Biannual Report to the Puget Sound Partnership
2009-2011

Invasive Species Tunicate and New Zealand Mudsnail Response Contract 09-1505

Prepared by the Washington Department of Fish and Wildlife
Aquatic Invasive Species Unit
June, 2011

Introduction

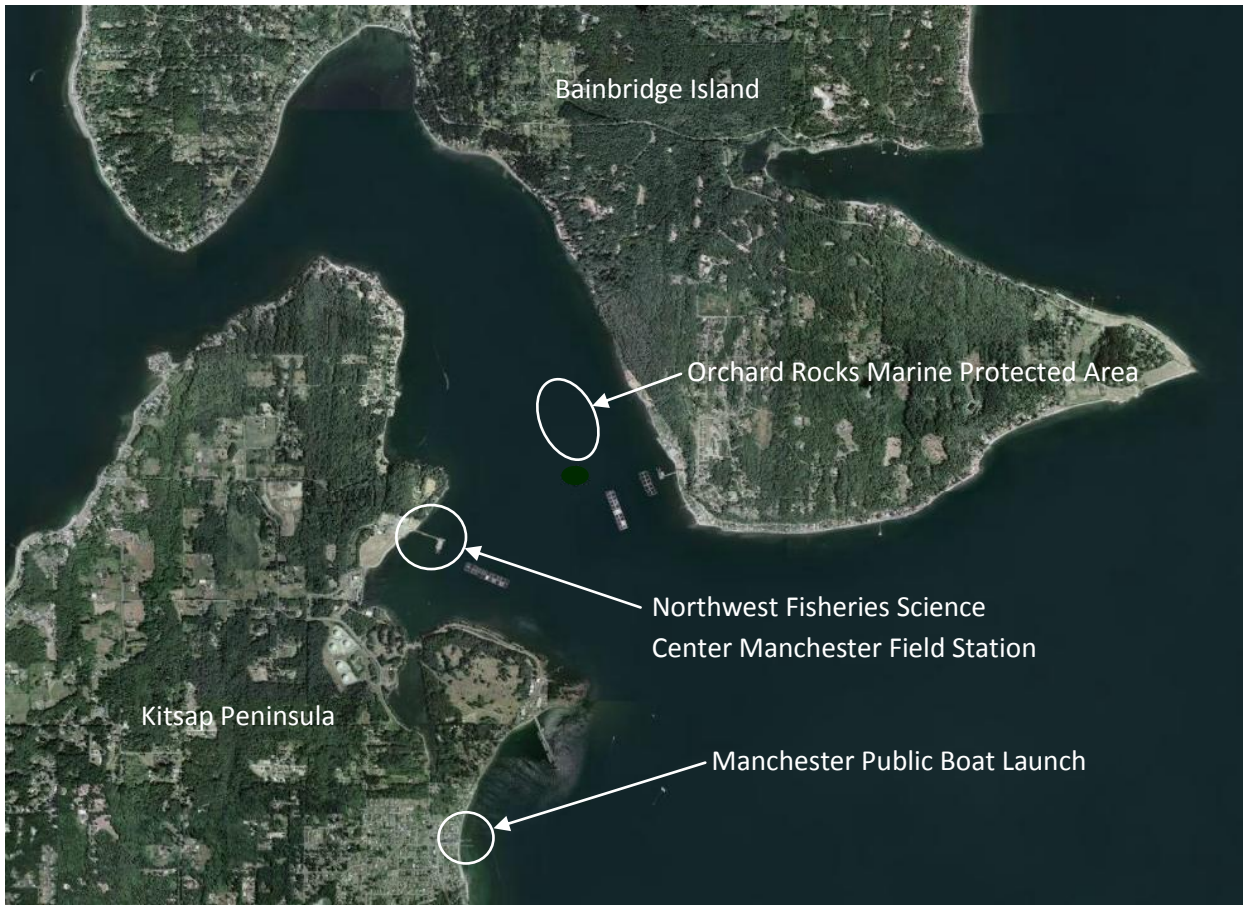
The Puget Sound Partnership (PSP) has contracted with the Washington State Department of Fish and Wildlife (WDFW) Aquatic Invasive Species Unit (AISU) to provide a continued response to the threat of non-native tunicates in Puget Sound and New Zealand mudsnails in Capitol Lake for the 2009 biennium. This report details AISU efforts toward meeting the goals and objectives of the contract.

Tunicate Rapid Response Summary

1) The AISU responded to a citizen report of *Styela clava* growing in abundance on commercial oyster cultch bags located near the mouth of Glencove in south Puget Sound's Carr Inlet. AISU divers conducted surveys of the area that included over 100 oyster cultch bags, derelict dock and barge structures, and nearly 5 square acres of adjacent natural substrate ranging in depth from 0-60 feet (Figure 1). Also, interviews were conducted with the commercial shellfish growers. No *S. clava* were found during the diver surveys and no information attained from the interviews suggested that there had been any presence of *S. clava* in the past.

Figure 1. Tunicate survey location of commercial oyster growing operations in Carr Inlet.

2) The AISU received a report from a commissioner at the Port of Manchester that *Didemnum vexillum* was growing on the Manchester Public Boat Launch dock. Divers from the AISU responded to the report by conducting a thorough survey of the dock and pilings and found no evidence of non-native tunicates. The divers also surveyed NOAA's Northwest Fisheries Science Center docks located approximately 2.5 watercourse miles NW of the boat launch, and the nearby Orchard Rocks Marine Protected Area (MPA) (Figure 2). The Center maintains the largest floating marine net-pen research complex on the West Coast and handles a large volume of boat traffic. Surveys included all of the net pens, all of the pilings supporting the 180 meter long main pier, and the majority of the docks. The Orchard Rocks MPA is an ecological preserve of primarily hard bottom substrate that could provide suitable habitat for non-native



tunicates of concern. No non-native tunicates were seen at either location.

Figure 2. Locations of tunicate survey sites near the Manchester Public Boat Launch.

3) The AISU received a report from the Lummi Nation Shellfish Hatchery that *S. clava* was present on its infrastructure docks which are very near the water intake source used to supply the upland hatchery facility. Staff from the AISU conducted a qualitative survey of the docks and inspected several sites along the approximately 3.5 mile long dike that encloses the adjacent 750

acre sea-pond, including the sea-gates that connect the pond to the surrounding bay and are used to control the water level within the pond. *Styela clava* was present in moderate abundance on the docks but no tunicates were detected within the sea-gates or at any of the inspected locations along the dike (Figure 3). Samples of the tunicates were sent to a local authority to confirm their identity and the results returned positive. Staff consulted with WDFW shellfish hatchery experts and the Lummi Hatchery managers to evaluate the hatchery's water intake filtration system and to ensure that it is adequately equipped to remove tunicate larvae from water that is pumped from

near the infested docks to the upland shellfish nursery facility. Staff remains in consultation with the Lummi Tribe to determine the best possible means of eradication.

Figure 3. a) Aerial view of the 750 acre sea pond impoundment at the Lummi Nation Shellfish Hatchery. b) *Styela clava* growing on a piece of rope suspended from a dock near the hatchery water supply intake screen. c) Photo of dock structure showing location of sea-gates that connect the sea-pond to the outer bay. d) Close up photo of sea-gates.

4) The AISU responded to a citizen report of *S. clava* growing on the hull of a privately owned motor boat at Sandy Point. Sandy Point is a small embayment containing two small marinas of 73 and 30 slips each, and 164 privately owned docks. It is located approximately 1.5 miles NE of the Lummi Hatchery sea-pond. A qualitative survey of the embayment determined that *S. clava* was present in low to moderate abundance on the docks and appeared to be uniformly distributed throughout the embayment (Figure 4). The site was scheduled for the removal of tunicates from all vessel hulls (see below).

Figure 4. a) Aerial view of Sandy Point. b) Relative location of Sandy Point to the Lummi Nation Shellfish Hatchery sea-pond. c) A privately owned vessel and dock, both infested with *Styela clava* at Sandy Point. d) A sample of the *Styela clava* removed from the vessel's hull.

5) The AISU received a call from a REEF diver that *Ciona savignyi* was present on the Dewatto Wall in southern Hood Canal. The AISU dive staff surveyed the area and confirmed that *C. savignyi* was present, though in low abundance, and was encountered most often on cobble and small boulders on either side of the mouth of Little Dewatto Creek (Figure 5). Some *C. savignyi*

was present on the shallowest sections of the Dewatto Wall, but not in numbers as great as those encountered during the ROV survey of 2009; however, due to SCUBA limitations, divers were not able to survey beyond the depth of the shallowest portion of the wall and densities may have been greater along the deeper parts of the wall where the ROV survey was conducted. No action was taken to remove *C. savignyi* at this location as the depths at which they occurred severely limited the amount of working bottom-time using conventional SCUBA.

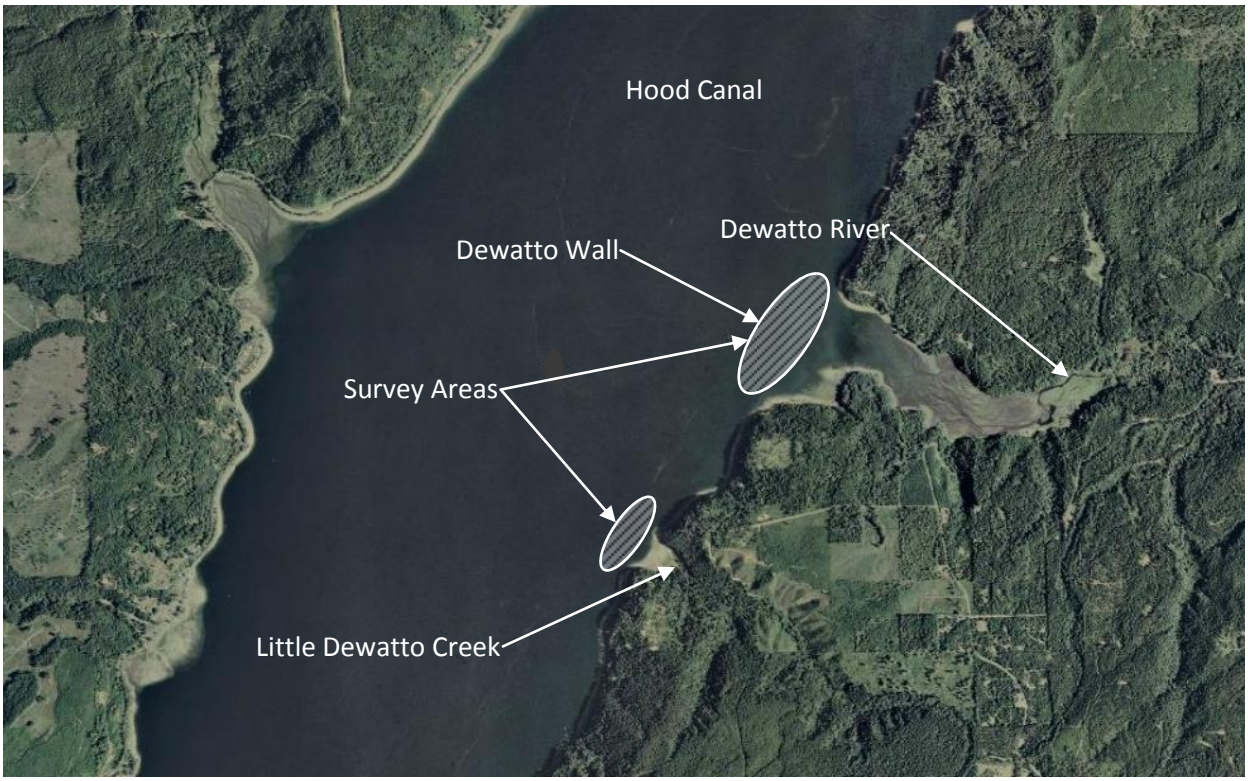


Figure 5. Location of survey areas at, and near the Dewatto Wall in southern Hood Canal.

6) The AISU received a report from a recreational diver that *D. vexillum* was present in abundance on the Shilshole marina boat launch breakwater (Figure 6). Divers were dispatched to the site and confirmed the findings. Due to the close proximity of the breakwater to the public boat launch, the breakwater was deemed a high priority for eradication. The AISU divers removed all visible traces of *D. vexillum* from the breakwater and disposed of the material at an upland facility. A survey was conducted along nearly the entire length of the Marina's outer breakwater and no non-native tunicates were found. A cursory inspection of pilings throughout the marina determined that low to moderate sized colonies of *D. vexillum* was present on many of the surveyed pilings.

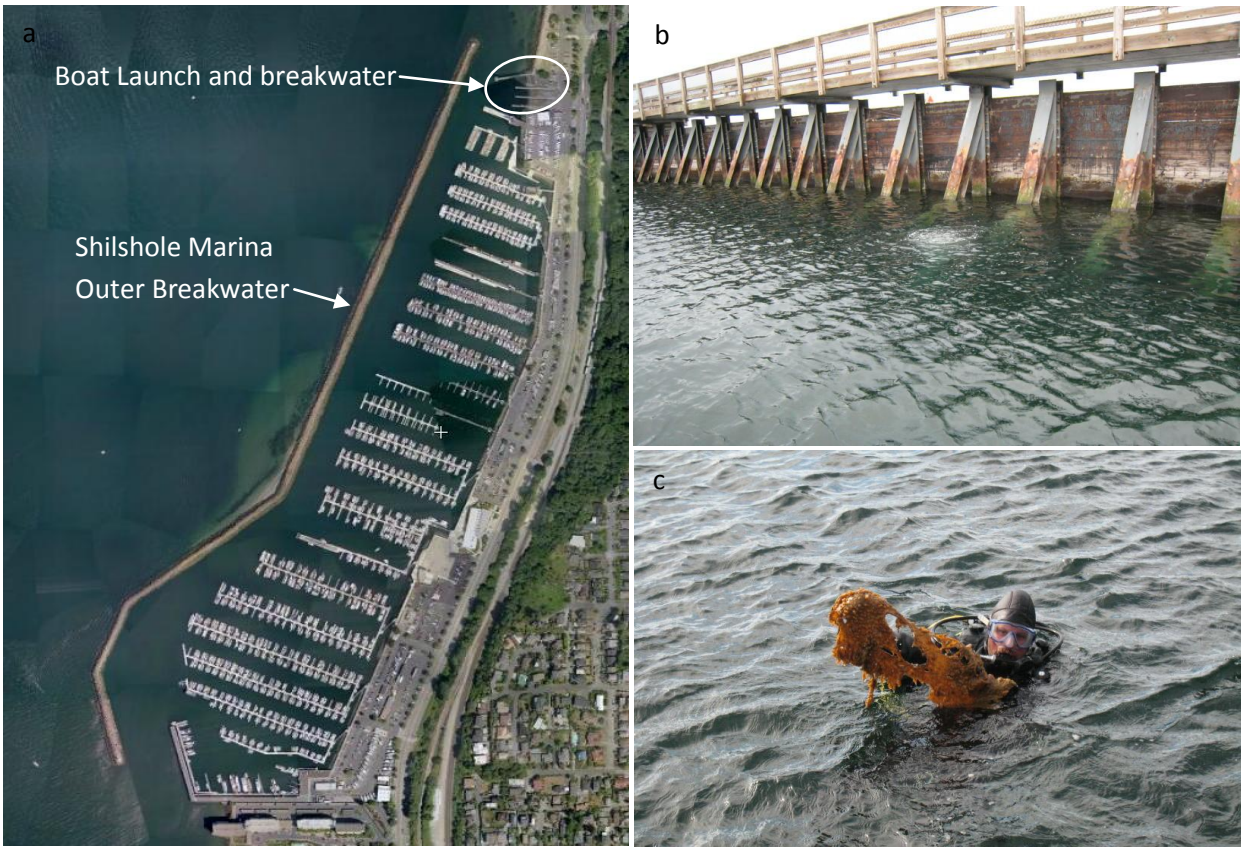


Figure 6. a) Aerial view of Shilshole Marina showing location of the public boat launch and breakwater and the surveyed outer breakwater. b) Boat launch breakwater. c) Example of *Didemnum vexillum* colony removed from the breakwater.

Sinclair Inlet

In the 2009 risk assessment survey (see below), *Ciona intestinalis* ranked among the top three species in an analysis of non-native tunicates posing the greatest risk to Puget Sound, and was given the highest priority for eradication in an analysis of preferred management actions by species. Two confirmed sightings of individual *C. intestinalis* had been previously reported from

Puget Sound (Sinclair Inlet) - one each in 2000 and 2006 (G. Lambert, Personal Communication). A qualitative dive survey of Sinclair Inlet that included five marinas, one natural substrate site (Waterman Wall), and one bridge (Manette) was conducted during the Spring of 2010 (Figure 7).

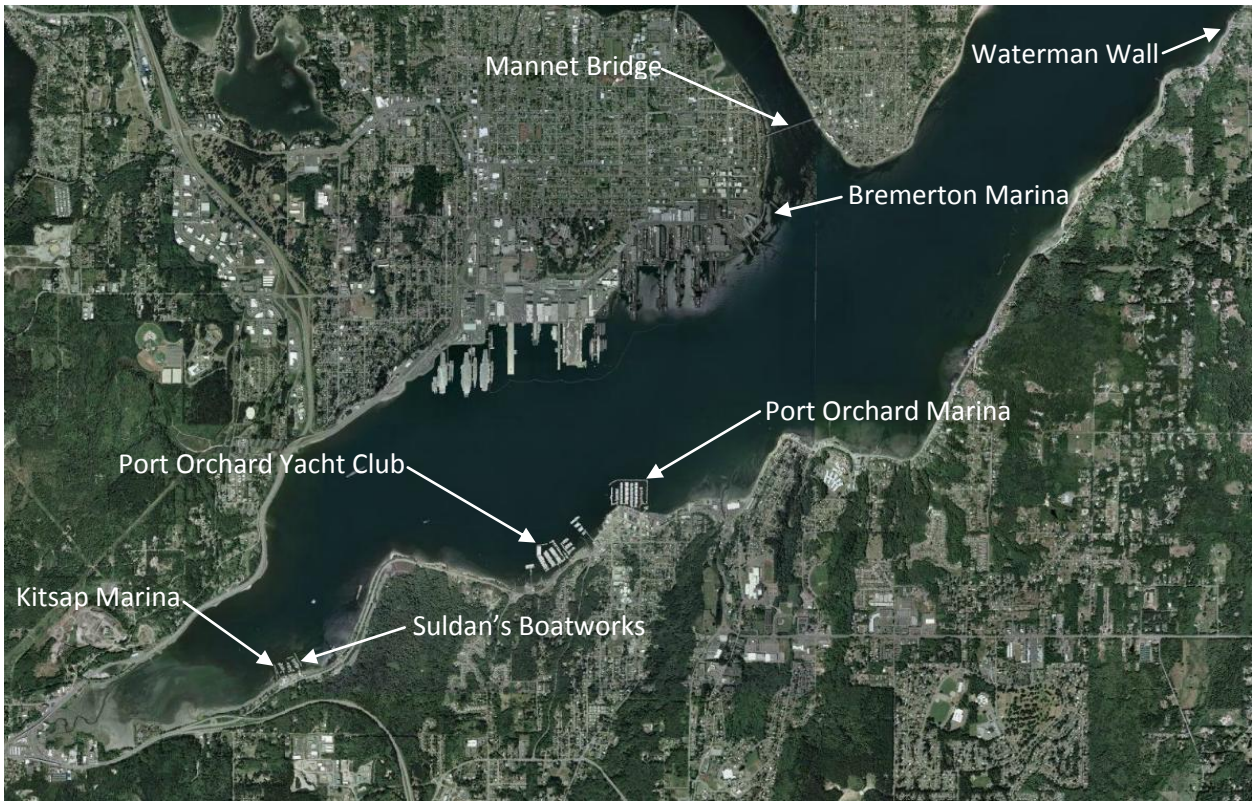


Figure 7. Aerial view of Sinclair Inlet showing survey locations for *Ciona intestinalis* and *C. savignyi*.

No *C. intestinalis* were found at any of the surveyed locations; however, *C. savignyi* was present at all but one of the marinas (Table 1). *Ciona savignya* densities at the infested marinas were subjectively characterized and ranged from low to moderate. Densities were notably higher under covered moorage docks and under the wide expanse of the breakwater dock at Bremerton Marina. The only surveyed marina where *C. savignyi* was not detected was the Suldan's Boatworks. The docks at that facility are buoyed by floating plastic pontoons and were relatively clear of dock-fouling organisms when compared to the other marinas which were constructed of concrete, wood, polystyrene billets, or foam-filled tires. In July, 2010, AIS divers returned to Sinclair Inlet to collect samples from previously identified *C. savignyi* infested sites in support of a University of Washington School of Aquatic Sciences and Fisheries study aimed at quantifying the effects of non-native tunicates on native fauna. No *C. savignyi* were located at any of the sites known from the Spring, 2010 survey to have been previously infested. This observation is consistent with the ephemeral nature of *C. savignyi* outbreaks that have occurred elsewhere in Puget Sound.

Table 1. Sinclair Inlet survey locations showing the presence/absence of four non-native tunicates.

Location	*Ciona savignyi*	*Didemnum vexillum*	*Ciona Intestinalis*	*Styela Clava*
Bremerton Marina	**present**	**present**	absent	absent
Port Orchard Yacht Club	**present**	**present**	absent	absent
Port Orchard Marina	**present**	**Present**	absent	absent
Suldan's Boat Works	absent	absent	absent	absent
Kitsap Marina	**present**	absent	absent	absent
Manette Bridge	absent	absent	absent	absent
Waterman Wall	absent	absent	absent	absent

Although no non-native tunicates were seen on or near the Manette Street bridge supports, a non-native bryozoan (*Watersipora sp.*) was present in abundance on the seabed adjacent to the supports. *Watersipora sp.*, commonly known as lace coral, produces ribbon-forming sheets of rigid mineralized exoskeleton that forms an excellent substrate for other non-native invertebrates including *Didemnum sp.* (Figure 8). It was first recorded on the west coast of the U.S. from southern California in the early 60's and has since expanded its range north, reaching central California in the 80's; Oregon in the 90's; and first reported in Puget Sound in 2008. It is known to be resistant to copper based antifouling paints and is believed to have been spread in the U.S. primarily by hull fouling, thus serving as a potential transport vector for other non-native hull fouling organisms that may not otherwise have been present on vessel hulls.

Figure 8. a) Lace coral, *Watersipora sp*. b) *Watersipora sp*. infested with *Didemnum sp*. from San Fransisco Bay.

Dockton Park

In May of 2008, all *D. vexillum* was removed from the docks and pilings at Dockton Park (see 2007-2009 biannual report). Thorough follow-up surveys of the facility conducted annually since then detected a few very small resurgent colonies. Each year, the colonies were removed where encountered. Annual removals of *D. vexillum* at this location have proved sufficient to prevent any further large-scale outbreaks and the logistics of the follow-up removals are easily manageable and conducted at low cost.

Pleasant Harbor Marina Acetic Acid Test Spray

Owing to an apparent effect on *D. vexillum* of an underwater spray application of 30% acetic acid at Dockton Park in 2008 (see 2007-2009 biannual report), a larger scale application was tested at Pleasant Harbor using both *S. clava* and *D. vexillum*. The application rate for *S. clava* was 2 liters per square meter over three 150 m^2 test sections and the pressure leaving the wand tip was adjusted to the lowest possible setting in order to avoid dislodging tunicates or other animals from the substrate. Densities of *S. clava* were determined in each of the three test sections and in each of three adjacent control sections of the same size prior to the treatment. Post treatment surveys were conducted one day, one week, and two weeks after the application. There were no significant changes in *S. clava* densities in any of the six sections (three treatment and three control) over time and no difference in the relative densities over time between the treatment and control sections. Thus, the underwater sprayed acetic acid treatment does not appear to be an effective means of control or eradication for *S. clava* at the tested application rates.

The spray application method was also tested on pilings that were infested with *D. vexillum* (Figure 9). Due to the mat-forming growth habit of *D. vexillum*, determining density by count, as was employed for *S. clava*, was not feasible. We chose six pilings that were all approximately equally infested with *D. vexillum* as determined by visual estimation of coverage. Three served as controls and three received an acetic acid treatment. Rather than expressing the application rate as liters/m^2, as was used with the *S. clava* experiment, we applied different quantities per unit time to the treated colonies for fixed periods. Three application rates were tested: 5, 10, and 15 L per minute for a period of five minutes per piling. The pilings were inspected one day, one week, and two weeks after the treatment. None of the three pilings showed any signs of mortality after just one day; however, all three pilings showed significant signs of mortality by the end of the first week and there appeared to be more of an effect on the pilings that received the heaviest application rates. Though the colonies that received the two higher application rates were substantially reduced in size by the end of two weeks, there were, nonetheless, still some small remaining patches of living colony. There were no significant changes in colony size on any of the three control pilings during the course of the experiment. Underwater spray

applications of acetic acid have a definite effect on *D. vexillum* survival; however, determining the most effective application rate will require further experimentation. If an application rate that achieves 100% mortality is found, an assessment of whether or not that rate is practical in terms of expense, logistics, and water quality effects will then need to be assessed.

Figure 9. a) Loading a commercial pressure washer onto a landing craft vessel in preparation for acetic acid test spraying. b) Landing craft showing pressure washer, acetic acid container, and spray wand positioned near a test treatment piling. c) Diver about to submerge with diver-held spray wand.

Tunicate Removals from Vessel Hulls

In an attempt to limit the spread of non-native tunicates from heavily infested marinas via vessel traffic, WDFW divers and various dive companies under contract to the AISU, have conducted hand removals from vessel hulls. Annual removals began in 2006 at those marinas previously known to be infested with *S. clava* (Pleasant Harbor, Home Port, Blaine, and Semiahmoo Marinas). Vessel hull removals were also conducted in 2011 at two additional sites that were recently discovered to be infested with *S. clava* (Sandy Point Bay and Birch Bay Marina). Annual removals of *C. savignyi* from Des Moines and Elliot Bay Marinas commenced in 2007 and concluded in 2009. Results from all vessel hull removal efforts, including 2009, 2010, and 2011 are summarized below by location and in Table 3 and Figure 10.

Pleasant Harbor

In May and June of 2006, the AISU contracted with the Skokomish Tribe and with Global Dive and Salvage, Inc. to survey vessel hulls and to remove, by hand, *Styela clava* from those vessels found to be infested at both Pleasant Harbor and Home Port Marinas, and from vessel hulls moored to private residence docks within the confines of Pleasant Harbor. During March and April of 2007, the survey and removal was conducted by WDFW Marine Resources Division divers. Biologists from the AISU completed the survey and removal during April and May 2008 and again in June 2009. During June 2010 and May 2011, the AISU contracted Ballard Diving and Salvage, Inc. to conduct the removals.

Des Moines Marina

In June, 2007, the AISU contracted with Seattle Diving Co. to remove, by hand, *C. savignyi* from the hulls of all vessels present at the marina during that time. Seventeen vessels were so severely infested that the divers determined that hand removal was not practical. These vessels did not appear to have left their moorings for an extended period of time and were designated extensions of the dock. Of the remaining vessels, 44 were infested and 598 individual *C. savignyi* were removed. The total number of vessels surveyed was not recorded. During May and March, 2008 and March and April 2009, the AISU contracted with Ballard Diving and Salvage, Inc. to conduct the hand removal. As in 2007, a small number of vessels were heavily infested and appeared not to have moved over an extended period, though in 2008 and 2009, efforts were made to remove all *C. savignyi* from their hulls.

On May 27, 2008, as a quality control measure, AISU divers identified 25 infested vessels prior to the contract diver's hand removal effort. On June 5, subsequent to the hand removal, the vessels were re-inspected and 11 remained infested. The contractor was notified and, upon review of their records, found that the same diver was assigned to nearly all 11 vessels. The contractor agreed to remove tunicates from all 25 vessels and further agreed to re-inspect and remove any additional tunicates from all other vessels assigned to that diver.

No removals were conducted after 2009 as results from the 2009 risk assessment (see below) placed a lower priority on the control and eradication of *C. savignyi*, and higher priority on *S. clava.*

Elliot Bay Marina

In June, 2007, the AISU contracted with Seattle Diving Co. to remove, by hand, *C. savignyi* from the hulls of all vessels present at the marina during that time. In June, 2008 and April, 2009, Ballard Diving and Salvage, Inc. was contracted to conduct the removals. As with Des Moines, no removals were conducted after 2009 as results from the 2009 risk assessment (see below) placed a lower priority on the control and eradication of *C. savignyi*, and higher priority on *S. clava.*

Blaine and Semihamoo Marinas

The AISU contracted with Natural Resource Consultants to conduct removals of *S. clava* from vessel hulls in Blaine and Semihamoo Marinas during May, 2006. In April and May 2007, Seattle Diving Co. was contracted to conduct the removals, and in June 2008, April 2009, June 2010, and May and June 2011, the contract was awarded to Ballard Diving and Salvage, Inc..

Sandy Point Bay

In November 2010, a citizen reported the presence of *S. clava* on a vessel in Sandy Point Bay (see above). The AISU contracted Ballard Diving and Salvage Inc. to conduct removals of *S. clava* from vessel hulls in Sandy Point Bay during June 2011.

Birch Bay

Because of its close proximity to Sandy Point (see above), Birch Bay was surveyed for S. clava and was found to be infested. The AISU contracted Ballard Diving and Salvage Inc. to conduct removals of *S. clava* from vessel hulls in June 2011.

Table 3. Vessel hull removal data by location and year.

Location/Species removed	Month(s), Year	# vessels surveyed	# vessels infested	# non-native tunicates removed	# non-native tunicates removed/# vessels surveyed	Contract Vendor/Agency
Pleasant Harbor Marina/*Styela clava*	May-June, 2006	169	24	917	5.4	Skokomish Tribe/Global Dive and Salvage, Inc.
	March-April, 2007	124	39	1,420	11.5	Washington Department of Fish and Wildlife
	April-May, 2008	168	48	803	4.8	Washington Department of Fish and Wildlife
	May, 2009	165	42	381	2.3	Washington Department of Fish and Wildlife
	June, 2010	146	29	1,037	7.1	Ballard Diving and Salvage, Inc.
	May, 2011	149	48	5254	35.3	Ballard Diving and Salvage, Inc.
Home Port Marina/ *Styela clava*	June, 2006	72	12	533	7.4	Skokomish Tribe/Global Dive and Salvage, Inc.
	April-May, 2007	52	10	97	1.9	Washington Department of Fish and Wildlife
	July, 2008	74	14	178	2.4	Washington Department of Fish and Wildlife
	May, 2009	65	10	55	0.8	Washington Department of Fish and Wildlife
	June, 2010	59	10	527	8.9	Ballard Diving and Salvage, Inc.
	May, 2011	63	15	530	8.4	Ballard Diving and Salvage, Inc.
Plesant Harbor Private Residence Docks/ *Styela clava*	June, 2006	13	4	0[1]	NA	Skokomish Tribe/Global Dive and Salvage, Inc.
	April-May, 2007	15	6	259	17.3	Washington Department of Fish and Wildlife
	July, 2008	10	1	2	0.2	Washington Department of Fish and Wildlife
	May, 2009	8	1	3	0.4	Washington Department of Fish and Wildlife
Des Moines Marina/ *Ciona savignyi*	June, 2007	NA[2]	44	598	NA	Seattle Diving Co.
	May, 2008	696	107	4,134	5.9	Ballard Diving and Salvage, Inc.
	March-April, 2009	642	142	4,043	6.3	Ballard Diving and Salvage, Inc.
Elliot Bay Marina/ *Ciona savignyi*	June, 2007	NA[2]	65	1,529	NA	Seattle Diving Co.
	June, 2008	1,029	141	5,291	5.1	Ballard Diving and Salvage, Inc.
	April, 2009	1,015	245	26,889	26.5	Ballard Diving and Salvage, Inc.
Blaine marina *Styela clava*	May, 2006	521	93	3,545	6.8	Natural Resources Consultants
	April-May, 2007	505	87	2,329	4.6	Seattle Diving Co.
	June, 2008	507	26	816	1.6	Ballard Diving and Salvage, Inc.
	April, 2009	533	13	41	0.08	Ballard Diving and Salvage, Inc.
	June, 2010	500	16	91	0.2	Ballard Diving and Salvage, Inc.
	May-June, 2011	518	37	1,282	2.5	Ballard Diving and Salvage, Inc.
Semiahmoo Marina/ *Styela clava*	May, 2006	211	17	82	0.4	Natural Resources Consultants
	May, 2007	211	19	281	1.3	Seattle Diving Co.
	June, 2008	195	0	0	0.0	Ballard Diving and Salvage, Inc.
	April, 2009	204	1	1	0.0	Ballard Diving and Salvage, Inc.
	June, 2010	231	3	7	0.0	Ballard Diving and Salvage, Inc.
	June, 2011	249	3	3	0.0	Ballard Diving and Salvage, Inc.
Birch Bay Marina/ *Styela clava*	June, 2011	111	15	160	1.4	Ballard Diving and Salvage, Inc.
Birch Bay Private Residence Docks/ *Styela clava*	June, 2011	15	1	12	0.8	Ballard Diving and Salvage, Inc.
Sandy Point Bay/ *Styela clava*	June, 2011	206	56	3337 [3]	16.2	Ballard Diving and Salvage, Inc.

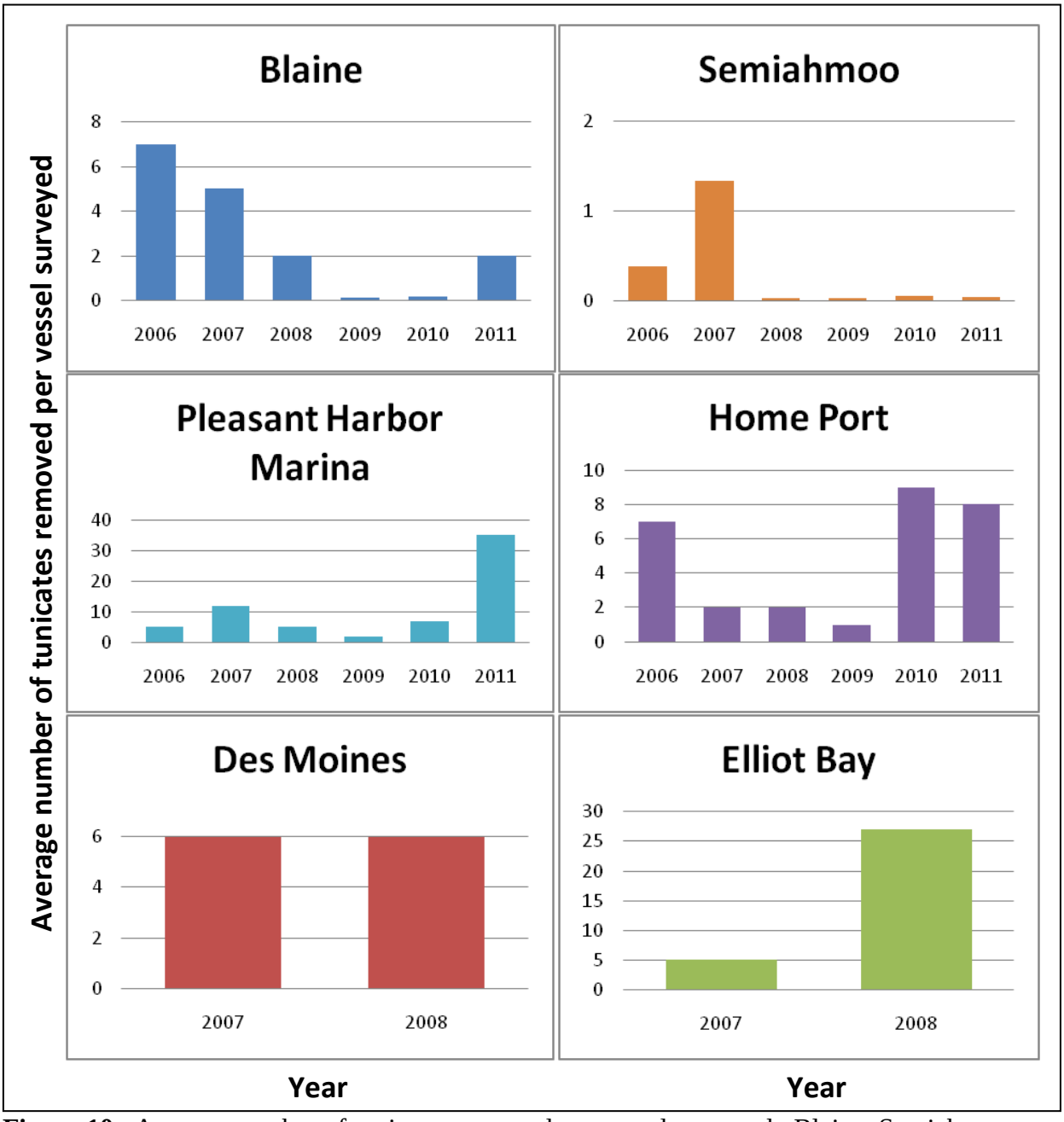


Figure 10. Average number of tunicates removed per vessel surveyed. Blaine, Semiahmoo, Pleasant Harbor Marina, and Home Port = *Styela clava*. Des Moines and Elliot Bay = *Ciona savignyi*.

Presence/Absence Field Surveys

In addition to the locations highlighted in the previous sections, 21 sites were surveyed for presence/absence at least once including five marine protected areas (Sunde Rock, Colvos Passage, Zee's Reef, and Titlow Beach) (Table 2). No non-native tunicates were observed at any of the MPAs. A small patch of *C. savignyi* was found near the base of the Dabob Bay Pinnacle in April of 2010. *Ciona savignyi* was not found at the same location in subsequent surveys conducted in May and June. *Didemnum vexillum* was found at Carlyon Beach and Breakwater Marinas. Otherwise, no non-native tunicates were seen at any of these additional survey sites.

Table 2. Presence/absence survey locations in Puget Sound.

Location	*Ciona savignyi*	*Didemnum vexillum*	*Ciona Intestinalis*	*Styela Clava*
Misery Point	absent	absent	absent	absent
Steamboat Island Wall	absent	absent	absent	absent
Black Point	absent	absent	absent	absent
Pullali Point	absent	absent	absent	absent
Dabob Bay Pinnacle	**Present**	absent	absent	absent
Anna Foss Wreck	absent	absent	absent	absent
Ketron Island (west shore)	absent	absent	absent	absent
Titlow Beach MPA	absent	absent	absent	absent
Point Heyer Artificial Reef	absent	absent	absent	absent
Colvos Passage MPA	absent	absent	absent	absent
Jarrell Cove Marina	absent	absent	absent	absent
Latimer's Landing Boat Launch	absent	absent	absent	absent
Harstene Island Bridge	absent	absent	absent	absent
Carlyon Beach Marina	absent	**Present**	absent	absent
Les Davis Artificial Reef	absent	absent	absent	absent
Saltwater Stae Park	absent	absent	absent	absent
Kopachuch Sate Park	absent	absent	absent	absent
Toliva Shoal	absent	absent	absent	absent
Taylor Bay Wreck	absent	absent	absent	absent
Breakwater Marina	absent	**Present**	absent	absent
Slag Pile	absent	absent	absent	absent

Density Surveys

In 2010, surveys were conducted to test for density changes in *S. clava* at Pleasant Harbor and Home Port Marinas, and *C. savignyi* at Des Moines Marina. The marinas were last surveyed in 2008. Counts were obtained using the same methodology and the same sample sections as were used in 2008 (see 2007-2009 biannual report), thus providing a robust comparison between years. The mean density averaged over all quadrats (40 sections, two quadrats per section) decreased at Des Moines Marina from a mean of 106/m^2 to 61/m^2. *Styela clava* densities at Pleasant Harbor increased from 18 m^2 in 2008 to 25 m^2 in 2010, and at Home Port Marina the density decreased from 3/m^2 in 2008 to 1/m^2 in 2010.

Risk Assessment Survey

During the 2009 6th International Marine Bioinvasion Conference in Portland, Oregon, the AISU and the Puget Sound Partnership surveyed a panel of ascidian experts from throughout the world to assess the relative risks of non-native tunicates in Puget Sound by species and to determine management priorities by species. The panel was convened after the survey to discuss the results. In summary, the panel determined that *D. vexillum*, *S. clava*, and *C. intestinalis* pose the greatest risk to Puget Sound based primarily on perceived and demonstrated threats to ecosystem health, aquaculture industries and wild-stock harvests, and physical infrastructures. *Ciona intestinalis* was identified as the species of highest priority for eradication, and *D. vexillum*, *S. clava*, and *C. savignyi* ranked highest for localized control efforts. Detailed results from the survey are presented in Appendix 1.

Cellulose Nanocrystals

Nanotechnology, though in a nascent stage, is currently one of the most promising arenas of technological development and is projected to have explosive growth in coming years. The most accepted definition is, "The creation of functional materials, devices and systems through control of matter on the nanometer length scale (1-100 nanometers), and exploitation of novel phenomena and properties (physical, chemical, biological) at that length scale". Because of their large surface area-to-volume ratio and aspect ratio, nanocrystals are predicted to have many potential applications in fields including electronics, materials science and medicine. The AISU is collaborating with materials engineers at Oregon State University to extract and refine nanocrystals from the tunic of *D. vexillum*, *S. clava*, and *C. savignyi*. Preliminary findings indicate that high quality crystals can be extracted from both *D. vexillum* and *S. clava* (Figure 11). *Ciona savignyi* remains under investigation.

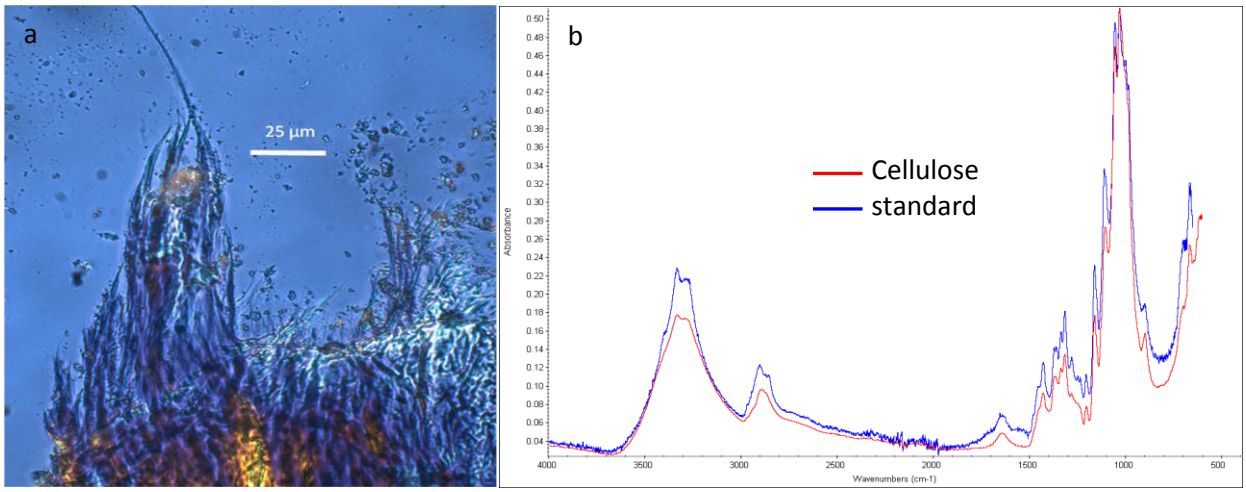


Figure 11. a) Optical microscope image of *Didemnum vexillum* from Shilshole Marina showing the fibrous structure remaining after protein digestion and bleaching. b) Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy comparison of processed *D. vexillum* from Shilshole Marina to a cellulose standard.

Tunicate Management Plan

The existing management plan has been reviewed and revisions are not deemed necessary at this time.

Environmental factors

The AISU has compiled a collection of nearly 100 reports and publications pertaining to limiting environmental factors, modes of distribution, and genetic connectivity of non-native tunicates. Most of these have been scanned and organized by author and year. This library will provide a valuable source of reference materials for any future experiments on non-native tunicates in Puget Sound.

The AISU has provided consultation and field-collected samples to a University of Washington study aimed at quantifying effects of non-native tunicate presence on native micro- and macroinvertebrates.

The AISU constructed six large (1m in diameter), multi-substrate settlement plates intended for deployment at Pleasant Harbor. The plates are designed to test substrate settlement preferences over five materials commonly used in marina dock construction (wood, concrete, polystyrene, rubber tire, and plastic), and oyster shell, which is the primary dock fouling organism at Pleasant Harbor and provides most of the substrate for *S. clava* at that location. The substrates will be retained for possible future deployment.

Geoduck/ *Ciona savignyi* Interactions

The AISU conducted limited searches for suitable study sites in the field to conduct investigations of the effects of *C. savignyi* on geoduck abundance and survival. Specifically, they searched for areas of high *C. savignyi* densities adjacent to areas harboring high numbers of geoduck. The searches were conducted in southern Hood Canal. No suitable study sites were found. The AISU consulted with the University of Washington School of Aquatic and Fisheries Sciences to explore the possibility of conducting laboratory experiments with *C. savignyi* and geoduck. Owing to the requirements of quarantined facilities to handle non-native tunicates in the laboratory and the unavailability of such facilities that are not committed to other ongoing investigations, laboratory investigations cannot be conducted at this time.

Genetic Identification of Tunicate Distribution Pathways

After an extensive review of the literature and consultation with WDFW Genetics Unit staff, a plan was developed to use variation in the cytochrome oxidase I genes for *D. vexillum* and existing primers for microsatellite loci in *S. clava* to trace the historical processes of dispersal in Puget Sound. By investigating the patterns of realized dispersal in Puget Sound and adjacent water bodies, we anticipate that some insight would be gained into identifying the most likely transport vectors, and how best to manage those vectors to limit or curtail further spread and introductions. All work would be carried out at the WDFW Genetics lab.

Web Page and Education and Outreach

Updates to the non-native tunicate distribution maps have been posted on the WDFW Invasive Species web site. Tunicate Response Advisory Committee meetings were held annually in 2009, 2010, and 2011. Posters describing risks posed by non-native tunicates and the need for hull cleaning to limit the risk of spread were posted at 23 marinas throughout Puget Sound. The AISU gave two presentations, one in 2010 and one in 2011, to local dive club chapters highlighting the risks of non-native tunicates and the importance of reporting non-native tunicate sightings.

New Zealand Mudsnails

The AISU has provided consultation with General Administration to design a long-term monitoring program for New Zealand mudsnails (NZMS) in Capitol Lake; and, reported on the effect and efficacy of two NZMS eradication methods employed at Capitol Lake – one for freezing temperatures and one for a controlled saltwater backflush. Results from the two experiments have been detailed in two manuscripts entitled, “A review of salinity tolerances for the New Zealand mudsnail (*Potamopyrgus antipodarum*, Gray 1843) and the effect of a controlled saltwater backflush on their survival in an impounded freshwater lake”, and “A

quantitative evaluation of the effect of freezing temperatures on the survival of New Zealand mudsnails (*Potamopyrgus antipodarum* Gray, 1843), in Olympia Washington's Capitol Lake". The former is under review with the *Journal of Shellfish Research* and was received with favorable feedback from the journal editor and the latter has been published in *Aquatic Invasions*. The manuscripts can be viewed in appendices 2 and 3, respectively, and each provides a thorough accounting of the work conducted, thus no further discussion is provided here.

Appendix 1

Summary of survey results from 15 respondents: WORLDWIDE

Survey Question	C. savignyi	S. clava	D. vexillum	B. violaceus	B. schlosseri	M. manhattensis	C. intestinaltis
1. Physical/geomorphic processes	UNK 62% NO 31%	UNK 62% NO 31%	UNK 54% NO 31%	UNK 62% NO 31%	UNK 62% NO 31%	UNK 62% NO 31%	UNK 54% NO 31%
2. Biological ecosystem processes	UNK 62% YES 31%	YES 85% NO 8%	YES 92% UNK 8%	YES 54% UNK 39%	UNK 46% YES 31%	UNK 85% NO 15%	YES 77% UNK 23%
3. Genetic integrity of native species	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%
4. Endangered, threatened, or other species/ecosystems of governmental concern	UNK 83% YES 8%	UNK 77% YES 15%	UNK 54% YES 46%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 77% NO 23%	UNK 70% YES 23%
5. Aquaculture/ wildstock fishery harvest yields	UNK 62% YES 23%	YES 92% UNK 8%	YES 92% UNK 8%	YES 69% NO 15%	YES 54% NO 23%	UNK 62% NO 31%	YES 85% UNK 15%
6. Physical infrastructure	UNK 50% NO 25%	YES 85% UNK 15%	YES 69% UNK 23%	UNK 46% YES 39%	UNK 46% YES 39%	UNK 62% NO 31%	YES 69% UNK 23%
7. Recreational sector	UNK 62% NO 31%	UNK 46% YES 31%	YES 46% UNK 35%	UNK 62% NO 39%	UNK 62% NO 39%	UNK 62% NO 39%	UNK 54% NO 31%
8. Human health	UNK 69% NO 31%	UNK 62% NO 31%	UNK 69% NO 31%	UNK 69% NO 31%	UNK 69% NO 31%	UNK 69% NO 31%	UNK 62% NO 23%

Cell shading legend: Orange = greater than 50% YES votes; Blue = greater than 25% YES votes; Green = greater than 15 and less than 25 YES votes

1. Greatest risks by category are to: a) aquaculture/wildstock fisheries yields (5 green) ; b) biological ecosystem processes(4 green, 2 blue; and c) physical infrastructure (3 green, 2 blue).
2. Greatest risks by species are: a) D. vexillum (3 green, 2 blue); b) S. clava (3 green, 1 blue), and c) C. intestinatlis (3 green).
3. Least likely risks are: a) physical/geomorphic processes; b) genetic integrity of native species; and c) human health.

Summary of survey results from 15 respondents: PUGET SOUND

Survey Question	C. savignyi	S. clava	D. vexillum	B. violaceus	B. schlosseri	M. manhattensis	C. intestinaltis
1. Physical/geomorphic processes	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 23%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%
2. Biological ecosystem processes	UNK 62% NO 15%	YES 46% UNK 31%	YES 54% UNK 31%	UNK 54% YES 23%	UNK 62% NO 31%	UNK 77% NO 8%	UNK 62% YES 23%
3. Genetic integrity of native species	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%
4. Endangered, threatened, or other species/ecosystems of governmental concern	UNK 75% YES 8%	UNK 69% YES 8%	UNK 62% YES 23%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% NO 15%	UNK 69% YES 8%
5. Aquaculture/ wildstock fishery harvest yields	UNK 69% YES 8%	YES 46% UNK 31%	YES 54% UNK 31%	UNK 46% YES 31%	UNK 46% YES 23%	UNK 62% NO 23%	UNK 54% YES 23%
6. Physical infrastructure	UNK 67% NO 17%	YES 46% UNK 31%	YES 46% UNK 31%	UNK 54% YES 15%	UNK 54% YES 15%	UNK 62% NO 23%	UNK 54% YES 15%
7. Recreational sector	UNK 62% NO 23%	UNK 69% NO 15%	UNK 69% YES 8%	UNK 62% NO 23%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%
8. Human health	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 54% NO 31%	UNK 62% NO 23%

Cell shading legend: Orange = greater than 50% YES votes; Blue = greater than 25% YES votes; Green = greater than 15 and less than 25 YES votes

1. Greatest risks by category are to: a) aquaculture/wildstock fisheries yields (1 green, 2 blue); b) biological ecosystem processes (1 green, 1 blue); and c) physical infrastructure (2 blue).

2. Greatest risks by species are: a) D. vexillum (2 green, 1 blue); b) S. clava (3 blue), and c) C. intestinatlis (1 blue).

3. Least likely risks are: a) physical/geomorphic processes; b) genetic integrity of native species; c) recreational sector, and d) human health.

Summary of survey results from 15 respondents: MANAGEMENT & SPECIES RISK PRIORITIES

Survey Question	C. savignyi	S. clava	D. vexillum	B. violaceus	B. schlosseri	M. manhattensis	C. intestinaltis
Management Priority*	**CON (31%)** RES (31%) UNK (23%)	**CON (46%)** PRE (23%) RES (23%)	**CON (46%)** PRE (15%) ERA (15%) RES (15%)	**RES (46%)** PRE (15%) UNK (23%)	**RES (46%)** PRE (23%) UNK (23%)	**UNK (46%)** RES (31%) NO (15%)	**ERA (39%)** PRE (31%) CON (23%)
Species Risk Priority - **Worldwide**	**3** (33%)	**3** (50%)	**1** (77%)	**4** (46%)	**5** (46%)	**7** (73%)	**3** (39%)
Species Risk Priority - **Puget Sound**	**3** (27%)	**2** (50%)	**1** (75%)	**4** (30%) **5** (30%)	**6** (50%)	**7** (70%)	**2** (25%)

*NO = No Action; PRE = Prevention; CON = Control; ERA = Eradication; RES = Research/Monitoring; UNK = Unknown

1. Greatest management priority actions by species are:
- a. Control actions for: C. savignyi, S. clava, D. vexillum
- b. Research/Monitoring for: B. violaceus and B. schosseri
- c. Eradication for: C.intestinalis

2. Greatest species risks Worldwide are:
- a. D. vexillum
- b. S. clava
- c. C. intestinalis

3. Greatest species risks for Puget Sound are:
- a. D. vexillum
- b. S. clava
- c. C. intestinalis

Appendix 2

A review of salinity tolerances for the New Zealand mudsnail (*Potamopyrgus antipodarum*, Gray 1843) and the effect of a controlled saltwater backflush on their survival in an impounded freshwater lake

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Abstract

The New Zealand mudsnail (NZMS) is an invasive first discovered in Olympia, Washington's Capitol Lake in 2009 and has since been detected in the lake at high densities. In this study, we review salinity tolerances from NZMS investigations conducted in the wild and in the laboratory. Based on the review, we backflushed Capitol Lake with saltwater introduced through a dam that connects the lake to the sea in order to examine the effect of raising the lake's salinity on NZMS survival. We present pre- and post-backflush survival rates from 26 sample stations, eight of which were supplemented with the topical application of salt. Raising the lake's salinity decreased survival and the topical application of salt increased the effect. Sample size and location had a significant effect on survival. We subjected pre- and post-backflush NZMSs to laboratory saltwater trials and used the data to construct a generalized linear model (GLM) to predict survival rates under various salinity exposure regimes. More NZMSs were killed in response to the backflush than was predicted by GLM. Water temperatures in the lake were lower than those used in the laboratory and this may have decreased the NZMS's resistance to increased salinities in the lake.

***Keywords**: salinity tolerance · New Zealand mudsnail · survival rate · generalized linear model.*

Introduction

The New Zealand mudsnail (NZMS) is a hydrobiid mollusk that is native to New Zealand. Its impact to native fauna and ecological processes outside its native range is not well understood (but see Schreiber et al., 2002; Kerans, 2005; Strzelec, 2005; Strzelec et al., 2006; Hall et al., 2006, 2003); however, its expansion over the last century to estuarine and freshwater environments around the world, and its ability to rapidly reach very high densities once established, has led to concerns of disruption to local food web and trophic dynamics, ecosystem functions, and native community structures (Hall et al., 2003). Developing strategies and methods to control and manage NZMS populations in the U.S. is listed as one of the objectives of the National Management and Control Plan for the New Zealand mudsnail (New Zealand Mudsnail Management and Control Plan Working Group, 2007) that was developed under the auspices of the intergovernmental national Aquatic Nuisance Species Task Force to address these and other concerns.

Thus far, NZMS management efforts in their non-native range have focused primarily on controlling their spread by limiting public access to infested water bodies, educating citizens through public awareness campaigns, and developing decontamination methods and protocols for recreationists and natural resource field workers (Richards et al., 2004; Hosea and Finlayson, 2005; Schisler et al., 2008). Large-scale *in situ* eradication has not been attempted (but see McMillin and Trumbo, 2009). In this study, we: 1) examined the effect on NZMS survival of backflushing a freshwater lake (Capitol Lake in Olympia, Washington) with saltwater introduced through an existing engineered tide gate dam that connects the lake to the sea (Puget Sound); 2) subjected pre-backflush and surviving post-backflush NZMSs to a laboratory conducted saltwater trial in order to evaluate the population's post-treatment response to increased salinity; 3) tested the effect of augmenting the saltwater backflush with topically applied rock salt, and; 4) used the data to construct a predictive model for determining the probability of NZMS survival under various salinity exposure regimes that can be used to inform managers who are considering saltwater treatments as an eradication or control measure against non-native NZMS infestations.

Literature Synopsis – Salinity Tolerances for NZMSs

Potamopyrgus antipodarum is variously referred to as *Hydrobia jenkinsi*, *Paludestrina jenkinsi*, and *Potamopyrgus jenkinsi* in the historical literature. All are synonymous and in the interest of clarity, the widely accepted vernacular NZMS will be used throughout this review. A summary of the salinity tolerances reported in this review is presented in Table 1.

The first recorded occurrences of the NZMS outside its native range were from estuarine environments in Western Europe, and only later were they noted from inland freshwater locations (reviewed by Bondesen and Kaiser, 1949; Lassen, 1978; and Hughes, 1996). Nicol (1936) reported finding NZMSs in salinity as high as 23‰ in the brackish-water marshes of

North Uist in Scotland's Outer Hebrides. Winterbourn (1970a) found NZMSs in salinities as high as 26‰ in the species' native New Zealand. Under laboratory conditions, the same author noted that after 24 hours, snails acquired from both fresh- and brackish water sources remained active in 17.5‰, exhibited reduced movement in 21‰, and withdrew completely into their shells and were inactive in higher salinities[1]. All of the snails resumed normal activity within 24 hours of being returned to low salinity water (3.5‰). Johnsen (1946) reported finding a single NZMS on the NE shore of Bornholm, Denmark near the mouth of the Baltic Sea in a 33‰ tide pool. The pool was described as having been part of a once larger pool that had been reduced in size through evaporation. Since the surface-water salinity of the Baltic Sea in the Bornholm Basin rarely exceeds 15‰, it would seem likely that the snail had arrived in the pool at a time when the water was less saline - prior to evaporation. The disposition of the snail at the time of discovery was not noted, though it is presumed to have been living. Thus, it may have been active, having acclimated to higher salinity over the time it took the pool to evaporate, or it may have retracted into its shell and become quiescent in response to rising salinity. Costil et al. (2001) examined the biodiversity of aquatic gastropods across several biotopes in the Mont St-Michel basin of northern France. They encountered NZMS over a wide range of salinities up to 28‰ and, of 59 stations sampled, those stations where NZMSs were found had the highest salinities; however, the authors did not indicate whether or not the snails were active at higher salinities. Gérard et al. (2003) studied the rate of trematode parasitism in relation to salinity and gastropod community structure in the same basin. The NZMS was the only hydrobiid mollusk encountered in the basin's polyhalinic (18-30‰) waters. The rate of infection by trematode parasites decreased with increasing salinity over all species examined and NZMSs were not infected at the highest salinities.

Jacobsen and Forbes (1997) subjected NZMSs sampled from six sites in Denmark to salinities of 0‰ and 10‰ (all six sites) and 5‰ and 15‰ (two of the six sites). The sites represented a mixture of the two most common morphologically distinguishable genetic strains (A and B) occurring in Britain and continental Europe (Hauser et al., 1992; Jacobsen et al., 1996). The A strain is most often associated with inland freshwater lakes and streams, while B is found in coastal estuarine environments. They tested the effect of salinity on four fitness-related traits (reproductive output, feeding rate, growth rate, and size at birth) and compared results between strains. All four traits were influenced by salinity in both strains; however, the authors concluded that NZMSs are able to feed, grow, and reproduce over a salinity range of 0-15‰ and that the general response to salinity of both strains suggested a salinity optimum of ≈5‰. Drown et al. (2011) compared six fitness-related traits (survival probability, probability of reproducing, growth rate, time to asymptotic shell size, time of first reproduction, shell size of first

[1] The New Zealand Mudsnail Management and Control Plan Working Group (2007) appears to have incorrectly ascribed Winterbourn's observation of complete withdrawal in salinities greater than 21 ‰ to Winterbourn (1970b), rather than Winterbourn (1970a).

reproduction, and individual fitness) from ancestral- and invasive-range lineages of NZMSs across a salinity gradient (0, 5, 10, and 15‰). Snails held at non-zero salinities were acclimated by increasing the salinity 5‰ every six hours until the desired treatment salinity was achieved. Snails were held at their respective treatment salinities for up to 230 days. The authors noted that attempts to acclimate snails to 30‰ resulted in high mortalities for some lineages and precluded their use for comparative analyses over all six examined traits, though they observed that only the invasive strains were successful at reproduction in 15‰ and 30‰. They concluded that invasive NZMS lineages are adapted to a higher salinity compared to ancestral lineages.

Consistent with Jacobsen and Forbes (1997), Muss (1963) listed 0-15‰ as being a rough estimate of the range over which NZMSs may be considered common in Denmark. In a later study, the same author noted that NZMSs were common in Kysing (Norsminde) Fjord, Denmark in salinities up to ≈22‰, and occurred sporadically in salinities as high as 24‰ near the seaward entrance to the fjord (Muss, 1967). This is similar to the findings of Siegismund and Hylleberg (1987), who described the distribution of the three most abundant hydrobiids (including NZMS) in the same fjord and assessed the factors leading to their coexistence. They found NZMSs at times and locations when, according to the hydrographic data they present, salinities would have been on the order of 20-22‰. In a different study, the same authors tested the effect of salinity in combination with temperature (Hylleberg and Siegismund, 1987). They reared NZMSs in salinities as high as 30‰ and concluded that NZMS tolerance to near-freezing temperatures decreased rapidly with increased salinity, and that the observation seemed to agree well with the temporal and spatial distribution of NZMSs in Kysing Fjord.

Todd (1964) tested the osmotic balance of strains A and B, and a less common European strain known as type C. Changes in internal osmotic concentration occurred rapidly as the snails were transferred from lower to higher salinities and all three strains maintained hyper-osmotic urine relative to the rearing medium over a range of salinities up to 32‰, the maximum salinity tested. The author also reported that NZMSs survived indefinitely in salinities up to 32‰ if conditioned first to lower salinities, but did not describe how the conditioning was achieved or if the snails were active at higher salinities. Similarly, Duncan (1967) tested the salinity tolerance of NZMS acquired from fresh- and brackish water sources in Poland over salinities ranging from freshwater to full seawater. They found that 100% of the snails tested from both sources survived for at least 24 hours after direct immersion in salinities up to 18‰, but, that the survival rate decreased rapidly with increased salinity above 18‰ to just 10% in full seawater. However, acclimatizing snails for up to two days in 18‰ before transferring them to higher salinities nearly doubled the survival rate. Further, they found that NZMSs acquired from both sources maintained hyper-osmotic haemolymph in the highest salinities tested.

Adam (1942) acclimated NZMSs obtained from a freshwater creek in Belgium by placing batches of 20 snails each in 14 ordinal salinities ranging from 0-24‰. At approximately one

month intervals, the surviving snails from each batch were moved to a slightly higher salinity. Snails that were placed directly in 22‰ or higher at the beginning of the experiment failed to survive the first month. Of the snails that were initially introduced to 20‰, only nine survived the first month; however, those nine survived the next seven monthly transfers up to a salinity of 34‰, after which they all died. Offspring were produced in salinities as high as 28‰, though the number of offspring was notably lower in higher salinities and, consistent with Jacobsen and Forbes (1997), was greatest in salinities less than 16‰. In similarly devised experiments Klekowski and Duncan (1966) measured respiration in juvenile NZMS acquired from a small (11-hectare) freshwater lake near Aberdeen, Scotland, and respiration and heart rate from juvenile NZMSs collected from a brackish-water marsh near Plymouth, England (Duncan and Klekowski, 1967). Some of the freshwater-derived snails survived in salinity as high as 64‰ when acclimated every two days by an increase of 2-3‰, and the authors noted that even at 58‰, a few snails were still capable of searching for food. The snails derived from brackish water were subjected to a more aggressive acclimatization process (8‰ increase every 24 hours) and did not survive beyond 58‰. The authors speculated that observed increases in respiratory rate with increased salinity may be the result of increased osmoregulatory demands. Boycott (1936) stated that, "…freshwater strains may easily be got to live and breed in sea water…", but did not provide any details.

The first recorded discovery of NZMS in North America occurred in 1987 in Idaho's Snake River (Bowler, 1991). Since then, NZMSs have been found in nine additional western states (Gustafson, 2002) and Dybdahl and Kane (2005) suggest that the western North American lineages may be genetically linked to Australia. The western North American and Australian lineages both appear to be genetically dissimilar to the European strains (M. Dybdahl, School of Biological Sciences, Washington State University, personal communication). Davidson et al. (2008) reported occurrences of NZMSs from several low-salinity estuarine locations along the Oregon coast, and from one estuarine location on the west coast of Vancouver Island, British Columbia. New Zealand mudsnails are now well established at many fresh- and brackish water sites throughout the lower Columbia River estuary (reviewed by Bersine et al., 2008). The highest salinity in which NZMSs have been documented in the lower Columbia River (Baker Bay) is 11‰ (Sytsma et al., 2004), and this is the highest salinity from which the NZMS has been recorded along the west coast of North America (T. Davidson, Aquatic Bioinvasion Research and Policy Institute, Portland State University, personal communication). However, salinity in the lower Columbia River fluctuates widely from 0-30‰, and it is likely that NZMSs in the lower-most reaches of the river experience at least intermittent exposures to higher salinities. The NZMS was first reported from Washington State's Capitol Lake in October, 2009 (B. Bartleson, Pacific Northwest Shell Club; E. Johannes, Deixis Consultants, personal communications) and densities of up to 20,000 per m^2 have since been detected (A. Pleus, Aquatic Invasive Species Unit, WDFW, personal communication). Invertebrate surveys of the lake conducted as recently as 2003 (reviewed by Hayes et al., 2008) did not detect the presence

of NZMS. The current infestation, therefore, is likely a recent phenomenon, having reached detectable levels sometime in the last eight years.

The published accounts of NZMS salinity tolerances and occurrences suggest the species is generally restricted to salinities ranging from fresh- to brackish water in the wild. Results from laboratory manipulations, however, indicate they may be acclimated to withstand hyper-saline water, likely due to their ability to osmoregulate over a broad range of environmental conditions, and that their maximum salinity tolerance is limited primarily by temperature and the rate of acclimatization. Based on Capitol Lake's freshwater hydrology and the predicted rate of seawater inflow from Puget Sound, we anticipated that we could rapidly achieve, and maintain for at least 48 hours, lake-water salinities of up to 24‰ in the lake's northern basin. This is close to the maximum salinities reported for most NZMSs collected from the wild elsewhere (Table 1).

Methods

Study area

Capitol Lake is a shallow manmade freshwater lake that is 3 km long, and covers an area of approximately 105 hectares. It was formed in 1951 when a constructed berm and dam enclosed a portion of Puget Sound's southernmost tidal basin (Budd Inlet) and enabled the retention of outflow from two adjoining streams (Deschutes River and Percival Creek) to permanently inundate the tidal flats (Figure 1). Puget Sound is a saltwater estuary fjord of mixed semi-diurnal tides. Salinity in Budd Inlet varies seasonally and is largely dependent on rainfall and input from adjoining rivers and streams, and storm water runoff from the city of Olympia (population ≈46,000). During the course of the backflush, surface salinity measurements taken at a station located near the entrance to Budd Inlet, approximately 14.5 watercourse km north of the dam, ranged from 27.3-28.3‰ (mean 27.8‰) and are typical for the months of February and March (Anonymous 2010). A salinity measurement taken from just seaward of the dam on the afternoon of March 1 during the initial phase of the backflush registered 28.7‰ (Hallock, 2010).

Unless otherwise noted, all depths and elevations in this study are reported relative to the National Geodetic Vertical Datum of 1929 (NGVD29). This datum is the benchmark elevation used by the City of Olympia and forms the basis for US Geodetic Survey quadrangle maps of the area. The mean winter elevation of Capitol Lake is 1.5 m. Mean sea level on the seaward side of the dam is approximately 0.3 m. This experiment took advantage of a spring tidal series that resulted in higher than usual tides in Puget Sound and enabled large volumes of saltwater to be backflushed into Capitol Lake through the dam. During the backflush, astronomically predicted high tides ranged from 2.38-2.74 m. Actual tide heights were not measured and may have differed somewhat from predicted heights due to meteorological effects (Anonymous, 2008). Air temperatures remained above freezing (3°C min., 12°C max.) during all phases of the experiment. Thus, exposure to sub-freezing temperatures during the drawdown phases of the experiment was not a factor affecting survival (Cheng and LeClair, 2011).

Field sampling

The dam is fitted with two steel radial arm gates that open upward so that the exchange or discharge of lake water occurs from beneath them. Freshwater was allowed to drain from the lake during low tides and the lake level elevation was kept lowered for a period of approximately 3 days prior to the backflush. This allowed some time for density re-stratification of the water on the seaward side of the dam to occur and reduced the potential for less dense freshwater to be refluxed into the lake during the backflush. The effect of the pre-backflush drawdown on NZMS survival was assumed to be insignificant due to the NZMS's known ability to survive unsubmerged in damp environments for long periods of time (Winterbourne, 1970a). Cloud cover, high humidity (100% max., 61% min.), low winds, and moderate temperatures prevented the exposed lake bed from drying out and all sampling occurred at locations that remained thoroughly moistened during the drawdown. The first lowering of the lake level commenced on February 26, 2010. The lake was rapidly refilled with saltwater during high tide on March 1 and lowered at the conclusion of the backflush on March 5. On March 6, the gates at the dam were closed and the lake was permitted to refill to the pre-backflush level (Figure 2).

During the time the lake level was lowered but prior to the backflush, fourteen stations were selected along the north shore near the dam where we judged maximum salinity would be achieved during the backflush, due to close proximity to the saltwater source and maximum distance from freshwater flowing into the lake from the two adjoining streams. Half of the stations were located upshore away from the water's edge (elevation > 0.5 m), and half were located near the water's edge (elevation < 0.5 m). Four more stations were selected along the south shore near the 1.5 m mean winter lake level isobath. An additional eight stations, four each at the north and south sample sites, were treated with a topical application of rock salt (Morton® White Crystal Rock Salt) applied at the rate of 1 kg/m^2 over an area of approximately 1 m^2. Each of the 26 stations (Figure 1) was marked with a numbered steel stake and NZMSs were sorted in the field from random substrate samples taken within 1 m of the stake. We aimed for a minimum sample size of 50 NZMSs from each station. At low density stations where it became apparent that the desired minimum sample size could not be achieved, we collected as many NZMSs as could be found in approximately 20 minutes of searching. All snails were immediately transported to a laboratory and examined microscopically. Any shells that did not contain a body were discarded. The remaining snails were examined for signs of movement and, arbitrarily, any snail that could not be induced to move after four hours was deemed dead. The same sampling procedure was repeated at each station immediately after the backflush during the second drawdown.

Salinity measurements, including one near-surface and one near-bottom, were taken at each of ten locations in the northern basin of the lake on March 2, shortly after the initial saltwater backflush phase of the experiment was begun. Near-surface salinities ranged from 7.5-14.2‰, and near-bottom salinities ranged from 12.7-24.9‰. The salinity measurements taken nearest the two NZMS sample sites (≈60 m distant) registered 10.5‰ and 12.4‰ (near-surface), and

24.8‰ and 22.8‰ (near-bottom), at the north and south sample sites, respectively. A weak halocline was evident at a depth below the surface of ≈1.5 m (not corrected to NGVD29) (Hallock, 2010).

Laboratory saltwater trial

In order to construct a predictive model for estimating the probability of NZMS survival under various salinity exposure regimes before and after the backflush, we placed 200 live adult snails collected from near the north sample site three days prior to, and five days after the backflush in each of five separate 15 L containers. The containers were filled respectively with: 1) fresh water from Capitol Lake; 2) brackish water (21‰) from Budd Inlet approximately 200 m north of the dam; 3) brackish water (24‰) produced by blending saltwater from the more saline entrance to Budd Inlet, approximately 11 km north of the dam, with freshwater from Capitol Lake; 4) brackish water (27‰) from the entrance to Budd Inlet, and; 5) saltwater (35‰) produced by mixing Instant Ocean® with fresh water from Capitol Lake. All of the containers were held at room temperature (≈ 25°C) to increase activity and expedite the identification of live snails.

We monitored survival of the snails from each container at timed intervals of 1, 24, 48, and 120 hours. At the prescribed times, all snails were removed from their containers and placed in fresh lake water. Snails that failed to show any signs of movement after four hours in freshwater were judged dead. All live snails were returned to their respective source containers once the number of dead snails had been determined.

Statistical analysis

We used a generalized linear model (GLM) (McCullagh and Nelder, 1989; Cheng and Gallinat, 2004) using the canonical link function for the binomial distribution (logit) to overcome problems associated with different sample sizes among various levels of predictors. For field observations, predictor variables were the sample site location (north vs. south), sample station elevation, sample size, presence or absence of topically applied rock salt, and the status of the experiment (i.e., before vs. after the backflush). For the laboratory saltwater trial, the predictor variables were time (hours), salinity (‰), and the status of the experiment (i.e., before vs. after the backflush). The response variable for both the field and laboratory experiments was the proportion of dead NZMSs. The chosen GLM sub-models for the field and laboratory experiments, using all of the respective predictor variables, were selected by both Akaike Information Criteria (AIC) (Akaike, 1974) and Bayesian Information Criteria (BIC) (Schwarz, 1978). The Student's t-test was used to test the significance of each predictor variable. Fitted values were plotted against observed values in order to compare how well the predictions compared with laboratory observations.

Results

Sample sizes, percent survival, and station elevations from field observations are presented in Table 2. The mean sample size averaged over all 26 stations was 107 (standard deviation = 69). The density of NZMS varied widely among stations, and higher density stations yielded greater sample sizes. We examined the effect of sample size on the modeled proportion of dead NZMSs by adding assumed sample sizes of 50 and 150. The smaller sample size resulted in a 9.9% and 5.7% increase in the proportion of dead NZMS at the north and south sites, respectively; while the larger sample size decreased the proportion by 7.5% and 3.8%, respectively. In order to standardize the effect of sample size, we modeled survival using a sample size of 100. With an assumed sample size of 100, the predicted proportion of dead NZMSs prior to the backflush was ≈0.5% at both sites. After the backflush, the predicted average proportion of dead NZMSs was 22.1% at the north sample site, and 10.2% at the south sample site. The application of topically applied rock salt added 4.3% and 2.4% to the predicted values for the north and south sites, respectively. This implies that topically added rock salt can increase the mortality of NZMSs; however, the relationship between added salt and mortality is non-linear and thus likely to be a function of other factors, as well.

The chosen sub-model for the field observations included the predictor variables sample site location ($P < 0.001$), sample size ($P < 0.0001$), presence or absence of topically applied rock salt ($P = 0.07$), and the status of the experiment ($P < 0.001$); sample station elevation was not included ($P > 0.2$). The fitted GLM results and the observed laboratory data are plotted in Figure 3. While the fitted GLM predicted values agreed well with the observed laboratory saltwater trial data; the predicted proportion of dead NZMSs under various salinity exposure regimes, based on the laboratory data, are slightly out of agreement with the mortality rate observed in the field.

The chosen sub-model for the laboratory saltwater trial used all three predictor variables (time, salinity, and status of the experiment), each of which was highly significant ($P < 0.001$), to predict the proportion of dead NZMSs (Figure 4). When standardized to a sample size of 100, the model indicates that prior to the backflush, at least 27‰ maintained over a period of five days would be necessary to achieve complete eradication. Substantial impacts to survival could be realized at 24‰ or less; however, the exposure time necessary to effect a complete eradication at any practically achievable concentration by backflush alone may be beyond reach in Capitol Lake due to constraints imposed by the lake system's local hydrology.

Results from the laboratory saltwater trial conducted with NZMSs sampled five days after the backflush showed a remarkable difference in salinity tolerance when compared with those NZMSs sampled prior to the backflush. After 120 hours in 27‰, 83% of the NZMSs sampled post-backflush were still alive, compared with just 7% of the snails sampled pre-backflush. While nearly all snails (pre- and post-backflush) were able to survive for 120 hours in 24‰, neither the pre- or post-backflush NZMSs survived the 120 hour immersion in 35‰.

Discussion

Temporarily raising the salinity of Capitol Lake impacted NZMS survival. Although we succeeded in achieving the maximum predicted salinity in the deeper water of the lake's north basin, freshwater input and the concomitant drop in lake-water salinity occurred more rapidly than anticipated. Measured surface salinities did not exceed 15‰ and the maximum achieved salinities recorded in deeper water were not sustained for 48 hours, as predicted. Maintaining the lake-level elevation below flood level during the backflush required that the dam be opened periodically in order to release excess water entering the lake via the two adjoining streams. Since the dam opens upward from the bottom, flood control releases would have consisted of denser (more saline) near-bottom water, and effectively increased the depth of the overriding mass of less saline water. Our study sites were located in the nearshore environment (i.e., shallower water), and probably were not exposed for appreciable lengths of time to the maximum salinities that were recorded at depth. Pumping surface water over the top of the dam, rather than releasing it from beneath, may have reduced the depth extent of the freshwater layer.

More NZMSs were killed in response to the backflush than was predicted by GLM. This may be due to lower temperatures in the lake (≈9°C throughout the course of the backflush) that decreased the NZMS's resistance to increased salinities as noted by Hylleberg and Siegismund (1987) (see Literature Synopsis). The water used for the laboratory saltwater trials was not chilled to the same temperature as the lake. The GLM predicted estimates of survival could therefore be viewed as conservative with saltwater treatments using cooler water. The pre-treatment mortality predicted by GLM (≈0.5%) could have been the result of natural mortality, induced handling effects, or some other factor(s).

The increased salinity tolerance of NZMSs collected from Capitol Lake after the backflush is likely due to acclimatization, and it is noteworthy that several live and actively crawling juvenile snails were observed in the post-backflush, 27‰ laboratory-reared sample after 216 hours (all other trials were terminated at 120 hours). This anecdotal observation agrees well with the findings of Adam (1942) and Drown et al. (2011) (see Literature Synopsis). Given that the pre- and post-backflush samples were taken less than a week apart, we assume that multiple cohorts were not sampled and that adaptability over multiple generations could not have occurred during the sampling period. During the backflush, the lake's salinity was increased gradually and, in light of the circumstances under which snails were able to survive in high salinities according to previous accounts, probably over a sufficient period of time for some snails to have successfully acclimated.

Determining to what extent the surviving NZMS population response was functionally adaptive would require further study. If it was largely adaptive we would expect the resiliency to be persistent with little or no impact to overall fitness. If, on the other hand, it was due primarily to

acclimatization mediated by non- or maladaptive phenotypic plasticity, we might predict that some cost to overall fitness would be incurred, and that the response would be ephemeral. Even if the tolerance were epigentically transmitted, the effect would likely be lost over the course of several generations. Among the concerns to managers responding to the Capitol Lake NZMS infestation is the potential for spread into the low salinity waters of adjacent southern Puget Sound and the threat that NZMSs may pose to the marine ecosystem there, including effects on the distribution and abundance of native littorinid snails. An adaptive NZMS population response to salinity, mediated by genetic variability, would increase the threat.

There are numerous potential transport vectors from Capitol Lake into Puget Sound. In addition to the direct outflow of water from Capitol Lake into Puget Sound through the dam, potential non-anthropogenic transport vectors include fecal deposits left by invertebrate-feeding fishes that pass through the dam. Aarnio and Bonsdorff (1997) studied the resistance to digestion of benthic prey organisms, including snails belonging to the same family as the NZMS, consumed by juvenile flounder (*Platichthys flesus*) in the Baltic Sea. They found that the snails could pass through the gut of juvenile flounder alive. *Platichthys flesus* is closely related to *P. stellatus* (Borsa et al., 1997), which is a common inhabitant of the waters just seaward of the dam and has been found in ichthyofauna surveys of Capitol Lake (Anonymous, 2004). Dean (1904) noted that NZMS can pass through perch (species not indicated) as intact shells, though the author did not indicate whether or not the snails were alive. Yellow perch (*Perca flavescens*) are known to be present in Capitol Lake (Hayes et al., 2008) and other species of perch occur in abundance on the seaward side of the dam. Bersine et al. (2008) documented the occurrence of NZMSs in the diet of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and determined that they could pass through the alimentary canal alive. Capitol Lake is a seasonal migration corridor for both juvenile and adult Chinook salmon on their way to and from Puget Sound. Lassen (1978) speculated that waterfowl may have been an important means of local, if not long range, dispersal of NZMSs in Europe. The snails may get caught in plumage, or adhere to feet and bills (Coates, 1922). There are several species of wading and diving birds that use the nearshore environment of both Capitol Lake and Puget Sound. Mammals, including pets, also pose a transfer risk. Potential human transport vectors include unintentional distribution through the movement of contaminated recreational, construction, and natural resource field sampling equipment.

Also of concern to lake managers is the impact that the backflush may have had on other resident benthic macroinvertebrate fauna. A pilot-scale study conducted by the Washington Department of Ecology using benthic samples acquired pre- and post-backflush showed that while the overall abundance of macroinvertebrates (including NZMSs), and the species diversity decreased after the backflush, the proportion of live NZMSs to the overall benthic macroinvertebrate community increased, and NZMS remained among the top five dominant species. As with NZMSs, the other benthic macroinvertebrates appeared to have sustained a greater impact at those sample stations that received a topical application of rock salt. Owing to the NZMS's high reproductive

potential, a reduction in numbers of resident competitors or predators could result in an increase in NZMS abundance if their ability to re-populate and exploit habitat and food resources outpaces that of other inhabitants (Adams, 2010). The rate of NZMS re-colonization following the backflush warrants further investigation, as does the extent and magnitude of collateral ecologic impacts to other species.

There are many water bodies that are at least partially amenable to controlled saltwater backflushes. For instance, navigation locks that connect inland freshwater lakes and canals to the sea are common and are often equipped with controllable saltwater barrier features designed to prevent excessive intrusion of seawater into freshwater ecosystems. Seagates are sometimes positioned along the perimeters of diked freshwater impoundments and may, under some circumstances, be used to alter the salinity of the contained water. The efficacy of saltwater treatments for controlling NZMS infestations at any location would depend on each system's unique hydrology and the ability of managers to control it. It is clear from the results of this study and previous accounts, that temperature and the rate at which maximum salinities are achieved are important factors in determining the outcome of a saltwater treatment. By incorporating predictions of maximum achievable salinities and durations, our GLM results can be used by managers to make informed decisions about the potential efficacy of eradicating or controlling localized infestations of NZMSs.

Table 1 References to salinity tolerances reported for the New Zealand mudsnail (*Potamopyrgus antipodarum)*.

Reference	Salinity (‰)	Wild	Laboratory
Adam (1942)	34		X
Costil et al. (2001)	28	X	
Drown et al. (2011)	30		X
Duncan (1967)	34		X
Duncan and Klekowski (1967)	58		X
Hylleberg and Siegismund (1987)	30		X
Jacobsen and Forbes (1997)	15		X
Johnsen (1946)	33	X	
Klekowski and Duncan (1966)	64		X
Muss (1963)	15	X	
Muss (1967)	24	X	
Nicol (1936)	23	X	
Siegismund and Hylleberg (1987)	22	X	
Todd (1964)	32		X
Winterbourn (1970a)	26	X	
Winterbourn (1970a)	21		X

Table 2 Sample size (*N*) and pre- and post-backflush percent survival at each of 26 sample stations. Stations S1 – S8 were supplemented with topically applied rock salt.

	Pre-backflush		Post-backflush		Elevation
Station #	*N*	% Live	*N*	% Live	(NGVD29[a])
1	141	100.00	55	69.09	0.74
2	200	100.00	237	89.03	0.79
3	146	100.00	130	45.38	0.79
4	200	100.00	122	72.13	0.28
5	28	100.00	73	90.41	0.91
6	200	100.00	184	97.83	0.91
7	48	100.00	37	48.65	0.36
8	79	100.00	116	69.83	0.36
9	244	98.36	207	96.14	0.89
10	119	100.00	73	79.45	0.74
11	100	100.00	258	99.22	1.47
12	40	97.50	114	88.60	1.75
13	127	100.00	100	100.00	1.75
14	92	97.83	23	78.26	1.17
15	119	100.00	105	94.29	0.23
16	207	99.52	125	92.80	0.23
17	100	100.00	71	76.06	0.28
18	169	100.00	61	70.49	0.38
S1	55	96.36	124	91.94	0.84
S2	72	100.00	133	78.95	0.66
S3	166	98.80	106	67.92	0.64
S4	30	100.00	3	66.67	0.81
S5	45	100.00	74	70.27	0.38
S6	2	100.00	5	80.00	0.41
S7	61	96.72	38	84.21	0.13
S8	2	100.00	17	76.47	0.46

[a] National Geodetic Vertical Datum of 1929.

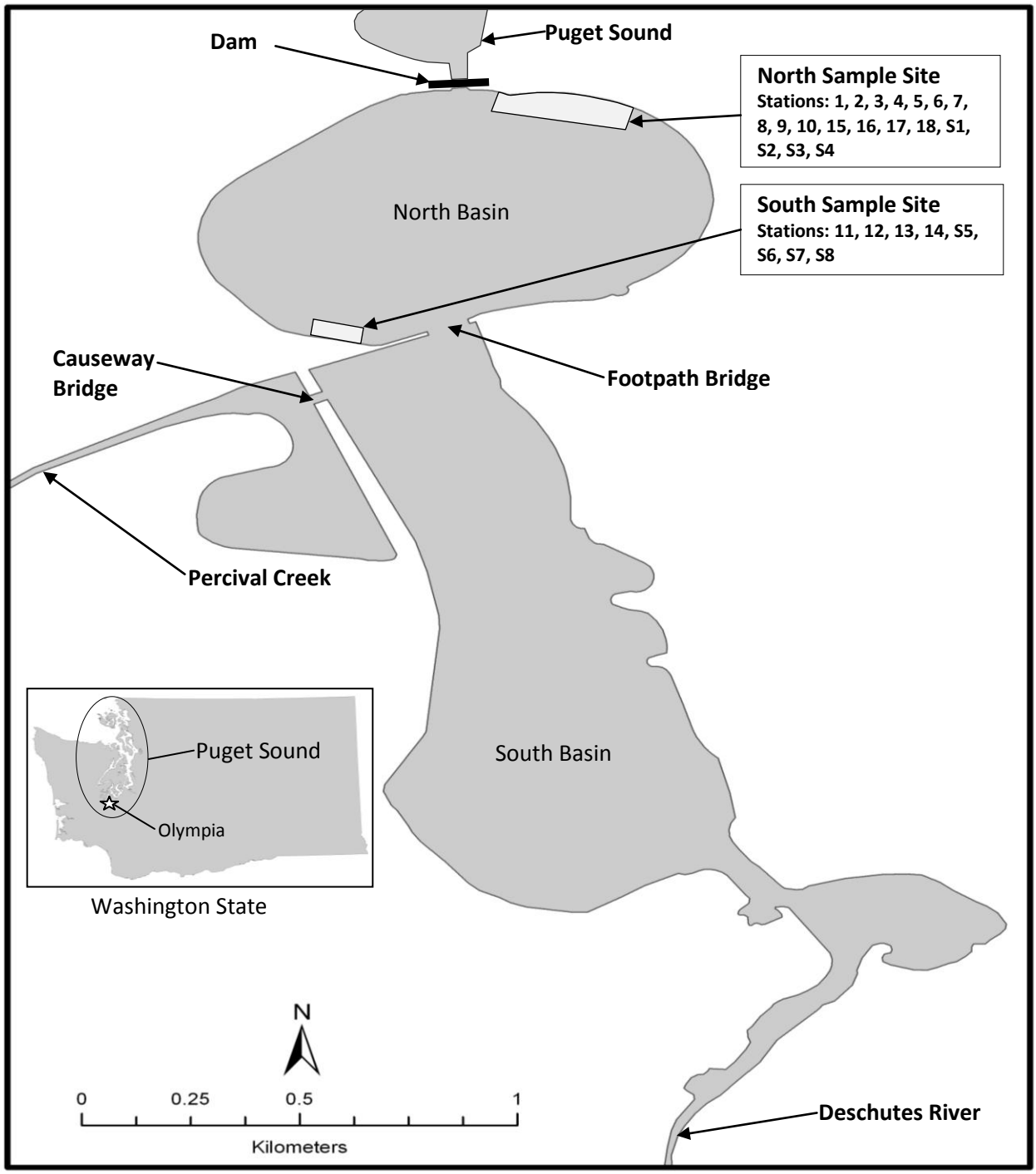

Fig. 1 Map of Washington State showing the location of Puget Sound and Olympia (Capitol Lake), and of Capitol Lake showing the two sample sites used to evaluate the effect of a saltwater backflush on New Zealand mudsnail survival.

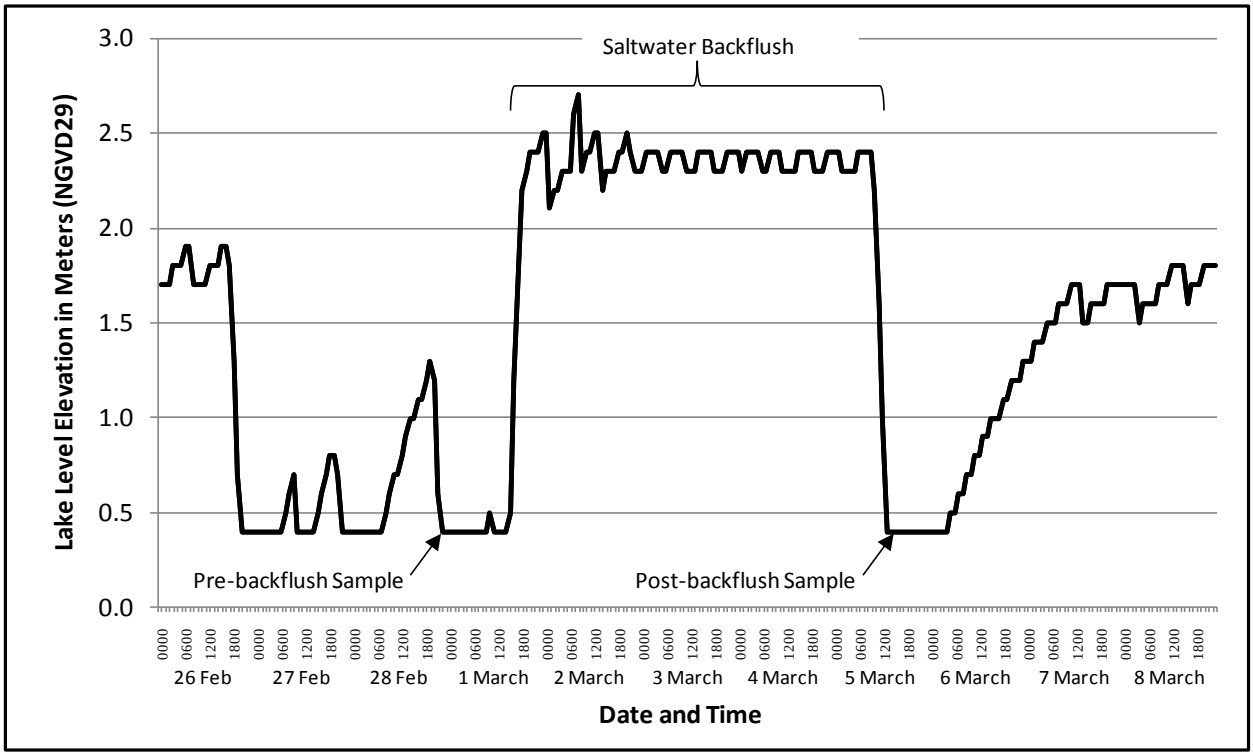

Fig. 2 Lake level elevations before, during, and after the saltwater backflush, and, pre- and post-backflush sample dates and times.

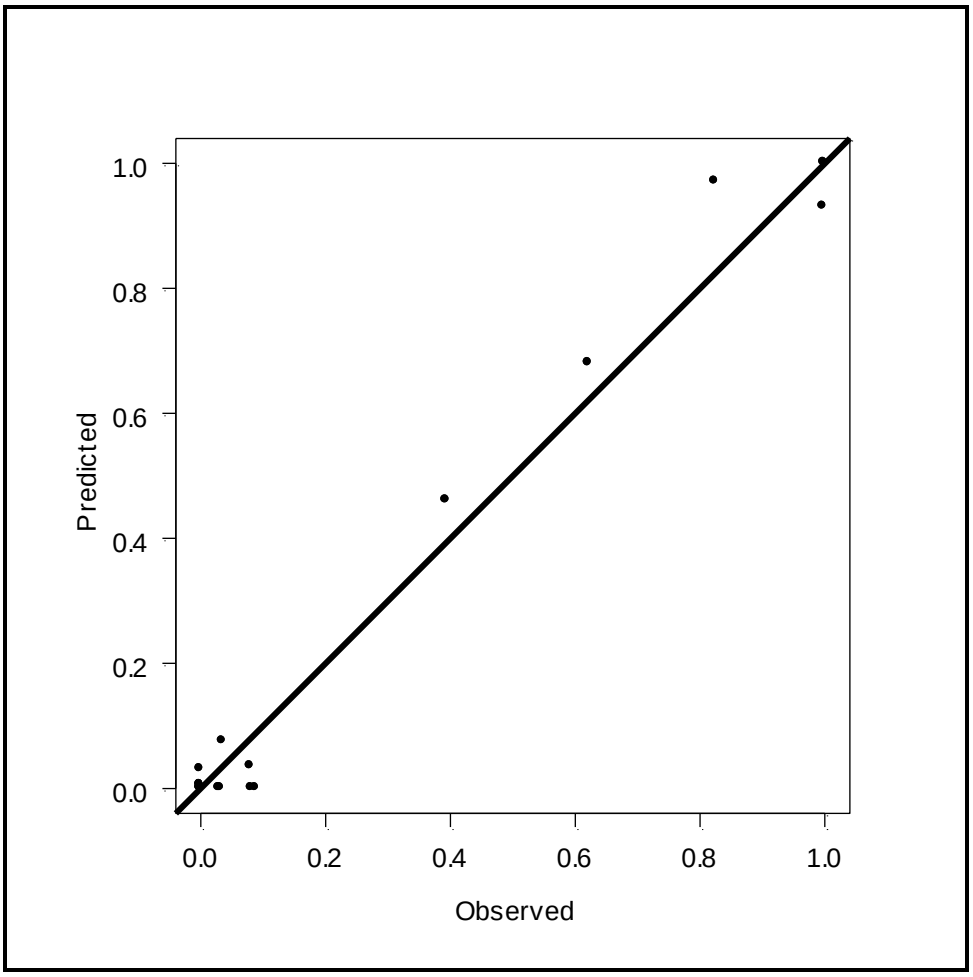

Fig. 3 Fitted GLM predicted and laboratory observed results. Axes represent percent survival.

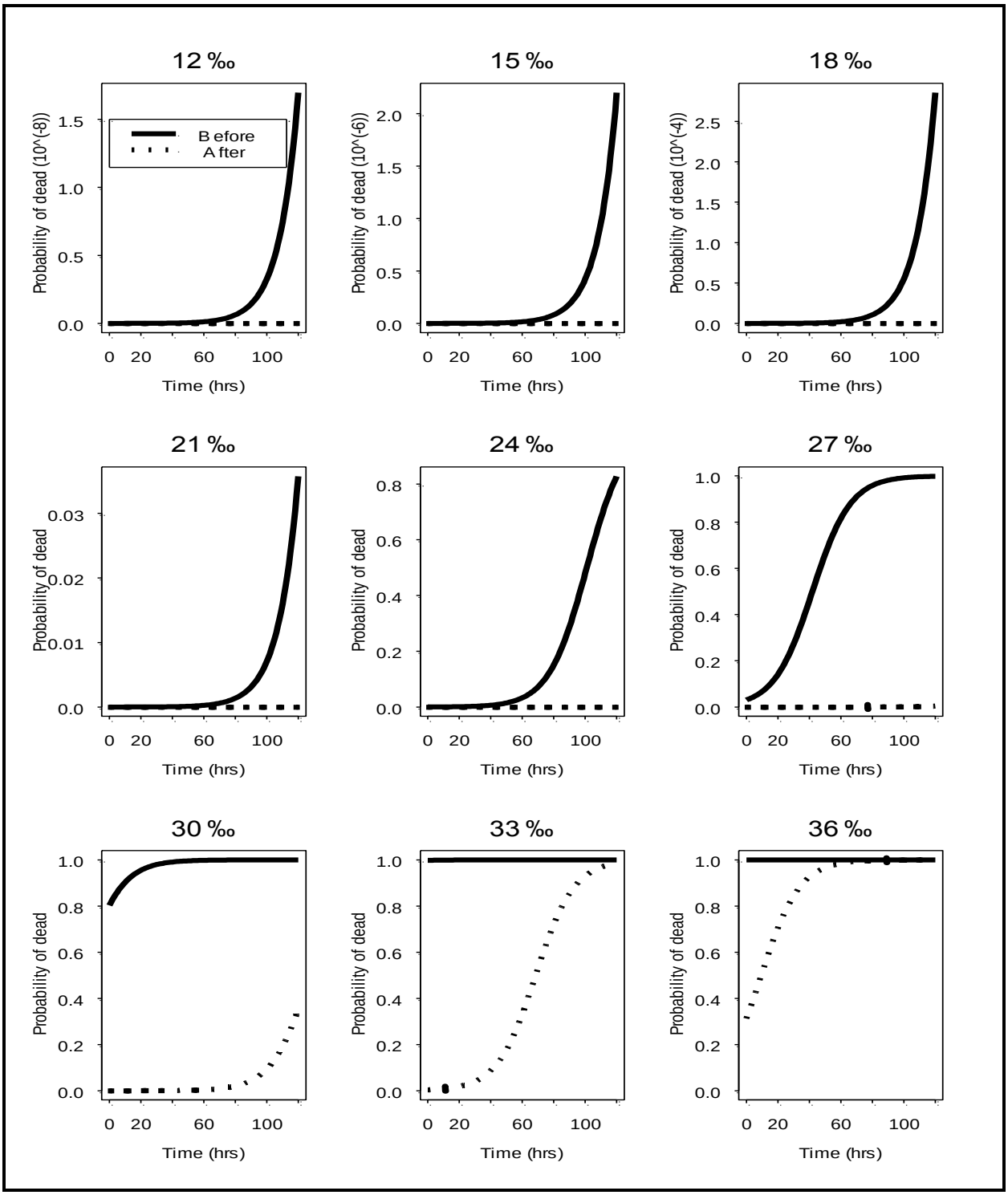

Fig. 4 GLM predicted mortality curves constructed from pre- and post-backflush laboratory saltwater trials. Note that the y-axis scales may differ among salinities.

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Appendix 3

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Open Access

Research Article

A quantitative evaluation of the effect of freezing temperatures on the survival of New Zealand mudsnails (*Potamopyrgus antipodarum* Gray, 1843), in Olympia Washington's Capitol Lake

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Abstract

First detected in the United States in Idaho's Snake River in 1987, the New Zealand mud snail (NZMS), *Potamopyrgus antipodarum*, was discovered in Olympia Washington's Capitol Lake in 2009. The snail is not native to North America and may be capable of adversely impacting native species diversity and food web dynamics in aquatic ecosystems. In this study, we evaluated the effect of lowering the lake level during freezing weather on the survival of NZMSs. Both generalized linear models with link function logit and nonlinear mixed effects models were used to investigate the rates of detection and survival with four temporal and environmental predictor variables. The rate of detection of NZMSs was affected by substrate depth and proximity to shore. The location of sample stations (upshore versus offshore), substrate depth, and elapsed time between collection from the field and laboratory processing did not affect survival rates. The survival rate of NZMSs decreased rapidly with time and the predicted survival rate at the conclusion of the freezing episode was 1.8%. The results indicate that lowering the water level during freezing weather can be a highly effective means for controlling the distribution and abundance of NZMSs and reducing the risk of their spread to other water bodies.

Key words: freezing weather, survival rate, generalized linear models

Introduction

The New Zealand mudsnail (NZMS) (*Potamopyrgus antipodarum* Gray, 1843) is a hydrobiid mollusk that is native to New Zealand, but occurs throughout eastern Australia and Europe where it was formerly known as *Potamopyrgus jenkinsi* Smith, 1889 (Ponder 1988). The first recorded discovery of the species in North America occurred in 1987 in Idaho's Snake River (Bowler 1991). Subsequently, five species of mollusks native to the Snake River drainage were listed under the U.S. Endangered Species Act as either "threatened" or "endangered", in part due to the proliferation of NZMSs (Richards 2002). Since then, the NZMS has been found in nine additional western states (Gustafson et al. 2002), and one western Canadian province (British Columbia) (Davidson et al. 2008), and five Great Lakes states and one eastern Canadian province (Ontario) (Benson 2010). In the absence of co-evolved predators and parasites, NZMSs can multiply to astounding numbers under favorable conditions. For instance, in less than a decade, snail densities have gone from undetectable levels to 229,000 snails per square meter of streambed in some rivers of Yellowstone National Park (Kerans et al. 2005).

Any new biotic component to an aquatic ecosystem, including non-native species such as the NZMS, must necessarily carve an ecological niche in order to survive. In doing so, the structure (i.e., species diversity) and function (i.e., energy flow) of the native food web may be disrupted. The non-native NZMS competes with native invertebrates, including native mollusks, for space and food resources. Because of their high reproductive potential, NZMSs can constitute up to 80% of the invertebrate biomass and consume more than 75% of the gross primary production (Hall et al. 2003). Thus, they have the potential to control the energy dynamics and nutrient cycling in an aquatic ecosystem. Adverse impacts to lower levels of the food web may have implications for organisms at higher

trophic levels, such as fish, which rely on lower-level organisms as a food source. The presence of NZMSs may reduce the availability of native invertebrate prey for fish such as salmonids and sculpins and at the same time, do not constitute a viable food source themselves. Their hard shell and resistance to digestion allow them to pass through fish without lending any nutritional value or caloric input to the consumer (Ryan 1982; Haynes et al. 1985; Aarnio and Bonsdorff 1997; Vinson and Baker 2008; Bruce et al. 2009). Bersine et al. (2008) documented the first occurrence of NZMSs in the diet of juvenile Chinook salmon (*Oncorhynchus tshawytscha* Walbaum, 1792). Seasonal migrations of both juvenile and adult Chinook salmon occur in Capitol Lake. The presence of NZMSs in the guts of fish, including salmon, may be an early indicator that an aquatic food web's dynamics are undergoing change due to the presence of NZMSs. Interestingly; few studies have investigated the negative impacts on entire fish populations in areas of high NZMS densities. While fish can still swim to un-invaded reaches to seek food, many biologists feel that it is only a matter of time until the NZMS spreads far enough within invaded streams to begin having a negative impact on fish growth. In general, it often takes decades for the impacts of a non-native species on native biota to fully manifest. Both positive and negative relationships between densities of native benthic invertebrates and NZMS have been observed (Cada 2004; Kerans et al. 2005; Schreiber et al. 2002).

The biology of the NZMS has been described in detail elsewhere (see Zaranko et al. 1997 and references therein) and only those attributes that are particularly relevant to the design and outcome of this study will be summarized here. In brief, they are tiny aquatic snails that reach a maximum length of about 6mm in their non-native range, but may attain twice that length in New Zealand. They have an annual life cycle and, age at maturity ranges from about three to nine months (Anonymous 2007). In New Zealand, females may reproduce either sexually or parthenogenically; however, in North America, known populations of NZMSs are composed almost entirely of parthenogenic females, therefore, colonization may occur from the introduction of a single female. In the western U.S., reproduction occurs in the spring and summer. Females brood embryos in a specialized brood pouch and release from 20 to 120 free crawling juveniles and may produce up to 230 offspring per year. Though optimal salinity for growth and reproduction is between 0 and 5 psu, they are considered euryhalinic and are capable of withstanding salinities up to 35 psu for short periods of time, and may thrive and reproduce at salinities as high as 15 psu (Jacobsen and Forbes 1997; Alonso and Camargo 2004). They tolerate a broad range of temperatures above freezing up to 34°C, but are not capable of surviving in temperatures at or below freezing (Zaranko et al. 1997; Cox and Rutherford 2000). Although they are resistant to desiccation and can survive up to 24 hours without water, and for weeks on damp surfaces, they are not tolerant of high temperatures.

The NZMS was first reported from Washington's Capitol Lake in October, 2009 (B. Bartleson and E. Johannes, personal communication). Shortly afterward, a rapid qualitative synoptic survey of the lake's nearshore environment and adjoining streams was conducted. The survey indicated a patchy distribution of snails throughout the lake with some areas of very high density, and, that the adjoining waterways were not infested. Capitol Lake is a shallow manmade lake that is 3 km long, and covers an area of approximately 105 hectares (Figure 1). It was formed in 1951 when a constructed berm and dam enclosed the southernmost tidal basin of Puget Sound and enabled the retention of outflow from two adjoining streams (Deschutes River and Percival Creek) to permanently inundate the tidal flats. The lake was originally conceived as an aesthetic accompaniment to the landscape and architecture of the State Capitol building. In recent years, the disposition of the lake has fallen under intense public scrutiny and debate, hinging on whether or not to return the lake, either partially or entirely, to a tidal estuary. The lake is managed by the Washington State Department of General Administration, which controls the water level by opening or closing spillways at the foot of the lake. As a first measure to stem the threat of transfer of the NZMS to other nearby water bodies, the lake was closed to public access in November, 2009.

The objectives of this study were to investigate the effect of freezing temperature on the mortality of NZMSs in situ and identify variation in the change of the density of NZMSs with substrate depth. During a seven day period beginning December 7, 2009, daily low

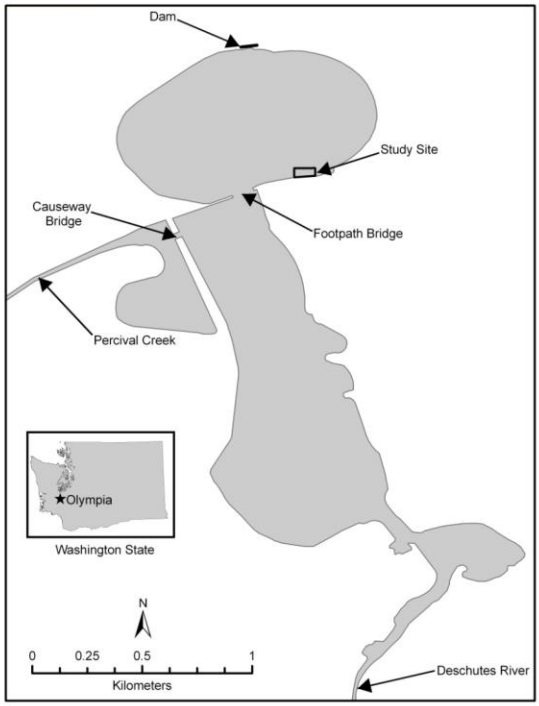

Figure 1. Map of Washington State showing the location of Olympia and Capitol Lake, and map of Capitol Lake.

temperatures in Olympia ranged from -8°C to -14°C and did not exceed 2°C. A rapid partial lowering of the lake level during this period enabled us to test the effect of freezing temperatures on nearshore NZMS survival and to evaluate the potential use of full lake draw-downs to control NZMSs in Capitol Lake. Specifically, we tested the following four hypotheses:

H1: The rate of detection of NZMSs varies with substrate depth from the surface to 70 cm;

H_2: The survival rate of NZMSs decreases with time under freezing temperatures;

H_3: The survival rate of NZMSs varies within sites and by proximity to shore; and

H_4: The survival rate of NZMSs varies among sample stations.

Methods

Field and laboratory

Lake draw-down commenced at 1500 on December 9. The lake level was lowered by 0.75 m below its normal winter level at a rate of 0.5 m per hour. At the time of the draw-down, the lake surface was frozen to a depth of approximately 7 cm, leaving the newly exposed shore blanketed in a layer of slab ice. Four transects running perpendicular to shore and spaced 10 m apart were established in an area known to harbor high densities of NZMSs. Two sample stations were positioned along each transect, one upshore at the pre draw-down water's edge, and one offshore located 1.5 m shoreward of the post draw-down water's edge. Approximately 4 sq. m of slab ice was cleared

from each sample station in order to fully expose the substrate and eliminate any possible insulating effect the ice may have provided against freezing temperatures. Field sampling commenced 18 hours after the completion of the draw-down. At each station, approximately 250 cm^3 of substrate was sampled from each of three substrate depth strata (surface, 30cm, and 70 cm) on three separate occasions (December 10, 11, and 14), except that a 70 cm sample was not taken on December 14. The temporal replicates from each station were excavated from immediately adjacent, but non-overlapping holes. The substrate samples were placed in plastic bags and transported to a laboratory for processing.

In the laboratory, each substrate sample was worked through a 0.425 mm stainless steel sieve in order to facilitate visual detection of the snails. Using forceps, each snail was carefully removed from the remaining substrate and placed in a petri dish filled with lake water, which was at room temperature. The snails were allowed to sit undisturbed in the petri dish for approximately 5 minutes before being examined under a dissecting microscope to determine if they were alive or dead. Snails were considered live if they emerged from their shells or if the operculum of non-emergent snails showed signs of movement when disturbed. Dead snails were further subcategorized as "recent dead" if one or more of the following conditions were met:

1) The periostracum appeared to be intact;
2) The operculum was present; or,
3) The body was present.

They were considered "long dead" if the periostracum, operculum, and body were all absent. Snails that were considered long dead were not included in the statistical analysis. All laboratory processing took place within 24-32 hours after collection from the field. Snail counts ranged from 0-55 individuals per substrate sample core.

Determining the minimum number of replicates

We used 5% and 20% probabilities for Type I (α) and Type II (β) errors, respectively, where $1-\beta$ is the power of the test. We assumed, conservatively, that the mean survival rate at days 0 and 4 were 0.5 (μ_{max}) and 0.05 (μ_{min}), respectively. Judging from a cursory visual exam of snail abundance at the study site, we deduced that at least 15 (m) NZMSs would be attainable from the surface at each sampling station. The minimum effective number of replicates (n) was determined by $\alpha, \beta, d,$ and k, where $d = (\mu_{max} - \mu_{min})/\sigma$, k is the number of treatments, and σ is the common population standard deviation estimated from a subsample of 15 (m) (Searcy-Bernal 1994). Each temporal sample (days 0, 1, and 4) is expected to affect the survival rate; therefore the number of treatments (k) is equal to 3. An estimation of the population standard deviation ($\hat{\sigma}$) can be produced by $\sqrt{2\bar{p}\bar{q}/m}$, where $\bar{p} = \frac{\mu_{max}+\mu_{min}}{2}$, and $\bar{q} = 1 - \bar{p}$ (Fleiss et al. 2003: pp 69-70). The effective size index (f) is equal to $d\sqrt{0.5/k}$ = 1.13. With known values of f, k, α, and $1-\beta$, we determine from Table A.2 of Searcy-Bernal (1994) that $n = 4$.

Statistical analysis

Analysis of variance of arcsine transformed data is a commonly used approach when the response variable from a Bernoulli trial and the number of trials are equal among treatments and replicates; however, the number of trials among treatments and replicates are often not equal. If there is a difference among the various levels of the predictor variables (treatments), additional tests are needed. A generalized linear model (GLM) (McCullagh and Nelder 1989; Cheng and Gallinat 2004) using the canonical link function for the binomial distribution (logit) can be used to overcome problems associated with different sample sizes among the various levels of predictors. The differences among all treatment levels when the response variable is from a Bernoulli trial can be tested with systematic changes of the control treatment in GLM. In addition, GLM can overcome the possible prediction of negative values produced by multiple regression tests.

The response variables are rate of detection and rate of survival for NZMSs. They are both Bernoullian with outcomes of either live or dead. The predictor variables are substrate depth (0 to 70 cm), station location (upshore and offshore), days (0, 1, and 4), and, elapsed time between field collection and laboratory processing (24 - 32 hrs). There are four replicates in the experiment.

The chosen GLM submodel was selected by both Akaike Information Criteria (AIC) (Akaike 1974) and Bayesian Information Criteria (BIC) (Schwarz, 1978), and was investigated with nonlinear mixed effects models (NLME) (Pinheiro 1994; Pinheiro and Bates 2000). NLME models compare individual curves and

group survival curves rather than group observations, as occurs with repeated measures analysis of variance (ANOVA) (Cheng and Kuk 2002; Purcell and Cheng 2010). The model accounts for the nonlinear survival rates, fixed effects from experimental factors and, random effects (Laird and Wares 1982) due to natural variation among sites. The likelihood ratio test was used to assess the significance of natural variation among sites. The *glm*() and *nlme()* functions in SPlus 2000 (Insightful Corporation) were used for the analyses.

Results

Rate of detection

The final submodel selected by both AIC (154.17) and BIC (161.66) was with the predictor variables substrate depth (estimated coefficient = 2.23, P<0.001, Student's t-test), station location (upshore versus offshore) (estimated coefficient = -0.89, P<0.01, Student's t-test) and intercept (estimated coefficient = 2.23, P<0.001, Students t-test) from the GLM fitted results (residual deviance = 41.23485, df = 45). The predicted mean rates of detection for upshore and offshore stations by substrate depth (0 to 70 cm) are plotted in Figure 2. The rate of detection was not affected by time or station location (P >0.5, Student's t-test). The estimated rates of detection of NZMS in upshore and offshore surface samples were 75% and 94%, respectively. The difference between upshore (67%) and offshore (27%) detection rates increased at a substrate depth of 30 cm. At 70 cm substrate depth, the estimated rates of detection up- and offshore were 5% and 21%, respectively.

Survival rate

The final GLM fitted submodel selected by both AIC (92.18) and BIC (95.23) is with the predictor variable day (estimated coefficient = -0.95, P<0.01, Students t-test) without intercept. The location of the sample stations (upshore and offshore), substrate depth, and elapsed time between collection from the field and laboratory processing did not affect the survival rates. Both the observed and predicted survival rates were plotted against time in Figure 3. On day 0, the predicted survival rate was 50% (s.e. = 0.08). On day 4 the survival dropped to 1.8% (s.e = 0.01). By day 4, the variation in survival rate was minimal.

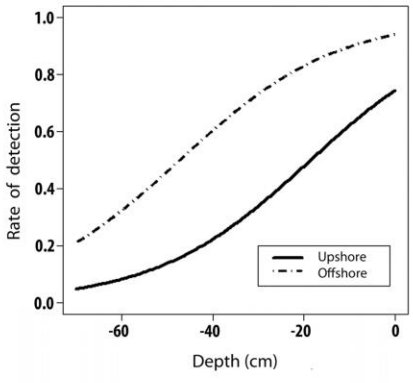

Figure 2. Plot of the expected rate of detection of New Zealand mudsnails against substrate depth at up- and offshore sample stations.

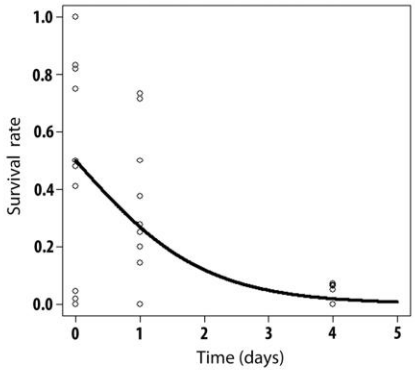

Figure 3. Plot of the expected survival rate of New Zealand mudsnails against time. The dots are raw data.

We modeled individual sample station variation with NLME on the selected submodel. We refitted the final submodel with non-linear regression (NLR) and NLME with the response variables equal to 1/(1+exp(-b*time)), where b is the unknown to be determined in NLR and random effects to be determined in NLME. The estimated coefficient of time from GLM, NLR, and NLME were -0.95 (s.e. = 0.42), -1.00 (s.e. = 0.38) and -1.00 (s.e. = 0.38), respectively. The

difference in results between GLM and the other two models may be due to the estimation methods used (e.g. fisher scoring, least square method), and the assumption of errors (e.g. non-normal distribution, normal distribution). In general, all three of them are very close. The estimated standard deviation of random effects was 0.00083. The random effects were not significant (P = 0.92, likelihood ratio test). The NLR fit was the preferred model by both AIC and BIC. Since all of the sample stations were located in close proximity to one another, we conclude that the variation within each sample station did not affect the survival rate over time.

Discussion

There were notable spatial differences in detection rate between the upshore and offshore stations. The detection rate decreased consistently with increased substrate depth both upshore and offshore; however, detection rates differed less consistently between upshore and offshore at the various substrate depths. At the surface and at 70 cm, the rate of detection was considerably higher offshore, near the water's edge, while at 30cm, the detection rate was much higher upshore. The higher offshore detection rate at the surface might be due to a hydrotactic response in order to escape the freezing temperature, differences between the upshore and offshore environments, or sampling error. Similarly, environmental factors (e.g. substrate type, wind exposure, shade, etc.) may account for the high variability in survival between day 0 and day 4, though efforts were made to ensure that the sample stations were similar in substrate type and atmospheric exposure.

NZMS density in one nearshore area of Capital Lake was estimated to be 20,000 per square meter, and may be higher in other areas (A. Pleus, personal communication). This suggests that NZMSs have been living in Capitol Lake for several years and that conditions are favorable for their proliferation. Judging from colonization events elsewhere, and, given their capacity for active and passive dispersal, their rate of reproduction, and the lack of predators and parasites in introduced populations relative to those found in New Zealand (Anonymous 2007), it seems likely that NZMSs will continue to spread throughout the lake and that their densities will increase if no action is taken to control them. Further, the likelihood that they will be transported to neighboring lakes and streams will also increase.

In Olympia, it is common to have 3 to 7 consecutive days with daily high temperatures of about 0°C between the months of December and February. In this study, we demonstrated that lowering the water level during a prolonged freeze can kill as many as 98% of the snails exposed to freezing temperatures. Assuming that each NZMS needs about 6 months to reach maturity and bears about 70 live offspring every three months, lowering the water level during freezing temperatures once per year would result in a substantial drop in productivity that would eventually reach a condition of equilibrium. This lower density equilibrium condition would lower the likelihood of transport through human or other animal contact, particularly in the nearshore environment where contact is most likely to occur. In Olympia, during the summer, there are occasionally days that exceed 34°C, and temperatures may reach as high as 39°C in some years. Given that NZMSs are not tolerant of extremely high temperatures, lowering the water level once during a summer heat wave may add an additional control measure by inducing further mortality during peak spawning season, though this treatment was not investigated here.

Owing to high labor costs, equipment purchases, and other project management expenses, controlling the population of any nonnative species is usually very expensive and time consuming. Controlling NZMSs in Capitol Lake through water level changes affords a rare opportunity to significantly reduce or eradicate the presence of an invasive species of high concern and decrease the likelihood of its spread to other water bodies at a very low cost. The impact of water level draw-downs on the lake's existing ecology, other aquatic species, and aesthetics would be dependent on the extent and duration of the treatment. The impact of partial draw-downs for short durations may be minimal, while complete draw-downs over protracted periods of time would probably result in more substantial impacts. In the case of Capitol Lake, balancing the risks posed by NZMSs and the means available to eradicate or stem their spread against other lake management concerns will likely present a challenge to managers. Although this study was geographically limited, it is highly doubtful that NZMS populations elsewhere would exhibit substantially different responses to freezing temperatures, and there are many places

that are at risk of invasion that could be subjected to this kind of control measure. Indeed, dozens of dammed lakes and reservoirs exist in Washington State and throughout the country where water levels can be manipulated and that experience periods of sub-freezing temperatures. Drawdowns are routinely used in Washington, Oregon, and Idaho to accommodate passage of migrating anadromous fish, thus the idea of using water-level manipulation as a biological management tool is one with which resource managers in the Pacific Northwest are well acquainted. Locks and reservoirs used to facilitate waterborne commerce exist across the country in climates subject to freezing temperatures and water level manipulations at these facilities could be used to control the spread of NZMSs should they become invaded.

This study did not address the rate at which the NZMS may recolonize the lakeshore subsequent to freezing. Given the NZMS's life history, and the physical and biotic complexity of the lakeshore environment, it is doubtful that a full eradication could be achieved at Capitol Lake. Knowing how quickly NZMSs can become reestablished in an area after a freeze treatment would help managers plan the frequency of drawdowns and should be examined in future studies.

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