are signs that gear has not been properly cleaned and mudsnails may have been retained.**
4. **Select a treatment method in addition to scrubbing and rinsing if mudsnails are present or suspected to be present.**

- **Freezing, hot water, or drying treatments** are recommended over chemical treatments because they are usually less expensive, more environmentally sound, and possibly less destructive to gear. However, most physical methods require longer treatment times and often cannot be performed in the field.
- **Chemical treatments** require a 10-minute soak in a special solution (see "CHEMICAL," page 5). After chemical treatment, gear must be rinsed thoroughly with tap water away from all bodies of water, and all soak solutions and rinse water must be properly disposed of.

PHYSICAL

These methods for cleaning gear are effective as well as environmentally sound. Use *one* of the following methods:

- **Freeze your gear for a minimum of 4 hours to kill all mudsnails. Freezer temperatures should be at 26°F (-3°C) or below.**
- **Soak gear in a bath of hot water (at least 120°F, 46°C) for 10 minutes. This method is not advised for Gortex.**
- **Dry your gear before reuse. A drying time of at least 48 hours under low humidity is recommended to remove all pockets of dampness. Gear must be completely dry for a minimum of 24 hours. Check to ensure that boots are totally dry.**

CHEMICAL

Common disinfecting cleaners containing quaternary ammonium compounds (e.g., alkyl dimethyl benzylammonium chloride [ADBAC]; diecyl dimethyl ammonium chloride [DDAC]) are effective for decontaminating gear. Disinfectants listed below will kill other aquatic invasive species but may not result in 100% mortality (see table on foldout page).

Gear should be soaked in *one* of the following solutions for 5 minutes and then rinsed thoroughly with tap water, away from the water body. Store and dispose of solution and used rinse water properly.

- **Commercial disinfectant solutions containing quaternary ammonium compounds (QAC) (e.g., Formula 409® Cleaner Degreaser Disinfectant, alkyl dimethyl benzylammonium chloride [ADBAC]; diecyl dimethyl ammonium**

The toe of this rubber wader boot has cracked after being exposed to repeated applications of benzethonium chloride. (Photo by Robert Hosea.)

A worker filters the cleaning solution after removing wading gear. (Photo by Robert Hosea.)

MUDSNAIL LOOK-ALIKES

Figure 1. *Comparison of the New Zealand mudsnail* (Potamopyrgus antipodarum) *with three freshwater snails native to the Pacific Northwest. From left to right: New Zealand mudsnail, a pond snail* (Family Lymnaeidae), *two pebblesnails* (Fluminicola sp.), *and two rock snails* (Juga plicifera).

Several freshwater snails native to the Pacific northwest are commonly misidentified as New Zealand mudsnails (*Potamopyrgus antipodarum*) (see Figure 1). "Pebblesnails" (*Fluminicola*) can be distinguished by its more-rounded, globose-shaped shell (vs. the conical New Zealand mudsnail) and a bottom whorl that is proportionally larger than its upper whorls (as compared to the New Zealand mudsnail, which tends to have more-uniform whorls). Air-breathing "pond" snails (*Lymnaeidae*) can also be very small, like New Zealand mudsnails, but they lack an operculum. "Rock" snails (*Juga sp.*) can be as small as New Zealand mudsnails when juveniles, but they grow to be much larger (up to 2.5 cm; New Zealand mudsnails are no larger than 6 mm). When small, *Juga plicifera* can be distinguished by its grooved whorls; however, other species of Juga such as *Juga silicula* can have smooth whorls similar to the New Zealand mudsnail.

Figure 2. *Comparison of the New Zealand mud snail (three snails on right) with* Assiminea parsitologica *(three snails on left). Both invasive snails might be found together in brackish-water estuaries.*

In estuaries, New Zealand mudsnail habitat overlaps with another nonnative invasive snail, *Assiminea parisitologica*, which is an intermediate host to the human lung fluke parasite, first discovered in Coos Bay, Oregon, in 2007. *Assiminea parisitologica*, native to Japan, occurs more frequently in higher saline to brackish waters, while the New Zealand mudsnail, native to New Zealand, occurs in brackish to freshwater environments. *Assiminea parisitologica* can be distinguished by its globose shape, larger bottom whorl, and a white tip (see Figure 2). *Assiminea parisitologica* is also a high-alert invasive species that should be reported. *Remember: report any species you suspect could be invasive.* It is better to have a suspected report than to miss a new infestation!

Aquatic Invasive Species of Concern, and Current Methods for Disinfection of Gear and Equipment

(Adapted from USDA Forest Service Region 4 Guidelines for disinfecting fire equipment, summarized by Cynthia Tait: http://www.fs.fed.us/r4/resources/aquatic/guidelines/aq_invasives_interim_fire_guidance08_final.pdf)

NOTE: A more complete—and continuously updated—table is available online at seagrant.oregonstate.edu/themes/invasives/

Decontam. Method	Whirling Disease	New Zealand Mudsnails	Chytrid Fungus	Zebra/Quagga Mussels	Didymo	Eurasian Watermilfoil
Hot water or freezing	90°C (195°F); 10 minutes	46°C (120°F); minimum of 5 minutes -3°C (27°F); > 4 hours	60°C (140°F); minimum of 5 minutes	≥ 60°C (140°F) water for minimum of 1 minute Freezing may be effective, but not tested	60°C (140°F); 1 minute	No data, but likely effective
Drying	Be dry for 24 hours, in sunlight best	Be dry for 48 hours, in sunlight best	Be dry for 3 hours, in sunlight best	3–5 days, in sunlight best	Be dry for 48 hours, in sunlight best	No data, but likely effective
Bleach (e.g., Clorox® or equivalent bleach product) 6% sodium hypochlorite (NaClO)	For 10 minutes: 1% bleach solution (500 ppm NaClO) • 1.1 liquid oz bleach per gallon water • 2.2 Tbsp liquid bleach per gallon water • 0.9 gallons each per 100 gallons water	Not effective at the necessary concentrations without risk of damaging gear and equipment	For 10 minutes: 7% bleach solution (0.4% NaClO) (>3,500 ppm NaClO) • 9 liquid oz bleach per gallon water • 7 gallons bleach per 100 gallons water	Gear rinsed with 0.5% bleach solution (250 ppm NaClO) • 0.6 liquid oz bleach per gallon water • 1.1 Tbsp liquid oz bleach per gallon water • 0.5 gallons bleach per 100 gallons water	For 1 minute: 2% bleach solution (800 ppm NaClO) • 1.8 liquid oz bleach per gallon water • 3.6 Tbsp liquid Clorox per gallon water • 1.4 gallons Clorox per 100 gallons water	No data, but likely effective
Quaternary ammonium compounds (QAC) (e.g., alkyl dimethyl benzylammonium chloride [ADBAC]; diecyl dimethyl ammonium chloride [DDAC])	15-minute exposure 4.4% Quat128® (1,500 ppm QAC active ingredient) • 6.1 liquid oz. Quat 128 per gallon of water OR 3.1% Sparquat 256® • 4.1 liquid oz. per gallon water	10-minute exposure 4.6% Quat 128 (1,570 ppm QAC active ingredient) • 6.4 liquid oz. Quat 128 per gallon of water OR 3.1% Sparquat 256 • 4.3 liquid oz. per gallon water OR Dilute 1 part Formula 409® Cleaner Degreaser Disinfectant to 1 part water	30-second exposure to 0.015% Quat 128 (5 ppm QAC active ingredient) • 1/8 tsp per gallon water	No published data, but likely effective	No published data, but likely effective	No data