Scientific literature on the origins of Didymo, felt soled wading boots, and vectors for some aquatic invasive species

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On the origins of Didymo in North America:

Bothwell, M. L., B. W. Taylor, and C. Kilroy. 2014. The Didymo story: the role of low dissolved phosphorus in the formation of Didymosphenia geminata blooms. Diatom Research 29(3):229-236.

http://www.dartmouth.edu/~btaylor/index/Publications_files/Bothwell,%20Taylor,%20and%20Kilroy%202014%20Diatom%20Research.pdf

Abstract: We outline, in chronological sequence, the events and findings over 25 years that have shaped our understanding of *Didymosphenia geminate* (Lyngbye) M. Schmidt blooms. Starting with the first appearance of *D. geminata* mats in streams on Vancouver Island in the late 1980s and followed years later by blooms in Iceland, South Dakota and Poland, *D. geminata* blooms were enigmatic for nearly 20 years. Early papers exploring whether blooms were caused by environmental change consistently failed to identify any specific factor(s) associated with their onset. Following the *D. geminata* outbreak in New Zealand in 2004 that seemed to result from an introduction of the species, the possibility that blooms that had previously occurred elsewhere in the world might also be explained by the introduction and movement among watersheds of a new variant with a bloom-forming tendency was touted and widely accepted. Now, however, the identification of very low soluble reactive phosphorus (SRP; below ~2 ppb) as the proximate cause of bloom formation, has led to the more likely explanation that *D. geminata* blooms are the result of large-scale human intervention in climatic, atmospheric and edaphic processes that favour this ultra-oligotrophic species. In this new view, blooms of *D. geminata* are not simply due to the introduction of cells into new areas. Rather, bloom formation occurs when the SRP concentration is low, or is reduced to low levels by the process of oligotrophication. Mechanisms that potentially cause oligotrophication on global and regional scales are identified.

Taylor, B. W; and M. L. Bothwell. 2014. The origin of invasive microorganisms matters for science, policy, and management: the case of Didymosphenia geminata. Bioscience 64:531-538.

http://legislature.vermont.gov/assets/Documents/2016/WorkGroups/House%20Fish%20and%20Wildlife/Bills/H.5/Witness%20Testimony/H.5~Eric%20Palmer~Origin%20of%20Invasive%20Microorganisms%20for%20Science,%20Policy%20and%20Mgmt~3-11-2015.pdf

Abstract: The value of distinguishing native from nonnative invasive species has recently been questioned. However, this dichotomy is important for understanding whether a species' successful dominance is caused by introductions, changing environmental conditions that facilitate an existing population, or both processes. We highlight the importance of knowing the origin of hard-to-detect invasive microorganisms for scientific research, management, and policy using a case study of recent algal blooms of the stalk-producing diatom Didymosphenia geminata. Nuisance blooms have been reported in rivers worldwide and have been hastily attributed to introductions. However, evidence

indicates that blooms are probably not caused by introductions but, rather, by environmental conditions that promote excessive stalk production by this historically rare species. Effective responses to invasive microorganisms depend on knowing whether their proliferation is caused by being nonnative or is the result of changing environmental conditions that promote invasive characteristics of native species.

Lavery, J. M.; J. Kurek; K. M. Ruhland; C. A. Gillis; M. F. J. Pisaric; and J. P. Smol. 2014. Exploring the environmental context of recent Didymosphenia geminata proliferation in Gaspesie, Quebec, using paleolimnology. Canadian Journal of Fisheries and Aquatic Sciences 71:1-11.

Abstract: Management authorities often perceive *Didymosphenia geminata* (didymo) as an invasive, nuisance diatom species that has been introduced to eastern Canada; however, observations from early 20th century diatom surveys challenge this characterization. We apply paleolimnological techniques to place recent didymo blooms from Gaspésie, Quebec, into historical and environmental context. Sedimentary diatom assemblages were examined from Lac Humqui (a headwater lake) and Lac au Saumon (a lake with an inflowing river currently supporting blooms). The Lac Humqui assemblage experienced a broad-scale shift in their dominant life strategy with declines in fragilarioid taxa and increases in planktonic diatoms (i.e., *Cyclotella–Discostella* species) that began_1970 and increased to modern abundances_1990. Strong relationships between this diatom shift and increases in regional air temperatures and earlier river ice-out dates are consistent with longer growing seasons and enhanced thermal stability in Lac Humqui. Didymo was observed throughout the Lac au Saumon core, demonstrating that it has been present in the region since at least _1970. Our paleolimnological evidence indicates that blooms likely form in response to regional consequences of climate warming, rather than human introduction.

Kilroy, C., and M. L. Bothwell. 2012. Didymosphenia geminata growth rates and bloom formation in relation to ambient dissolved phosphorus concentration. Freshwater Biology 57:641-653.

https://www.researchgate.net/publication/263198176_Didymosphenia_geminata_growth_rates_and_bloom_formation_in_relation_to_ambient_dissolved_phosphorus_concentration

Summary
1. The bloom-forming freshwater stalked diatom Didymosphenia geminata is unusual among algae in that nuisance growths occur almost exclusively in oligotrophic waters. Current hypotheses to explain this phenomenon have assumed supplemental acquisition of phosphorus from novel sources within the stalk ⁄mat matrix.
2. We carried out a synoptic survey of river sites in the South Island, New Zealand, to determine whether D. geminata cell division and stalk development (measured as mat coverage or standing crop) were related to ambient phosphorus concentrations in the overlying river water.
3. High coverage (>50%) by D. geminata was largely concentrated at sites with mean dissolved reactive phosphorus (DRP) <2 mg m) in the overlying water. Didymosphenia geminata was present at only one site with DRP >4 mg m)3, with very low coverage. Cell division rate (measured as the frequency of dividing cells, FDC) was positively correlated with mean DRP suggesting that division rates were controlled by the available phosphorus concentration in ambient river water.
At the same time, FDC was negatively correlated with D. geminata standing crop (measured as an index incorporating percentage cover and mat thickness).
4. In a single river reach with a stable cross-channel gradient of total dissolved phosphorus (TDP) caused by inflows from a high-nutrient tributary, again we observed a negative correlation between percentage cover by D. geminata and concentrations of TDP.

5. Finally, we made a series of observations on D. geminata-dominated communities that had been exposed to water enriched with NO_3 -N and PO_4 -P for 4 weeks, followed by exposure to unenriched water. After 2 weeks of nutrient deprivation, D. geminata cell division rates declined by 60%, mean stalk length increased by 250%, and total carbohydrate quadrupled relative to initial values. The appearance of the community changed from a dark brown mat to a thick pale mat typical of D. geminata blooms.
6. All these results indicate that D. geminata cell division rates are actively controlled by concentrations of available phosphorus in the overlying water and that stalk production (represented by mat thickness and extent, stalk length and total carbohydrate) is inversely related to D. geminata cell division rates. They thus support an explanation for D. geminata blooms in oligotrophic rivers tied to enhanced stalk production in nutrient-poor waters, rather than through acquisition of additional phosphorus through recycling processes within the mat.

Kilroy, C., and M. L. Bothwell. 2011. Environmental control of stalk length in the bloom-forming, freshwater benthic diatom Didymosphenia geminata (Bacillariophyceae). Journal of Phycology 47:981-989.

Abstract: Blooms of the freshwater stalked diatom Didymosphenia geminata (Lyngb.) M. Schmidt in A. Schmidt typically occur in oligotrophic, unshaded streams and rivers. Observations that proliferations comprise primarily stalk material composed of extracellular polymeric substances (EPS) led us to ask whether or not the production of excessive EPS is favored under nutrient-limited, high-light conditions. We conducted experiments in outdoor flumes colonized by D. geminata using water from the oligotrophic, D. geminata–affected Waitaki River, South Island, New Zealand, to determine the relationship between D. geminata stalk length, cell division rates, and light intensity under ambient and nutrient enriched conditions. Stalk lengths were measured in situ, and cell division rates were estimated as the frequency of dividing cells (FDC). FDC responded positively to increasing light intensity and to nutrient additions (N+P and P). Under ambient conditions, stalk length increased as light level increased except at low ambient light levels and temperature. Nutrient enrichment resulted in decreased stalk length and negative correlations with FDC, with this effect most evident under high light. Our results are consistent with the hypothesis that extensive stalk production in D. geminata occurs when cell division rates are nutrient limited and light levels are high. Thus, photosynthetically driven EPS production in the form of stalks, under nutrient-limited conditions, may explain the development of very high biomass in this species in oligotrophic rivers. The responses of FDC and stalk length under nutrient replete conditions are also consistent with occurrences of D. geminata as a nondominant component of mixed periphyton communities in high-nutrient streams.

Bothwell, M. L., and C. Kilroy. 2011. Phosphorus limitation of the freshwater benthic diatom Didymosphenia geminata determined by the frequency of dividing cells. Freshwater Biology 56:565-578.

https://www.researchgate.net/publication/230353517_Phosphorus_limitation_of_the_freshwater_benthic_diatom_Didymosphenia_geminata_determined_by_the_frequency_of_dividing_cells

Summary
1. Unlike other nuisance algal species, the freshwater benthic diatom Didymosphenia geminata typically forms blooms in low-nutrient rivers. The negative association between D. geminata blooming behaviour and nutrient levels appears at both catchment and smaller scales. We conducted a series of trials in streamside experimental channels colonised with D. geminata using water from the D. geminata-

affected, oligotrophic Waitaki River, South Island, New Zealand to determine how elevated nitrate and phosphate concentrations affected D. geminata cell division. Because D. geminata blooms are typically most pronounced in unshaded waters, we also investigated the growth response to shading. In all experiments, we used the frequency of dividing cells (FDC) as a metric of cell division.
2. Concentrations of nitrate and dissolved reactive phosphorus (DRP) in the Waitaki River were very low (4 mg m)3 NO_3 -N and <1 mg m)3 DRP). In pilot trials, substrata colonized by D. geminata were subjected to enrichment by either switching the water source to N- and P-rich spring water or by adding a stock NO_3 _ PO3_4 solution. Both trials resulted in periods of rapid cell division lasting at least 8 days.
3. Experimental addition of NO_3 alone triggered an initial cell division which was not sustained. However, addition of PO3_4 alone or together with NO_3 resulted in prolonged elevation in cell division indicating that the cell division rate was P-limited.
4. Reduced light levels resulted in decreased FDC in D. geminata in both ambient and N,
N + P and P-enriched river water.
5. Stimulation of D. geminata division rate by addition of PO3_4 above ambient levels confirms that, while blooming behaviour is often associated with oligotrophic rivers, the cells divide faster with greater levels of phosphorus enrichment.

If Didymo weren't already present everywhere, this is how it might spread:

Bothwell, M. L., D. R. Lynch, H. Wright, and J. Densinger. 2009. On the boots of fishermen: the history of Didymo blooms on Vancouver Island, British Columbia. Fisheries 34(8):382-388.

http://www.env.gov.bc.ca/wat/wq/studies/didymo-blooms.pdf

Abstract: In 1989 blooms of the river benthic diatom *Didymosphenia geminata* (didymo) first appeared and rapidly spread among rivers on central Vancouver Island, covering the bottoms with thick, woolly-looking mats. Although didymo is native to North America, extensive field surveys of rivers on Vancouver Island and other data indicate that didymo blooms are new. No known environmental changes were associated with the onset of didymo blooms. However the pattern of didymo spread among rivers on Vancouver Island correlates with the activity of fishermen and the commercial introduction and widespread use of felt-soled waders in the late 1980s. Since 1994 nuisance blooms of didymo have appeared in numerous other places in the Northern Hemisphere and South Island, New Zealand, all areas frequented by fishermen. Actions by government agencies to educate the public and restrict the use of felt-soled waders have been undertaken in some jurisdictions and at least one commercial manufacturer of waders will discontinue production of felt-soled models in the near future.
NOTE: Bothwell later proved his own theory to be wrong (see above).

Kilroy, C., A. Lagerstedt, A. Davey, and K. Robinson. 2007. Studies on the survivability of the invasive diatom Didymosphenia geminata under a range of environmental and chemical conditions. National Institute of Water and Atmospheric Research Ltd, Christchurch, New Zealand, report CHC2006-116.

http://www.biosecurity.govt.nz/files/pests/didymo/didymo-survival-dec-06-rev-may-07.pdf

Executive Summary

- Since it was first identified in New Zealand in October 2004, the invasive freshwater diatom *Didymosphenia geminata* (a single-celled alga) has been recorded in at least seven catchments in the South Island. To date, the species has not been recorded in the North Island.

- In an effort to contain the spread of *D. geminata*, within two months of the discovery of the alga in New Zealand, preliminary conservative decontamination methods based on the microbial biosafety literature were identified and promoted by Biosecurity New Zealand for decontaminating risk goods that may have come into contact with the alga. Two months later, in February 2005, the decontamination methods were amended and re-issued based on initial results from experimental studies. In November 2005, an extensive "Check Clean Dry" behaviour change campaign was launched to further increase public awareness of the decontamination methods.

- The study reported here is part of Biosecurity New Zealand's ongoing effort to provide validated information to enable freshwater users to reduce the spread of *D. geminata*. After previously determining how to quickly kill the alga with decontamination treatments, the next priority was to determine how long *D. geminata* cells might survive if removed from a river and left untreated.

- As part of an evaluation of the risk associated with transport of *D. geminata* by a range of potential vectors, this laboratory-based study assessed the survivability of *D. geminata* under a range of temperature, light and moisture conditions. Once the optimum temperature–light–moisture regime was estimated, trials to determine the survivability of the alga under different water quality conditions (salinity, pH, municipal water, dilutions of detergent/cleaning agents) were conducted under these optimum survival conditions to develop worst-case scenarios for risk of survival outside waterways. The information has been used to augment the current decontamination recommendations because the "Check, Clean, Dry" campaign has necessitated cleaning on a much larger scale than when the original decontamination measures were initially validated, and more options are required.

- The work was further extended to include an assessment of one specific item that has been identified as a prime candidate for transfer of *D. geminata* between rivers and possibly into New Zealand: felt-soled wading boots.

- In all trials, cell viability in *D. geminata* was assessed using the neutral red staining technique. Live cells take up this stain in cytoplasmic vacuoles (appearing as deep red spots under the microscope). Dead cells do not take up the stain. For every sample tested, we counted at least 100 cells, distinguished stained cells (assumed viable) from unstained or empty cells (assumed dead), and then calculated the proportion of stained cells. A review and evaluation of the neutral red staining technique is included as an Appendix. The risk of false negatives (counting cells as non-viable when in fact they are viable) is present, but can be avoided. We conclude that the method is appropriate and cost-effective in the present context.

- The primary temperature–light–moisture experiment was undertaken in a controlled temperature unit with temperature set at 28, 20, 12 and 5 °C. Different covers over *D. geminata* samples provided light treatments ranging from full fluorescent lighting to complete darkness. Wet treatments were kept immersed in river water (topped up as necessary). In damp treatments, *D.*

geminata pieces were drained, then allowed to dry out as the experiment progressed. Survival in extreme temperatures (40 °C water and <0 °C) was assessed in separate experiments.

- Survival of *D. geminata* was strongly dependent on light, temperature and moisture. The rate of decline in cell viability was faster at higher temperatures. In all cases, cells held in the dark died faster than those receiving some light, and survival of cells in damp colonies declined faster than in wet colonies.

- At 28 °C, no viable cells remained in any treatment after three days. Survival at lower temperatures was much longer than anticipated, and for practical reasons all three trials had to be terminated before 100% mortality was achieved. Models fitted to the data for each treatment combination enabled predictions to be made of the time required to decline to 5% survival. These times range from just over 1 day (damp colonies at 28 °C in the dark) to over 8 months (wet colonies at 5 °C in low light). Lower rates of survival in damp colonies were due to desiccation, which occurred faster at higher temperatures.

- The extreme temperatures tested were rapidly lethal to *D. geminata*. Mat fragments soaked in 40 °C water required between five and 20 minutes exposure for 100% cell mortality. To translate these experimental results into operational recommendations, we allowed for temperature differentials which can occur at the interface of a cold item soaked in hot water. We recommend that the temperature for hot tap water used to decontaminate gear be no less than 45 °C, with soaking times of at least 20 minutes for non-absorbent items or of at least 20 minutes plus whatever additional time is required for thorough saturation of absorbent items. Domestic water heating cylinders often deliver water to outlet taps at between 45 and 55 °C, but this may vary, so those wishing to use hot water treatment to decontaminate gear should check the temperature at the beginning and end of the treatment to ensure that it remains at no less than 45 °C. Previous recommendations for using very hot water for rapid treatment (at least 60 °C for at least one minute) still apply, where practical, for temperature resilient items and where temperature compliance can be assured.

- Freezing caused 100% mortality and there was no difference in response between freezing at -2 °C and -15 °C, although the time taken for cells to freeze solid occurs faster in lower temperatures.

- For practical reasons, the temperature trials in the primary experiment had to be run consecutively and the unexpected duration of the trials meant that optimum conditions for all subsequent trials had to be estimated. We determined that a temperature of 9 °C in high light would be near optimal. Survival rates for the first 30 days of the 5 and 12 °C trial (wet treatments, all light levels except dark) were similar, hence this estimate is considered reasonable. All remaining trials on the effects of water quality conditions were therefore undertaken at 9 °C in high light.

- In tests on a range of seawater dilutions, full-strength seawater (3.1% w/v salt) was lethal to all *D. geminata* cells in the samples tested after 4 hours. Half-strength seawater killed most cells within one day but residual, possibly viable, cells persisted for at least 30 days. Greater dilutions were even less effective, with 10% seawater yielding better survivability than river water. For effective decontamination, immersion in full-strength sea water for at least 4 hours is recommended. Immersion in estuarine water of 50% seawater or less cannot be recommended

as a reliable decontamination method. Immersion times required for 100% mortality in seawater between half and full strength are likely to be too long to be practical.

- A suite of pH levels was made up in river water by adding lime (for high pH) and hydrochloric acid (for low pH). As expected, *D. geminata* did not withstand exposure to very low or very high pH (pH 1 and pH 11), but survived well in the range between pH 4 to 9.5, which covers the entire pH range of natural freshwaters in New Zealand. Extrapolating the result that survival occurs at pH 4 suggests that cells may withstand pH levels in bird guts from time to time, however the accompanying conditions in bird guts (high temperatures and darkness) are extremely unlikely to support live *D. geminata* cells passing through. Transport of *D. geminata* by birds is considered more likely to be on feathers or feet than in the gut, although the relative risk of such transport between catchments is estimated to be small compared to transport by humans for the following reasons: the natural tendency of birds to groom and clean their feathers, the natural tendency of feathers to shed water and debris and the estimated rapid desiccation of cells during flight under dry weather conditions.

- Under optimal survival conditions, 14 products were tested for their effectiveness in killing *D. geminata* cells over a range of dilutions and contact times. Products included "generic" constituents of household cleaners, products recommended for general decontamination purposes at biosecurity checkpoints at New Zealand's border, products currently recommended for decontamination of *D. geminata*, cleaners marketed as environmentally friendly, and commonly available detergents (dishwashing liquids with no special environmental claims). The aim was to identify concentrations required for 100% mortality of *D. geminata* at a range of contact times from 1 minute to 1000 minutes (16 h 40 mins) and thus provide options for longer soaking times at reduced concentrations of products, especially for high volume/commercial users who wish to soak gear overnight at less expense to themselves, their business and the environment.

- Three constituents of common cleaning products [borax, sodium percarbonate and sodium dodecyl sulphate (SDS, an anionic surfactant)] were less effective than commercial detergents containing mixed ingredients that increase product effectiveness. For example, 5% nappy cleaner (main active ingredient sodium percarbonate) killed all *D. geminata* cells within one minute, but the equivalent concentration of the pure active ingredient did not. Borax was ineffective at the concentrations tested and SDS required high concentrations for complete mortality.

- Concentrations of sodium metabisulphite recommended for use at the border were not fully effective for *D. geminata*. A disinfectant currently in use at the border (Virkon®) had been found in a previous test to be effective in one minute at the recommended concentration.

- The current recommendation for household bleach as a *D. geminata* decontaminant was re-confirmed (i.e., a 2% solution for 1 minute). Longer contact times with lower concentrations were not fully effective, indicating that the low concentrations of chlorine in swimming pool water or treated tap water should not be recommended for treatment of contaminated items.

- A swimming pool cleaner (active ingredients, quaternary ammonium compounds – quats, which are cationic surfactants) required high concentrations for complete mortality of *D. geminata*, even at long exposure times. Five hundred times the stated recommended dose for killing algae

was needed to kill all *D. geminata* cells in one minute. Quat compounds are known to be toxic against micro-organisms, but specific information about concentrations in quat-based products is needed before their effectiveness can be assessed.

- Ethanol was tested because this is used as a preservative for *D. geminata* genomic material. The recommended concentration of 70% was completely effective within 10 minutes. Lower concentrations were also effective over longer times. We conclude that use of ethanol for *D. geminata* preservation poses minimal risk of spreading live cells.

- Cleaning products marketed with environmental claims were less effective for killing *D. geminata* than commonly available detergents (dishwashing liquids). For example, 2% solutions of the former were needed for 100% mortality within 1000 minutes, whereas 0.1 or 0.5% solutions of the latter were effective. Very high concentrations (>50%) of the environmentally friendly products were needed for a one minute kill, and one product was not fully effective at 100%. The original finding that exposure to 5% regular dishwashing liquid for one minute was 100% lethal to *D. geminata* was confirmed. The difference between the two groups of products may simply reflect lower concentrations of active ingredients in the "non-toxic" group, rather than different, less toxic ingredients.

- To assist Biosecurity New Zealand in providing practical recommendations for decontaminating risk goods against *D. geminata*, a summary table was provided ranking all effective products and methods based on their relative effectiveness, and the following additional factors: availability, cost, toxicity/irritation to humans, corrosiveness, possible effect on other organisms, and biodegradability. It is recognised that not all methods will be practical in all situations and users must exercise judgement. Our recommendation is to select the highest ranked methods that are practical for the situation. Regardless of rank, all products and methods recommended in the table are effective provided that the specified contact times and concentrations (if applicable) are used. This means that all of the potentially contaminated material (interior as well as exterior) must be in direct contact with the decontamination agent for the full required contact time. Such contact may not be easily achieved for absorbent materials such as felt soles, foam, etc., and therefore soaking for prolonged times will be necessary. Because *D. geminata* mortality increases with temperature, the effectiveness of all the chemical treatments is likely to increase with temperature.

- The question of longevity of decontamination baths was considered, i.e., how often should decontaminants be replenished? It is concluded that a precautionary approach is desirable, since the active ingredients are "used up" with use, and also biodegrade over time. Household bleach solutions should be changed daily, and more often with heavy use. Other products recommended for decontamination will generally be biodegradable, a process that starts as soon as they are in solution and in contact with particulate organic matter. Therefore solutions other than bleach should be renewed at least every other day, and preferably daily.

- Trials on the efficacy of wading boots as vectors for *D. geminata* showed that in the short term (four to five hours), felt soles, leather boot tops and neoprene waders *all* present a high risk of transferring cells if they are not decontaminated. However rubber gumboots present a much lower risk. In the longer term (36 hours), felt soles harbour live *D. geminata* cells much more successfully than the other materials tested.

- Trials to compare the efficacy of decontamination methods on felt soles indicated that solutions containing surfactants (nappy cleaner, dishwashing liquid) soaked into felt soles faster than those containing bleach, though neither type of solution appeared to soak to their maximum potential depth within one minute. Spraying the felt soles was much less effective than soaking and is not recommended for any porous or absorbent material.

- It was not feasible to experimentally test if the depth to which decontamination solutions penetrate felt soles would be sufficient to kill every *D. geminata* cell which could potentially be forced deep within the soles from the weight of footsteps in affected rivers. However, the finding that decontamination solutions at ambient temperatures do not passively soak quickly into felt soles coupled with the finding that felt soles remain damp for long periods and harbour relatively large amounts of live *D. geminata* cells indicates the need for precaution. Combining the power of heat with the power of detergent is highly recommended for decontaminating felt soles. The entire felt sole needs to be completely immersed for 30 minutes in hot tap water at no less than 45 °C (uncomfortably hot to touch) containing 5% dishwashing liquid or nappy cleaner. If hot water alone is used, careful attention is needed to ensure the temperature of the water is maintained at no less than 45 °C for 40 minutes to ensure the interior of the felt reaches a sufficient temperature. Alternatively, freezing felt-soled waders until solid is also effective.

- Relying on ambient drying as a stand-alone treatment for decontaminating highly absorbent risk goods such as felt-soled waders is not recommended in situations where use between waterways is frequent (daily, weekly or even monthly). Dessication of *D. geminata* mat fragments to a water content of less than 83% has previously been shown to be 100% lethal, however cells kept damp and cool (< 20 °C) can remain viable for months. Felt soles that were dried at ambient conditions for 36 hours and were barely wet to the touch still harboured viable cells. Drying should only be relied upon as a decontamination treatment if great care is taken to actively and completely dry the felt (such as by using a heat source where temperatures around the felt are assured of reaching 30 °C). Once the felt appears dry, complete dryness must be confirmed by a tactile inspection of the felt pile to the base of the fibres. Once completely dry, items must remain dry for at least 48 hours before use in another waterway. These findings for felt soles can be extrapolated to other absorbent materials.

Kilroy, C. 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with Didymosphenia geminata. National Institute of Water and Atmospheric Research Ltd., Christchurch, New Zealand NIWA Project: MAF05501.

http://www.biosecurity.govt.nz/files/pests/didymo/didymo-decon-feb-05-rev-aug-06.pdf

Executive Summary:

• NIWA was requested by Biosecurity New Zealand to test the effectiveness of a range of products as decontaminating agents for materials (clothing/equipment) that may be contaminated with the invasive diatom *Didymosphenia geminata*. Currently the species appears to be confined to the Mararoa and lower Waiau Rivers in Southland, and measures are in place to try to prevent its spread into other rivers.

• Trials were conducted to find a method for distinguishing live and dead diatom cells. A staining technique using neutral red dye was considered to be the best general method available. Neutral red dye is taken up by the cytoplasm of live cells, but not dead cells. Observations on the microscopic appearance of cells can provide supplementary evidence of the effectiveness of some treatments.

• In preliminary tests carried out on 22 December, the neutral red staining technique indicated that 5% solutions of nappy cleaner and two household antiseptic liquids (active ingredients chlorhexidine and chloroxylenol) rapidly killed cells of *Cymbella kappii* (a diatom related to *D. geminata*).
• The tests were repeated on 18-20 January 2005 using *D. geminata* mats collected on 16 January. Salt solutions, detergent, and bleach were also tested. The earlier results were confirmed for <1 min treatment with 5% nappy cleaner and chlorhexidine antiseptic. Treatment with 5% (v/v) salt solution for 1 min resulted in very few dyed cells, either outside or inside the mat. A longer treatment time (10+ min) was more effective, and a 1% solution less effective. Mats treated for <1 min with 5% detergent, chloroxylenol antiseptic and bleach also appeared to be effective, however the results were inconclusive because very low concentrations of dyed cells in control (untreated) mats indicated that the *D. geminata* mats were deteriorating rapidly in the laboratory.
• Further *D. geminata* colonies were collected on 3 February and transported to the laboratory the same day. To try to enhance their survival, colonies were left attached to rocks from the river bed, were immersed in at least 20 times their volume in river water, and were maintained at a cooler temperature. The tests were repeated on these colonies, this time with replication.
• These repeat tests confirmed that immersion in 5% solutions of nappy cleaner, chlorhexidine and chloroxylenol antiseptic liquids, and detergent rapidly (30 sec) killed *D. geminata* cells. In addition, a 1% solution of household bleach was found to be equally effective. Five percent salt solution was effective, but a 2% solution was slightly less reliable. All solutions were made up by volume. Tests to determine the effectiveness of methods for sanitising materials contaminated with *Didymosphenia geminata* ii
• The tests with salt solution indicated that prolonged immersion in sea water (*at least* 1 hour) may be an effective method of decontaminating larger equipment such as the outer surfaces of boats and kayaks, but this remains to be tested.
• The effects of desiccation on *D. geminata* colonies were also investigated. After 15 h desiccation of a large mat in partially sunny conditions few live cells remained either outside or inside the mat. After 48 h the mats had dried completely and no live cells were found.
• In a more detailed desiccation test, no live cells were found in mats that had dried to a water content of less than 83%. The time taken to reach this point would vary according to initial water content, but in the warm (~20 °C), shaded conditions of the present trial, took up to three days.
• Heat treatment was not tested directly in these trials, however rapid heating to 60 °C is a standard laboratory method for killing algal cells.
• The rapid response of cells to a mild dishwashing detergent, along with the poor viability of *D. geminata* colonies in warm laboratory conditions (>22 °C) suggest that *thorough* shampooing and rinsing in warm to hot water, will be effective in killing any cells lodged in hair following swimming, particularly if the hair is dried before shampooing.
• Based on these results, recommendations for decontaminating materials that may have come into contact with *D. geminata* are:

o Soak and scrub in a 5% (v/v) solution of either salt, nappy cleaner, household antiseptics (chlorhexidine or chloroxylenol based) or detergent for about 1 minute.

o Or, soak and scrub in a 2% solution of household bleach for 1 min.

o Or, immerse contaminated items in hot water for 2 min, to a final temperature of at least 60 °C.

o After swimming in an affected waterway, allow hair to dry completely, then shampoo for *at least* 1 minute, and rinse thoroughly using warm to hot water.

o For large items of equipment, desiccate completely by drying for an extended period of time (e.g., 48 hours) *after* all parts of the item (and therefore algal mats on or within it) appear to be dry.

On felt-soled boots as a vector for aquatic invasive species:

Stockton, K. A.; and C. M. Moffitt. 2013. Disinfection of three wading boot surfaces infested with New Zealand Mudsnails. North American Journal of Fisheries Management 33:529-538.

https://pubs.er.usgs.gov/publication/70042270

Abstract: New Zealand mudsnails *Potamopyrgus antipodarum* (NZMS) have been introduced into many continents and are easily transported live while attached to wading and other field gear. We quantified the relative attachment by different life stages of NZMS to felt, neoprene, and rubber-soled boots exposed to two densities of NZMS in experimental exposure totes. Attachment by NZMS occurred on boots of all surfaces, but the highest numbers of all life stages occurred on boots with felt surfaces.We found a 15–20-min bath application of 20 g/L Virkon Aquatic was a reliable tool to disinfect boot surfaces infested with NZMS and other aquatic invertebrates. Our studies support that spray application of this disinfectant was not reliable to provide complete mortality of attached adult NZMS or neonates. Wading gear surfaces exposed to repeated bath disinfections showed little deterioration. Our results provide strong evidence that bath disinfections with Virkon Aquatic are helpful to assure biosecurity in field and hatchery settings, but applications should be coupled with cleaning procedures to remove organic materials that can deactivate the reagent.

Gates, K. K.; C. S. Guy; A. V. Zale; and T. B. Horton. 2008. Adherence of Myxobolus cerebalis myxospores to waders: implications for disease dissemination. North American Journal of Fisheries Management 28:1453-1458.

http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.568.7144

Abstract: The vectors involved in the spread of whirling disease, which is caused by Myxobolus cerebralis, are only partly understood. However, the parasite has rapidly become established in many regions, suggesting that it is easily disseminated. We gained insight into transport vectors by examining the surface porosity of common wading equipment materials and the adherence of M. cerebralis myxospores to them. Interstitial spaces within rubber, felt, lightweight nylon, and neoprene were measured on scanning electron microscope images. Myxospores were applied to each material, the material was rinsed, and the myxospores recovered to assess adherence. The mean interstitial space size of rubber was the smallest (2.0 lm), whereas that of felt was the largest (31.3 lm). The highest recovery rates were from rubber and the glass control. Percent myxospore recovery varied by material, the recovery from felt being lower than that from all other materials. The potential for felt to carry even small numbers of myxospores suggests that the introduction of M. cerebralis by felt-soled wading boots is possible.

Amphibian Vectors:

Picco, A. M., and J. P. Collins. 2008. Amphibian commerce as a likely source of pathogen pollution. Conservation Biology 22(6):1582-1589.

Abstract: The commercial trade of wildlife occurs on a global scale. In addition to removing animals from their native populations, this trade may lead to the release and subsequent introduction of nonindigenous species and the pathogens they carry. Emerging infectious diseases, such as chytridiomycosis caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), and ranaviral

disease have spread with global trade in amphibians and are linked to amphibian declines and die-offs worldwide, which suggests that the commercial trade in amphibians may be a source of pathogen pollution. We screened tiger salamanders involved in the bait trade in the western United States for both ranaviruses and Bd with polymerase chain reaction and used oral reports from bait shops and ranavirus DNA sequences from infected bait salamanders to determine how these animals and their pathogens are moved geographically by commerce. In addition, we conducted 2 surveys of anglers to determine how often tiger salamanders are used as bait and how often they are released into fishing waters by anglers, and organized bait-shop surveys to determine whether tiger salamanders are released back into the wild after being housed in bait shops. Ranaviruses were detected in the tiger salamander bait trade in Arizona, Colorado, and New Mexico, and Bd was detected in Arizona bait shops. Ranaviruses were spread geographically through the bait trade. All tiger salamanders in the bait trade were collected from the wild, and in general they moved east to west and north to south, bringing with them their multiple ranavirus strains. Finally, 26–73% of anglers used tiger salamanders as fishing bait, 26–67% of anglers released tiger salamanders bought as bait into fishing waters, and 4% of bait shops released tiger salamanders back into the wild after they were housed in shops with infected animals. The tiger salamander bait trade in the western United States is a useful model for understanding the consequences of the unregulated anthropogenic movement of amphibians and their pathogens through trade.

Aquatic vegetation vectors:

Brukerhoff, L., J. Havel, and S. Knight. 2015. Survival of invasive aquatic plants after air exposure and implications for dispersal by recreational boats. Hydrobiologia 746:113-121.

Abstract: Recreational boating is widely recognized as an important vector for overland transport of invasive aquatic plants. Since their dominant form of recruitment is vegetative reproduction, entangled fragments on boats and trailers can establish new populations. The effectiveness of recreational boats as transport vectors relies on the resistance of macrophytes to air exposure. During the summers of 2012 and 2013, we conducted five field experiments in northern Wisconsin to assess air tolerance of Eurasian water-milfoil (*Myriophyllum spicatum*) and curly-leaf pondweed (*Potamogeton crispus*). We simulated conditions that these plants would experience when ensnared on boats and trailers by testing viability after drying of single stems, coiled stems, and vegetative buds (turions). Single stems of M. spicatum and *P. crispus* were viable for up to 18 and 12 h of air exposure, respectively. Coiling extended the viability of *M. spicatum* to 48 h of air exposure. Turions of *P. crispus* successfully sprouted after 28 days of drying. The fact that recreational boaters in the region typically visit multiple lakes within a few days suggests that most lakes are susceptible to introduction of viable plants, and so lake managers should continue to focus attention on boat cleaning.

Rothlisberger, J. D., W. L. Chadderton, J. McNulty, and D. M. Lodge. 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries 35(3):121-132.

https://www.researchgate.net/publication/230778933_Aquatic_Invasive_Species_Transport_via_Trailered_Boats_What_Is_Being_Moved_Who_Is_Moving_It_and_What_Can_Be_Done

Abstract: Trailered boats have been implicated in the spread of aquatic invasive species. There has been, however, little empirical research on the type and quantity of aquatic invasive species being transported, nor on the efficacy of management interventions (e.g., inspection crews, boat washing). In a study of small-craft boats and trailers, we collected numerous aquatic and terrestrial organisms, including some species that are morphologically similar to known aquatic invasive species. Additionally, a mail survey of registered boaters (*n* = 944, 11% response rate) and an in-person survey of boaters in the field (*n* = 459, 90% response rate) both indicated that more than two-thirds of boaters do not always take steps to clean their boats. Furthermore, we used a controlled experiment to learn that visual inspection and hand removal can reduce the amount of macrophytes on boats by 88% ± 5% (mean ± SE), with high-pressure washing equally as effective (83% ± 4%) and low-pressure washing less so (62% ± 3% removal rate). For removing small-bodied organisms, high-pressure washing was most effective with a 91% ± 2% removal rate; low-pressure washing and hand removal were less effective (74% ± 6% and 65% ± 4% removal rates, respectively). This research supports the widespread belief that trailered boats are an important vector in the spread of aquatic invasive species, and suggests that many boaters have not yet adopted consistent and effective boat cleaning habits. Therefore, additional management efforts may be appropriate.

Blumer, D. L., R. M. Newman, and F. K. Gleason. 2009. Can Hot Water Be Used to Kill Eurasian Watermilfoil? Journal of Aquatic Plant Management 47:122-127.

http://www.apms.org/japm/vol47/v47p122_2009.pdf

Abstract: Hot water has been used as a safe and efficient strategy to control unwanted pest species in row crops and to control zebra mussel infestations of water intake and discharge pipes. The goal of this study was to determine if hot water can be used as an effective prevention strategy for killing an aquatic invasive plant species like Eurasian watermilfoil (*Myriophyllum spicatum* L.) attached to watercraft. In 2006, 20-cm Eurasian watermilfoil fragments with and without an apical meristem were exposed to six different water temperatures ranging from 45 to 80 C for 2-, 5-, and 10-min intervals to see if they could be killed. Fragment viability after treatment was determined in three ways: (a) the presence of living tissue at the end of a 30-day observation period; (b) change in biomass from before treatment to after treatment over the 30-day observation period; and (c) enzyme activity within fragments immediately after heat treatment. The 20-cm fragments were killed at temperatures ≥60 C. All fragments at temperatures ≥60 C lost mass, and enzyme activity was significantly reduced at temperatures ≥ 60 C for fragments without an apical meristem. Temperature was the most important variable causing death, and time of exposure had little effect at the tested temperatures. These results suggest that using hot water alone to kill fragments of Eurasian watermilfoil attached to watercraft may not be feasible because of the high water temperatures required. More research into additives, such as chlorine or bromine, may reveal ways to reduce the temperature of water necessary to kill plant fragments and make hot water control feasible.

Crayfish Vectors:

DiStefano, R. J., M. E. Litvan, and P. T. Horner. 2009. The bait industry as a potential vector for alien crayfish introductions: problem recognition by fisheries agencies and a Missouri evaluation. Fisheries 34(12):586-597.

http://www.mostreamteam.org/Documents/Research/AquaticInverts/Pages%20from%20DiStefano_et_al._Fisheries_12-09.pdf

Abstract: "Bait-bucket introductions" related to the fishing bait industry are the suspected primary cause of alien (non-indigenous) crayfish introductions that have damaged North American aquatic ecosystems.

Our 2008 survey of U.S. and Canadian fisheries agencies revealed that 49% of respondents reported aquatic resource problems that were believed to have been caused by bait-bucket introductions of alien crayfishes. Most respondents reported existing regulations designed to address those problems; however, only 4% prohibited the use of live crayfish bait. Our 2002-2007 examination of Missouri bait shops revealed sales of illegal and invasive alien crayfishes by bait shop proprietors who could not identify the species they were selling. Fisheries agencies should consider more effective bait regulations and education to prevent negative impacts to aquatic biodiversity, habitat, and fisheries that can result from alien crayfish introductions.

Peters, J. A., and D. M. Lodge. 2009. Invasive species policy at the regional level: a multiple weak links problem. Fisheries 34(8):373-381.

http://www.fishsciences.net/reports/2009/Fisheries_34_373-380.pdf

Abstract: Policy is used to prevent the introduction and spread of invasive species. For aquatic invasive species that can easily cross political boundaries, regional policies are needed. A weak link problem occurs when regulations of individual jurisdictions increase the region-wide risk of species introductions, especially in adjacent jurisdictions. Such cross-jurisdictional weak links may be compounded by another sort of weak link within jurisdictions: inconsistent regulation among multiple vectors that may introduce the same species. We used crayfish as a model system to study regulations for anglers, bait dealers, the pet trade, and aquaculture across the Great Lakes region. We identified a continuum of regulations ranging from no regulations to those that prohibit all use of crayfish. Furthermore, regulations differed depending on state and vector. Many states had regulations that specifically targeted the invasive rusty crayfish (*Orconectes rusticus*). However, these regulations were enacted reactively only after rusty crayfish had become established in the state. The lack of regulatory consistency among the Great Lakes jurisdictions is creating a multiple weak links problem and making success unlikely in efforts to slow the spread of crayfishes and other invasive species throughout the region.

Fish Vectors:

Johnson, B. M., R. Arlinghaus, and P. J. Martinez. 2009. Are we doing all that we can to stem the tide of illegal fish stocking? Fisheries 34(8):389-394.

http://warnercnr.colostate.edu/~brett/lab/documents/2009.Johnsonetal.Fisheries.pdf

Abstract: They've been called "bucket biologists" or "midnight managers" but these nicknames belie the ignorance and selfishness of individuals who engage in illegal stocking. Their actions are defeating multi-million dollar native fish recovery projects, damaging sustainable recreational fisheries worth billions of dollars, threatening native species with extinction, and diverting dwindling agency resources away from programs that benefit fishing and aquatic resources into expensive and often perpetual remediation programs. Illegal stocking is creating a growing burden on agencies and on society, and its impacts can be irreversible. The problem is global and yet there appears to be little collaboration across jurisdictions to seek solutions. We contend that the fisheries profession can and must do a better job of preventing illegal fish stocking through more strategic education programs, proactive regulatory policy, universally severe penalties, an international reward pool, and inter-jurisdictional collaboration.

Sharma, S., L. Herborg, and T. W. Therriault. 2009. Predicting introduction, establishment and potential impacts of smallmouth bass. Diversity and Distributions 15:831-840.

https://www.researchgate.net/publication/227732904_Predicting_introduction_establishment_and_potential_impacts_of_smallmouth_bass_Divers_Distrib

Abstract: The introduction of non-indigenous species has resulted in wide-ranging ecological and economic impacts. Predictive modelling of the introduction and establishment of non-indigenous species is imperative to identify areas at high risk of invasion to effectively manage non-indigenous species and conserve native populations. Smallmouth bass (*Micropterus dolomieu*), a warm water fish species native to central North America has negatively impacted native fish communities, including cyprinids and salmonid populations, as a result of intentional introductions. We predicted the introduction risk; species establishment based on habitat suitability; identified lakes at high risk of invasion; and finally assessed the consequential impacts on native salmon, trout and cyprinid populations. Classification tree and logistic regression models were developed and validated to predict the introduction and establishment of smallmouth bass for thousands of lakes. Densely human populated areas and larger lake surface areas successfully identify lakes associated with the introduction of smallmouth bass (introduction model) in British Columbia. Climate, lake morphology and water chemistry variables were the driving environmental parameters to define suitable smallmouth bass habitat (establishment model). A combination of the introduction and establishment model identified 138 lakes that are currently at risk in British Columbia to the introduction and establishment of smallmouth bass. Of these 138 high-risk lakes, 95% of them contain at least one species of salmon, trout or cyprinid, thereby increasing the potential impact of an invasion by smallmouth bass.
Our framework can be applied to other terrestrial and aquatic species to obtain a better understanding of the potential risk posed by a nonindigenous species to an ecosystem. Furthermore, our methodology can be used to focus management efforts on areas at higher risk (e.g. number of potential releases, more favourable habitats) to control future introductions of nonindigenous species, thereby conserving native populations.

Fish Disease Vectors:

Phelps, N. B. D., M. E. Craft, D. Travis, K. Pelican, and S. M. Goyal. 2014. Risk-based management of viral hemorrhagic septicemia virus in Minnesota. North American Journal of Fisheries Management 34:373-379.

https://www.researchgate.net/publication/261885190_Risk-Based_Management_of_Viral_Hemorrhagic_Septicemia_Virus_in_Minnesota

Abstract: Viral hemorrhagic septicemia virus (VHSV; strain VHSV-IVb) is an emergent and serious disease of fish in the Great Lakes region of North America. In addition to the numerous large-scale mortality events in wild fish, the emergence of VHSV has resulted in a major regulatory response to protect both farm-raised and wild fish populations. However, characterizing and mitigating risk factors for the continued transmission of VHSV is a difficult task. A semi-quantitative risk assessment model was used to focus VHSV management efforts in Minnesota. The risk of VHSV introduction into major watersheds in Minnesota was directly correlated to their proximity to Lake Superior, the only VHSV-positive body of water in the state. Although the current regulations are uniform across Minnesota, the risk varied for specific locations within the watersheds. For example, the introduction of game fish for stock enhancement (a common fisheries management practice) is a significant factor in determining the risk

of VHSV introduction into public waters, as is the movement of baitfish. In this analysis, aquaculture facilities with strict biosecurity programs and frequent health inspections received the lowest risk scores and were largely considered as protective or at low risk for VHSV introduction. These results suggest that the current management strategy, based on political boundaries, should be reevaluated. We recommend the creation of a risk-based management strategy based upon the identification of higher-risk watersheds and specific bodies of water, thus allowing managers to efficiently target surveillance and response activities in Minnesota.

Getchell, R. G., E. R. Cornwall, G. H. Groocock, P. T. Wong, L. L. Coffee, G. A. Wooster, and P. R. Bowser. 2013. Experimental Transmission of VHSV Genotype IVb by Predation. Journal of Aquatic Animal Health 25:221-229.

Abstract: Preliminary surveillance of wild baitfish during the 2006 viral hemorrhagic septicemia virus genotype IVb (VHSV IVb) outbreaks indicated Emerald Shiners *Notropis atherinoides* and Bluntnose Minnow *Pimephales notatus* were infected with high levels of VHSV without showing clinical signs of disease. The movement and use of baitfish was recognized as the most probable vector for the introduction of VHSV to inland waters, such as Conesus Lake and Skaneateles Lake in New York, Budd Lake in Michigan, and Little Lake Butte des Morts and Lake Winnebago in Wisconsin. While numerous government agencies implemented restrictions to stop the movement of potentially infected baitfish into new waters and prevent the spread of VHSV IVb, until now, studies to investigate whether these initial introductions were by an oral route of infection have not occurred. Our studies identified infected Fathead Minnow *Pimephales promelas* as suitable vectors for transmitting VHSV IVb when fed to Tiger Muskellunge (Northern Pike *Esox lucius* × Muskellunge *Esox masquinongy*) during laboratory trials. Six of 16 Tiger Muskellunge were infected with VHSV IVb after consumption of infected Fathead Minnows when assayed with quantitative reverse transcriptase polymerase chain reaction and viral isolation in cell culture. Weekly sampling of water and feces from these Tiger Muskellunge individually reared showed intermittent shedding of VHSV IVb. Those exposed to similarly VHSV IVb-inoculated fathead minnows by cohabitation only became infected in 1 case out of 16. A similar trial of 12 Tiger Muskellunge fed Round Goby *Neogobius melanostomus* that survived a VHSV IVb immersion challenge did not result in infection. Overall, our findings imply that consumption of infected wild baitfish may be a risk factor for introduction of VHSV.

Koel, T. M., B. L. Kerans, S. C. Barras, K. C. Hanson, and J. S. Wood. 2010. Avian piscivores as vectors for Myxobolus cerebralis in the greater Yellowstone ecosystem. Transactions of the American Fisheries Society 139:976-988.

https://www.aphis.usda.gov/wildlife_damage/nwrc/publications/10pubs/hanson101.pdf

Abstract: Myxobolus cerebralis, the cause of whirling disease in salmonids, has dispersed to waters in 25 states within the USA, often by an unknown vector. Its incidence in Yellowstone cutthroat trout Oncorhynchus clarkii bouvieri within the highly protected environment of Yellowstone Lake, Yellowstone National Park, is a prime example. Given the local abundances of piscivorous birds, we sought to clarify their potential role in the dissemination of M. cerebralis. Six individuals from each of three bird species (American white pelican Pelecanus erythrorhynchos, double-crested cormorant Phalacrocorax auritus, and great blue heron Ardea herodias) were fed known-infected or uninfected rainbow trout O. mykiss. Fecal material produced during 10-d periods before and after feeding was collected to determine whether M. cerebralis could be detected and, if so, whether it remained viable

after passage through the gastrointestinal tract of these birds. For all (100%) of the nine birds fed known-infected fish, fecal samples collected during days 1–4 after feeding tested positive for M. cerebralis by polymerase chain reaction. In addition, tubificid worms Tubifex tubifex that were fed fecal material from known-infected great blue herons produced triactinomyxons in laboratory cultures, confirming the persistent viability of the parasite. No triactinomyxons were produced from T. tubifex fed fecal material from known-infected American white pelicans or double-crested cormorants, indicating a potential loss of parasite viability in these species. Great blue herons have the ability to concentrate and release viable myxospores into shallow-water habitats that are highly suitable for T. tubifex, thereby supporting a positive feedback loop in which the proliferation of M. cerebralis is enhanced. The presence of avian piscivores as an important component of aquatic ecosystems should continue to be supported. However, given the distances traveled by great blue herons between rookeries and foraging areas in just days, any practices that unnaturally attract them may heighten the probability of M. cerebralis dispersal and proliferation within the Greater Yellowstone Ecosystem.

Kim, R., and M. Faisal. 2010. Comparative susceptibility of representative Great Lakes fish species to the North American viral hemorrhagic septicemia virus Sublineage IVb. Diseases of Aquatic Organisms 91:23-24.

http://www.int-res.com/articles/dao_oa/d091p023.pdf

Abstract: The present study compared the susceptibility of representative Laurentian Great Lakes fish species to the emerging viral hemorrhagic septicemia virus (VHSV) Genotype IVb. The median lethal dose of infection by intraperitoneal injection (IP-LD50) was obtained from fish that were experimentally infected with the MI03 index strain of VHSV-IVb. Fish were injected at doses ranging from 7×10^{7} to 7×10^{-2} plaque-forming units (pfu) and maintained at 12 ± 1°C. The infection trials identified species of high, medium, and low susceptibility based on the IP-LD50 values. Pathogenicity of VHSV-IVb was highest in largemouth bass *Micropterus salmoides*, which resulted in an IP-LD50 of 1.5×10^{2} pfu, while also demonstrating the clinical diathesis of VHSV-infected fish. The virus was moderately pathogenic in yellow perch *Perca flavescens* (IP-LD50 of 2.5 ??105 pfu), but also showed the classical signs of VHSV infection. Salmonids were the least susceptible to VHSV-IVb with IP-LD50 values of no less than 1.4×10^{6} pfu; however, in fish that succumbed to infection, characteristic VHSV lesions were observed. Histopathologic alterations were most profound in gill, skin, muscle, gonads, and liver of largemouth bass and yellow perch, while in salmonids, hemorrhages in the swimbladder and/or degenerative changes in the liver were the most common lesions noticed. VHSV was isolated from infected fish, and its identity was confirmed by the reverse transcriptase polymerase chain reaction. These results highlight the variations among fish species susceptibility to this emerging strain of VHSV and offer insights into the potential impact of VHSV-IVb on the Laurentian Great Lakes fish community.

Faisal, M., and C. A. Schulz. 2009. Detection of Viral Hemorrhagic Septicemia virus (VHSV) from the leech Myzobdella lugubris Leidy, 1851. Parasites & Vectors 2009, 2:45.

https://www.researchgate.net/publication/26852703_Detection_of_Viral_Hemorrhagic_Septicemia_virus_VHSV_from_the_leech

Abstract: The leech *Myzobdella lugubris* is widespread in the Lake Erie Watershed, especially Lake St. Clair. However, its role in pathogen transmission is not fully understood. In this same watershed, several widespread fish mortalities associated with the Viral Hemorrhagic Septicemia virus (VHSV) were

recorded. Viral Hemorrhagic Septicemia is an emerging disease in the Great Lakes Basin that is deadly to the fish population, yet little is known about its mode of transmission. To assess the potential role of *M. lugubris* in VHSV transmission, leeches were collected from Lake St. Clair and Lake Erie and pooled into samples of five. Cell culture and reverse transcriptase polymerase chain reaction (RT-PCR) were used to determine the presence of the virus and its identity. Results showed that 57 of the 91 pooled leech samples were positive by cell culture for VHSV and 66 of the 91 pooled leech samples were positive by RT-PCR for the VHSV. Two representative virus isolates were sequenced for further genetic confirmation and genotype classification. VHSV detected within *M. lugubris* was homologous to the Great Lakes strain of VHSV genotype IVb. This is the first record of the VHSV being detected from within a leech, specifically *M. lugubris*, and suggests the potential of *M. lugubris* being involved in VHSV transmission.

Bartholomew, J. L., and P. W. Reno. 2002. The history and dissemination of whirling disease. American Fisheries Society Symposium 29:3-24.

ABSTRACT. The explosion of information on the distribution and impacts of whirling disease in the United States during the last decade has changed the way in which we view *Myxobolus cerebralis*. However, even a cursory review of whirling disease literature reveals that many of our concerns today have been expressed at some previous time in the history of our experience with this parasite. From the first description of M. *cerebralis* in Germany in 1893, it was recognized that whirling disease could severely affect the growing trout farming industry. During the first half of this century M. *cerebralis* was disseminated throughout Europe, especially following WWII when live rainbow trout *Oncorhynchus mykiss* were transferred freely. Between 1950 and 1970, the parasite began to appear at trout farms on other continents, and it was in the late 1950s that whirling disease first emerged in the United States. Nearly all reports of detection, both here and in Europe, were associated with artificial rearing facilities. Until the 1980s, the only references reporting infections in natural populations of salmonids are from Finland, Russia, and Michigan, and the reported infections were usually light. Clinical whirling disease was largely associated with culture of trout in earthen ponds, where the infective agent concentrated. In the period between 1970 and 1990, there were increasing reports of the parasite in hatcheries throughout Europe and the United States. In Europe, the perspective after many years of living with whirling disease was that eradication was not possible in most cases, but that we knew enough to reduce infection levels below the point where clinical disease occurs. In the United States, reports of whirling disease in hatcheries were often followed by destruction of any fish on the facility, but as it became apparent that proper management could reduce infection levels, and as there appeared to be no effects outside the bounds of the hatchery, these standards were relaxed. However, in the 1990s, clinical whirling disease was reported in free-ranging trout populations in Colorado and Montana, causing us, once again, to rethink how this disease can be controlled and managed.

Bergersen, E. P., and D. E. Anderson. 1997. The distribution and spread of Myxobolus cerebralis in the United States. Fisheries 22(8):6-7.

Abstract: Myxobolus cerebralis, the causative agent of salmonid whirling disease, was first found in the United States in Pennsylvania in 1956, and has since spread to most regions of the country having self-sustaining trout populations. By fall 1996, 21 states had reported the presence of *M. cerebralis.* How far the organism will spread is not known.

El-Matbouli, M., and R. W. Hoffmann. 1991. Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of Myxobolus cerebralis spores. Journal of Aquatic Animal Health 3:260-262.

https://www.researchgate.net/publication/250020231_Effects_of_Freezing_Aging_and_Passage_through_the_Alimentary_Canal_of_Predatory_Animals_on_the_Viability_of_Myxobolus_cerebralis_Spores_1

Abstract: In transmission experiments with tubificids *Tubifex tubifex* as primary hosts and fry of rainbow trout *Oncorhynchus mykiss* as secondary hosts, it was shown that *Myxobolus cerebralis* spores can tolerate freezing at -20°C for at least 3 months, aging in mud at 13°C for at least 5 months, and passage through the guts of northern pike *Esox lucius* or mallards *Anas platyrhynchos* without loss of infectivity.

Mollusk vectors:

Sousa, R., C. Antunes, and L. Guilhermino. 2008. Ecology of the invasive Asian clam Corbicula fluminea (Muller, 1774) in aquatic ecosystems: an overview. International Journal of Limnology 44(2):85-94.

https://www.researchgate.net/publication/41698742_Ecology_of_the_invasive_Asian_clam_Corbicula_fluminea_Muller_1774_in_aquatic_ecosystems_an_overview_Annales_de_Limnologie_-_International

Abstract: The Asian clam *Corbicula fluminea* is one of the most invasive species in freshwater aquatic ecosystems. The rapid growth, earlier sexual maturity, short life span, high fecundity and its association with human activities makes *C. fluminea* a non-indigenous invasive species likely to colonize new environments. This species, originally distributed in Asiatic ecosystems, is now a common inhabitant of American and European freshwater habitats. The present paper reviews the information related to the life cycle, ecology and potential ecological and economic impacts caused by *C. fluminea* in the invaded habitats. Furthermore, this paper also proposed future works that may be implemented in order to increase our general knowledge about the ecology of this bivalve.

Davidson, T. M., V. E. F. Brenneis, C. de Rivera, R. Draheim, and G. E. Gillespie. 2008. Northern range expansion and costal occurrences of the New Zealand mud snail Potamopyrgus antipodarum (Gray, 1843) in the northeast Pacific. Aquatic Invasions 3(3):349-353.

http://www.fws.gov/answest/Projects/AI2008Davidsonetal.pdf

Abstract: The New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843) is a common invasive species in fresh and brackish water ecosystems in Europe, Australia, Japan, and North America. In some invaded habitats, *P. antipodarum* can reach high densities (over 500,000 snails m-2) and dominate the biomass of the benthos, leading to detrimental impacts to native biota and changes in ecosystem dynamics. We report the previously unpublished occurrence of *P. antipodarum* in thirteen fresh and brackish water systems adjacent to the Pacific coast of North America including a new northern range for *P. antipodarum*: Port Alberni, Vancouver Island, British Columbia, Canada (49.2479º, -124.8395º). We hypothesize the snail was spread from the Columbia River Estuary to Port Alberni via recreational watercraft or infected fishing equipment. Its discovery in Port Alberni reveals the potential for other aquatic nuisance species in the lower Columbia River to spread to British Columbia. Resource managers

on the Pacific coast should remain vigilant and educate the public to prevent the further spread of the *P. antipodarum* as well as other aquatic invaders.

Loo, S. E., R. M. Nally, and P. S. Lake. 2007. Forecasting New Zealand mudsnail invasion range: model comparisons using native and invaded ranges. Ecological Applications 17(1):181-189.

https://www.researchgate.net/publication/6350406_Forecasting_New_Zealand_mudsnail_invasion_range_Model_comparisons_using_native_and_invaded_ranges

Abstract: Evaluations of the potential distribution of invasive species can increase the efficiency of their management by focusing prevention measures. Generally, ecological models are built using occurrence data from a species' native range to predict the distribution in areas that the species may invade. However, historical and geographical constraints can limit a species' native distribution. Genetic Algorithm for Rule-set Production (GARP), an ecological niche modeling program, was used to predict the potential distribution of the invasive, freshwater New Zealand mudsnail, *Potamopyrgus antipodarum*, in Australia and North America. We compared the strength of the predictions made by models built with data from the snail's native range in New Zealand to models built with data from the locations invaded by the species. A time-series analysis of the Australian models demonstrated that range-of invasion data can make better predictions about the potential distribution of invasive species than models built with native range data. Large differences among the model forecasts indicate that uncritical choice of the data set used in training the GARP models can result in misleading predictions. The models predict a large expansion in the range of *P. antipodarum* in both Australia and North America unless prevention measures are implemented rapidly.

Hosea, R. C., and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails on wading gear. California Department of Fish and Game, Rancho Cordova, California, USA.

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

McMahon, R. F. 2002. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Science 59:1235-1244.

Abstract: Invasive species have been characterized as tolerant of environmental extremes. This hypothesis was evaluated for invasive aquatic species in North America, particularly Asian clams, *Corbicula fluminea*, and zebra mussels, *Dreissena polymorpha*. Both species have rapid growth, early maturity, short life spans, and elevated fecundity, allowing rapid population recovery after reductions by rarefractive, environmental extremes. Extensive resistance capacities offer little adaptive value to invasive, *r*-selected species, because population reductions occur in their unstable habitats regardless of degree of stress tolerance. Thus, both species have relatively poor physiologic resistance, depending

instead on elevated growth and fecundity for rapid population recovery. In contrast, native North American bivalve species are often adapted to stable habitats where perturbation is infrequent (i.e., freshwater unionoidean bivalves). They are characterized by slow growth, extended life spans, and low effective fecundities, slowing population recoveries (*K*-selected), and have evolved extensive resistance adaptations to avoid extirpation during environmental extremes. Review of resistance adaptations in other North American aquatic invaders revealed poorer or equivalent physiological tolerance relative to taxonomically related native species, suggesting that extensive physiological tolerance is not required for invasive success.

Zooplankton Vectors:

Yan, N. D., B. Leung, M. A. Lewis, and S. D. Peacor. 2011. The spread, establishment and impacts of the spiny water flea, Bythotrephes longimanus, in temperate North America: a synopsis of the special issue. Biological Invasions 13:2423-2432.

https://era.library.ualberta.ca/downloads/vt150j467

Abstract: More than most sub-disciplines of ecology, the study of biological invasions is characterized by breadth rather than by depth. Studies of expanding ranges of invaders are common, as are post-invasion case studies, but we rarely have a deep understanding of the dynamics and regulators of the processes of invasion and resultant ecological transformations. This is unfortunate because such depth may well be needed to develop targeted, knowledge-based, management plans. In this collection we provide this needed depth of study of the key aspects of the invasion process for the spiny water flea, Bythotrephes longimanus. We do so by presenting the results of the work conducted by researchers in the Canadian Aquatic Invasive Species Network (CAISN), and several of their American and European collaborators over the past half-decade. Given its rapid spread in the Great Lakes basin in North America, and the decreases in pelagic biodiversity that have ensued, the last decade has witnessed a surge of research on Bythotrephes. In this collection we learn much about mechanisms and dynamics of its spread, about the key role of humans in that spread, about the importance of Allee effects to establishment and persistence, about choices and parameterization of risk assessment models, about the value of comparing ‘‘effects’’ in native and invaded regions, about complex probable interactions of the invasion with impending changes in the climate, and about the regulators of the invader’s abundance and impacts. There should be much of interest in the collection for aquatic ecologists and invading species biologists alike.

General Aquatic Invasive Species Spread:

Drake, D. A. R., and N. E. Mandrak. 2010. Least-cost transportation networks predict spatial interaction of invasion vectors. Ecological Applications 2286-2299.

http://www.caisn.ca/publications/Spatial.pdf

Abstract: Human-mediated dispersal among aquatic ecosystems often results in biotic transfer between drainage basins. Such activities may circumvent biogeographic factors, with considerable ecological, evolutionary, and economic implications. However, the efficacy of predictions concerning community changes following inter-basin movements are limited, often because the dispersal mechanism is poorly understood (e.g., quantified only partially). To date, spatial-interaction models that predict the movement of humans as vectors of biotic transfer have not incorporated patterns of human movement

through transportation networks. As a necessary first step to determine the role of anglers as invasion vectors across a land-lake ecosystem, we investigate their movement potential within Ontario, Canada. To determine possible model improvements resulting from inclusion of network travel, spatial interaction models were constructed using standard Euclidean (e.g., straight-line) distance measures and also with distances derived from least-cost routing of human transportation networks. Model comparisons determined that least-cost routing both provided the most parsimonious model and also excelled at forecasting spatial interactions, with a proportion of 0.477 total movement deviance explained. The distribution of movements was characterized by many relatively short to medium travel distances (median = 292.6 km) with fewer lengthier distances (75th percentile = 484.6 km, 95th percentile = 775.2 km); however, even the shortest movements were sufficient to overcome drainage-basin boundaries. Ranking of variables in order of their contribution within the most parsimonious model determined that distance traveled, origin outflow, lake attractiveness, and sportfish richness significantly influence movement patterns. Model improvements associated with least-cost routing of human transportation networks imply that patterns of human-mediated invasion are fundamentally linked to the spatial configuration and relative impedance of human transportation networks, placing increased importance on understanding their contribution to the invasion process.

Marsden, J. E., and M. Hauser. 2009. Exotic species in Lake Champlain. Journal of Great Lakes Research 35:250-265.

https://www.researchgate.net/publication/232696717_Exotic_Species_in_Lake_Champlain

Abstract: The Lake Champlain basin contains substantially fewer exotic species (N=48) than the Great Lakes (NN180), in part due to its isolation from commercial traffic. Exotic species have been introduced by authorized and unauthorized stocking, bait buckets, use of ornamental plants, and through the Champlain and Chambly canals that link the lake to the Hudson River, Mohawk River, Erie Canal, and the Great Lakes. Several species, such as water chestnut and zebra mussels, have had severe ecological, economic, and nuisance effects. The rate of appearance of new species increased in the 1990s, potentially as a result of increasing activity in the basin, improved water quality in the Champlain Canal, and increased sampling. Efforts to slow the introduction of new species have focused on public education and legislation to reduce bait bucket introductions and quarantine undesirable plants; however, the major remaining vector for introductions is the Champlain Canal. An estimated 20 species have entered the lake via canals, of which at least 12 used the Champlain Canal, and numerous species in the connected drainage systems could still enter via this route; some are already in the Erie Canal. Most recently (2008), the Asian clam was discovered two locks below Lake Champlain. The Lake Champlain canals also function as a conduit for exotic species exchange between the Hudson River, St. Lawrence River, and Great Lakes. The potential for future introductions could be reduced by a biological barrier on the Champlain Canal, and additional emphasis on public education.

Vander Zanden, M. J., and J. D. Olden. 2008. A management framework for preventing the secondary spread of aquatic invasive species. Canadian Journal of Fisheries and Aquatic Sciences 65:1512-1522.

Abstract: Biological invasions continue to accelerate, and there is a need for closer integration between invasive species research and on-the-ground management. In many regions, aquatic invasive species have established isolated populations, but have not yet spread to many sites that provide suitable habitat. In the Laurentian Great Lakes region, several Great Lakes invaders such as zebra mussel (*Dreissena polymorpha*), rainbow smelt (*Osmerus mordax*), and spiny water flea (*Bythotrephes*

longimanus) are currently undergoing secondary spread to the smaller inland lakes and streams. This paper describes recent advances in forecasting the secondary spread of aquatic invasive species and presents a framework for assessing vulnerability of inland waters based on explicit assessment of three distinct aspects of biological invasions: colonization, site suitability, and adverse impact. In many cases, only a fraction of lakes on the landscape are vulnerable to specific invasive species, highlighting the potential application of this type of research for improving invasive species management. Effective application to on-the-ground resource management will require that research aimed at assessing site vulnerability be translated into management tools.

Ricciardi, A. 2007. Are Modern Biological Invasions an Unprecedented Form of Global Change? Conservation Biology 21:329-336.

https://www.researchgate.net/publication/51375732_Are_Modern_Biological_Invasions_an_Unprecedented_Form_of_Global_Change

Abstract: The uniqueness of the current, global mass invasion by nonindigenous species has been challenged recently by researchers who argue that modern rates and consequences of nonindigenous species establishment are comparable to episodes in the geological past. Although there is a fossil record of species invasions occurring in waves after geographic barriers had been lifted, such episodic events differ markedly from human-assisted invasions in spatial and temporal scales and in the number and diversity of organisms involved in long distance dispersal. Today, every region of the planet is simultaneously affected and modern rates of invasion are several orders of magnitude higher than prehistoric rates. In terms of its rate and geographical extent, its potential for synergistic disruption and the scope of its evolutionary consequences, the current mass invasion event is without precedent and should be regarded as a unique form of global change. Prehistoric examples of biotic interchanges are nonetheless instructive and can increase our understanding of species-area effects, evolutionary effects, biotic resistance to invasion, and the impacts of novel functional groups introduced to naıve biotas. Nevertheless, they provide only limited insight into the synergistic effects of invasions and other environmental stressors, the effect of frequent introductions of large numbers of propagules, and global homogenization, all of which characterize the current mass invasion event.

Simberloff, D. 2003. How Much Information on Population Biology Is Needed to Manage Introduced Species? Conservation Biology 17:83-92.

http://unalmed.edu.co/~poboyca/documentos/documentos1/Biolog%EDa_Conservacion/03_2008/Mancera/Noviembre_7/1Simberloff2003.pdf

Abstract: Study of the population biology of introduced species has elucidated many fundamental questions in ecology and evolution. Detailed population biological research is likely to aid in fine-tuning control of widespread and/or long-established invasions, and it may lead to novel control methods. It will also contribute to an overall understanding of the invasion process that may aid in the formulation of policy and help to focus attention on invasions that are especially prone to becoming problematic. But the importance of intensive population biological research in dealing with introduced species, especially those recently introduced, is often limited. In the worst instances, the absence of population biological data can be an excuse for inaction, when a prudent decision or quick and dirty operation might have excluded or eliminated an invader. The most effective way to deal with invasive introduced species, short of keeping them out, is to discover them early and attempt to eradicate or at least contain

them before they spread. This approach has often been successful, but its success has usually relied on brute-force chemical and mechanical techniques, not on population biological research.

Johnson, L. E., A. Ricciardi, and J. T. Carlton. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecological Applications 11:1789-1799.

https://www.researchgate.net/publication/228863745_Overland_Dispersal_of_Aquatic_Invasive_Species_A_Risk_Assessment_of_Transient_Recreational_Boating

Abstract: Predictions of the geographic spread of introduced species are often limited by a lack of data on their mechanisms of dispersal. We interviewed boaters and inspected boating equipment at public boat launches on Lake St. Clair (Michigan, USA) to assess the potential for the zebra mussel, an invasive bivalve, to be dispersed overland to inland waters by transient recreational boating activities. Several mechanisms associated with recreational boating were found to be capable of transporting either larval or adult life stages. Larvae were found in all forms of water carried by boats (i.e., in live wells, bilges, bait buckets, and engines) but were estimated to be 40-lOOx more abundant in live wells than other locations. Dilution in receiving waters should, however, greatly reduce the risk of establishing new populations by the introduction of larvae. Contrary to common belief, mussel dispersal from these boat launches did not occur by direct attachment to transient boats. Rather, adult and juvenile mussels were transported primarily on macrophytes entangled on boat trailers and, less frequently, on anchors (5.3% and 0.9% of departing boats, respectively). Combining these data with estimates of survival in air and reported boater destinations, we predict that a maximum of 0.12% of the trailered boats departing these access sites delivered live adult mussels to inland waters solely by transport on entangled macrophytes. While this is a small probability, high levels of vector activity resulted in a prediction of a total of 170 dispersal events to inland waters within the summer season from the primary boat launch studied. Many other potential vectors remain to be assessed, but the dispersal of zebra mussels by trailered boats, particularly by "piggybacking" on macrophytes entangled on the trailers, must be controlled in order to limit further range expansion of the zebra mussel within North America.