Biol Invasions (2018) 20:3167–3178
https://doi.org/10.1007/s10530-018-1766-4

ORIGINAL PAPER

Invertebrates associated with aquatic plants bought from aquarium stores in Canada and New Zealand

Ian C. Duggan · Paul D. Champion · Hugh J. MacIsaac

Received: 5 March 2018 / Accepted: 21 May 2018 / Published online: 25 May 2018

Abstract Invertebrate species carried incidentally (i.e., 'hitchhikers') in the aquarium trade have gained increasing attention in recent years, but factors affecting the movement of species from stores to homes are poorly understood. We aimed to determine how macrophytes bought from stores act as vectors for transport of non-indigenous invertebrate species. We tested whether incidental invertebrate faunas carried on macrophytes vary internationally by comparing the New Zealand and Canadian trades, and if macrophyte species with different morphologies carry different risks. We recorded a large variety of invertebrate species associated with *Vallisneria* spp., Sword plants (*Echinodorus* spp.) and *Elodea canadensis* bought from stores, including species non-indigenous to both countries. Community composition of incidental fauna differed significantly between New Zealand (primarily domestically cultivated) and Canadian (primarily imported) bought macrophytes. Differences in composition between different macrophyte species were only statistically significant between wild-collected *E. canadensis* and the cultured species in New Zealand. Behaviours observed in stores, such as the amount of time macrophytes were removed from water before being placed in plastic bags for transport, did not affect the abundances or richness of incidental invertebrates transported, and thus did not appear to be effective in reducing invasion risk. We therefore recommend chemical treatment for removal of invertebrates from macrophytes at or pre-border, and from tanks containing plants at culture facilities and in stores. Such management will reduce the probability of introduction of hitchhikers to home aquaria, from which risk of release to natural waters is greatest.

Keywords Incidental fauna · Hitchhikers · Biological invasions · Ornamental plants · Aquarium trade · Copepoda

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-018-1766-4) contains supplementary material, which is available to authorized users.

I. C. Duggan (✉)
Environmental Research Institute, School of Science, The University of Waikato, Private Bag 3105, Hamilton, New Zealand
e-mail: ian.duggan@waikato.ac.nz

P. D. Champion
National Institute of Water and Atmospheric Research Ltd. (NIWA), PO Box 11-115, Hamilton 3200, New Zealand

H. J. MacIsaac
Great Lakes Institute for Environmental Research, The University of Windsor, Windsor, ON N9B 3P4, Canada

Introduction

For the successful invasion of any species, individuals must pass through a series of sequential steps: entrainment, transportation, introduction and establishment. Understanding biological invasions is best achieved by examining each component phase separately (Carlton 1996; Kolar and Lodge 2001). In the aquarium trade, the transportation phase can itself be divided into multiple sub-steps, typically including the movement of individuals from their native range to overseas aquaculture facilities, to wholesalers and stores, then homes, before eventual possible release into waterways (Duggan 2011). As such, full comprehension of invasions via the aquarium trade requires not only an understanding of the dynamics of species between transitions, but also of factors occurring within the transportation phase.

For species carried intentionally in the aquarium trade (e.g., fish, macrophytes, crayfish and some snail species), exporters, importers, sellers and buyers all will want to maximise the survivability of species from the native ranges to homes. Nevertheless, while it is likely only a small subset of individuals in home aquaria that are transported to natural waters, invasions from the aquarium trade are still commonplace (Duggan et al. 2006; Cohen et al. 2007; Strecker et al. 2011). For fauna carried incidentally or accidentally in the aquarium trade (i.e., 'hitchhiking' species; including invertebrates such as snails, copepods and rotifers), no such care will be taken during transportation (Patoka et al. 2016b). Greater variability thus likely exists among each of the sub-transitions during transportation, dependent on (for example) the behaviours of the exporters, management of aquaculture facilities, pre-border and border management of aquarium fauna, flora and water, and of store workers selling the products to the aquarium owners. Duggan (2010) found a variety of invertebrates living in the water and, in particular, bottom sediments of home aquaria in New Zealand, including copepods, ostracods, cladocerans, mites, flatworms and nematodes; among these were known established, non-indigenous species, and species not previously recorded from New Zealand. While little is known of the movement of incidental fauna within the aquarium trade, interest in this area is growing rapidly. Buczyński and Bielak-Bielecki (2012), for example, recorded an Asian dragonfly in an aquarium store in Poland, from an aquarium containing recently imported macrophytes. Patoka et al. (2016a) recorded a variety of invertebrates entering the Czech Republic associated with water hyacinth imported from Indonesia, for the pond trade, including rotifers, nematodes, gastropods, annelids, cladocerans, copepods and insects. Ng et al. (2016), Patoka et al. (2017) and Yanai et al. (2017) all examined molluscs in aquarium trade wholesalers in the Singapore, Czech Republic, and Israel, respectively, and each recorded a variety of snail species not intentionally carried. Finally, Abe et al. (2017) recorded a non-indigenous nereidid polychaete in a private goldfish aquarium in Japan. Nevertheless, besides Duggan (2010), such papers have primarily recorded the presence of hitchhiking species, and have not sought to examine patterns regarding hitchhiking taxa that may aid in risk management.

A major finding by Duggan (2010) was that there was a greater likelihood of non-indigenous invertebrates occurring in home aquaria if they had aquatic macrophytes introduced to them. As such, macrophytes have been indirectly identified as responsible for the movement of non-indigenous species to homes, though plants from aquarium stores have yet to be assessed directly for the presence of these invertebrates. Further, that study sampled organisms from the water column and bottom sediments of home aquaria but, due to the destructive nature of this sampling, did not sample from the macrophytes themselves. Different countries commonly source macrophytes from different regions. For example, in an analysis of European countries, most aquatic plants sold in the aquarium trade were imported from Singapore, Indonesia and Thailand (Brunel 2009), while in New Zealand importation has been limited since the 1990s and most macrophytes are domestically cultivated (Champion and Clayton 2001). Such differences in source material may provide variability in the species transported to stores and homes in association with macrophytes globally. Risk may also vary depending on the macrophyte species bought. For example, more structurally complex macrophytes may carry higher species richness and greater abundances of invertebrates than those that are less complex (Lucena-Moya and Duggan 2011), and thus vary in their risk. Such factors have not been systematically investigated for the aquarium trade.

We surveyed small invertebrates from aquatic macrophytes sold in aquarium stores in the Great Lakes region of Canada, and New Zealand. Both areas have a history of aquatic invasions, and recent invaders in both locations have primarily been small invertebrate species (e.g., Grigorovich et al. 2003; Connolly et al. 2017; Duggan and Collier 2018). Since the presence of non–native invertebrates in home aquaria is associated with the presence of store-bought macrophytes (Duggan 2010), our first major aim was to determine whether macrophytes bought from stores act as vectors for movement of incidental fauna, including of non-indigenous species. Secondly, as the relative importance of importation and domestic cultivation of macrophytes for the aquarium trade varies among countries, we determined whether differences exist between the incidental fauna carried between two distinct geographical regions, New Zealand and Canada. Thirdly, as field observations have found that some macrophyte species have different species richness and abundances of invertebrates than others, we aimed to determine whether different species of macrophytes bought from aquarium stores carry different species. Further, practices that varied among stores that may influence the abundances of incidental taxa were examined, such as whether fish were kept in tanks with macrophytes, the amount of time taken to move macrophytes from tanks to plastic bags, and whether water was added to plastic bags for transportation.

Methods

Sampling of Canadian macrophytes from stores was undertaken between 28 October and 12 November 2010, and from New Zealand stores between 22 and 28 February 2016. Stores in both countries ranged from small, privately owned retailers to large chains. In Canada, 15 stores were visited, located in Ontario, between Windsor, Toronto and Niagara Falls (all in close proximity to the Great Lakes). Sixteen New Zealand stores were visited, located between Auckland and Wellington. Macrophytes were chosen that had grossly different architecture. In the Canadian stores, various 'Sword plants' (*Echinodorus* spp.) were bought from ten stores, and *Vallisneria* (*Vallisneria* spp.) were bought from nine. Although Sword plants and *Vallisneria* may have comprised of more than one species each, the general morphology was similar within each taxon; *Vallisneria* spp. have long flat narrow leaves, while Sword plants have larger, denser lanceolate leaves. In New Zealand, Sword plants were bought from 14 stores, *Vallisneria* from six, and Canadian pondweed (*Elodea canadensis*) from seven; *Elodea* (all *E. canadensis*) is the only oxygen weed allowable for sale in New Zealand and has a greater relative complexity than the other macrophytes bought, with leaves arranged in whorls of three around a central stem. Where different macrophytes were bought from the same store, they were either bought at separate times, or it was requested that they be put in separate bags, to avoid cross-contamination of samples. Within any store, macrophytes were typically stocked in tanks designated for communities of aquatic plant species. It is unknown how long any individual macrophyte was stocked in the store before purchase.

When buying the macrophytes, actions of store workers were noted that might influence the number of invertebrates being transported with the plants. Specifically, we considered the time required to move each plant from the water before it was placed in a plastic bag, and whether any subsequent water was added (on the eight occasions that this occurred, water was added from aquaria seven times, and from the tap once). It was also noted whether fish were stocked with the plants that were purchased. After leaving the store, volumes of any water added (if added intentionally) to the Canadian bags was measured, while the volumes of all water in bags was measured for the New Zealand stores. Although water temperature is likely an important variable determining invertebrate species composition and diversity, this variable could not generally be measured due to the very low volumes of free water contained in the bags with macrophytes (median = 3 ml). Macrophytes were placed into 200 or 400 ml PET containers, the insides of plastic bags washed into the containers, and ethanol added to preserve the invertebrates.

On return to the laboratory, plants were washed gently by rubbing the leaves in water, and the washings passed through a 40 μm mesh to collect invertebrates of all size ranges. Material retained was again preserved. Wet weights of macrophytes were measured following the drying of plant surfaces with a paper towel, to determine whether relationships exist between weights of macrophytes sold and richness and abundances of invertebrates moved to homes. All invertebrates were counted in all samples. For identification, particular attention was paid to microcrustaceans and rotifers, as (1) these groups were found to be well represented among non-indigenous species in home aquaria in New Zealand by Duggan (2010), and (2) a number of non-indigenous crustaceans are known from the Great Lakes and New Zealand for which no vector is known (National Research Council 2011). Further, 84% of individuals collected in this survey belonged to these groups. Snails, although commonly moved via plants in the aquarium trade, were typically found on macrophytes as eggs or juveniles, making identification based on morphology generally impossible.

Analyses were conducted on a dataset including all of the invertebrates collected, not only the non-indigenous species, as we believe reducing the prevalence of all individuals carried is the best method for managing the movement of species by this vector. We utilised a t-test to determine whether differences existed between New Zealand and Canadian stores with respect to the amount of time taken for plants to be removed from aquaria to being placed in plastic bags. We used a Fishers Exact test to determine whether the proportion of macrophyte tanks that held fish differed between New Zealand and Canada. Relationships between the number of individual invertebrates collected, and of the species richness, with country and macrophyte type, were analysed using Kruskal–Wallis tests. Multiple regression analyses were used to determine whether the number of individual invertebrates or richness could be predicted based on wet weight of plants, the amount of time plants were removed from the water before being placed in bags, and of the volume of water found within bags. Simple linear regression was used to determine if there was a relationship between the number of individuals collected per bunch of macrophyte and the species richness; this was done to determine whether efforts to reduce the number of individuals transported would also be effective in reducing the variety of species transported. All univariate analyses were undertaken using Statistica 13 (StatSoft, Tulsa, OK). Time taken for macrophytes to be placed in bags, volumes of water in the bags, and the number of individual invertebrates collected were log transformed for parametric analyses to improve normality.

Non-metric multi-dimensional scaling (MDS) was used to visually explore patterns in invertebrate composition among countries and plants (Clarke and Warwick 1994). MDS produces a two-dimensional representational plot of all samples based on their similarity to each other, based on an underlying similarity matrix. Two-way permutational multivariate analysis of variance (PERMANOVA) was used to determine whether the effects of plant type and country on invertebrate community composition, as observed in the MDS ordination, were statistically significant. Due to *Elodea* only being sampled in New Zealand, only Sword and *Vallisneria* were used in our analysis of the influence of plant type and country on invertebrate composition. To include *Elodea*, Analysis of Similarities (ANOSIM) was subsequently used to test for plant effects on invertebrate community composition on *Vallisneria* spp., Sword plants and *Elodea* for the New Zealand data. MDS, PERMANOVA and ANOSIM analyses were based on Bray–Curtis dissimilarities calculated on $\log(x + 1)$ transformed data; this transformation was undertaken to reduce any undue contribution to the analysis of species with high abundances. Fourteen species were removed from the multivariate analyses that comprised only single individuals from single samples; this was done to remove any undue influence of rare taxa that may have been sampled only by chance in some samples but not others. Further, indeterminate copepod juveniles and nauplii were also removed due to these taxa possibly representing a variety of species, leaving 51 taxa. All multivariate analyses were performed on communities on a ‘per bunch’ basis (i.e., per unit of macrophyte sold by the store), as we were interested in trends relating to what is carried home by the buyer. For all multivariate tests, 999 permutations (i.e., random restarts to the analyses) were executed. Multivariate analyses were undertaken using PRIMER (ver. 6.1.13; PRIMER-E, Plymouth, UK).

Results

Environmental conditions

Median wet-weights of macrophytes bought were 3.8 g for *Vallisneria* from New Zealand, 4.7 g for *Vallisneria* in Canada, 7.9 g for Sword in New

Zealand, 9.9 g for Sword in Canada and 18.3 g for *Elodea* in New Zealand. Nevertheless, Kruskal–Wallis and associated multiple comparisons tests indicated significant weight differences existed only between *Vallisneria* from both Canada and New Zealand with *Elodea* sold in New Zealand ($P < 0.005$). Canadian stores remove plants from the water for 12.2 s on average, while this value was much lower for New Zealand stores (3.8 s); emersion time for New Zealand plants was significantly shorter (*t* test, *P* value = 0.002). For seven of the 19 plants bought in Canada, between 250 and 800 ml of water were added to the plastic bag from the tank (average 493 ml). This practice was not done for any of the 27 plants bought in New Zealand. Eight of the nineteen plants bought in Canada had fish swimming among the plants in stores, while nine of twenty-six plants bought in New Zealand did (data in one New Zealand store was not recorded; Fishers Exact test, two-tailed *P* value = 0.757).

Invertebrates

A total of 61 taxa were identified, including 30 rotifer, four cladoceran and seven copepod species (Table 1). A variety of snails were also found, but confident identifications could not be made due to them comprising largely juveniles. Cnidarians (*Hydra* and polyps of *Craspedacusta sowerbii*), insect larvae, leeches, oligochaetes, Bryozoa, mites, nematodes, tardigrades and flatworms were also observed. In Canada, 19 rotifer taxa, two cladocerans, and five copepod taxa were identified, while in New Zealand, 24 rotifer, three cladoceran and four copepod taxa. A number of potentially non-indigenous species were identified. From New Zealand aquarium plants, we recorded the rotifers *Lecane acuelata*, *L. monostyla*, *L. quadridentata*, *L. shieli*, *L. signifera*, and the copepods *Elaphoidella sewelli* and *Nitokra pietshmanni*, which are currently not known from this country outside of aquaria. Species not native to the Great Lakes and found associated with the Canadian aquarium plants were the rotifers *Cephalodella hoodi*, *Dicranophorus epicharis*, *Lecane monostyla, Nitokra pietshmanni,* the copepod *Schizopera* sp., gastropod *Melanoides tuberculata* and cnidarian *Craspedacusta sowerbii.*

Multiple regression analyses indicated no significant relationships between richness ($P = 0.543$) or abundance ($P = 0.791$) with wet weight of plants, time taken for plants to be moved from the aquaria to plastic bags, or the volumes of water moved. Kruskal-Wallis tests indicated no significant differences occurred between richness ($P = 0.209$) or abundance ($P = 0.098$) of invertebrates and the plant taxon or country. Simple regression indicated that there was a significant positive relationship between the number of individuals collected on plants and the richness of invertebrates collected ($P < 0.001$).

The MDS analysis undertaken for Canadian and New Zealand *Vallisneria* and Sword plants exhibited some separation of invertebrate community composition by country, with Canadian samples primarily occurring on the left and New Zealand samples on the top right of the ordination (Fig. 1). Some separation also seemed to be apparent between plant type, with *Vallisneria* samples more commonly at the bottom of the ordination, and Sword at the top. Two factor PERMANOVA indicated that community composition significantly differed between the countries ($P = 0.001$), but not by plants ($P = 0.081$); the interaction term was not significant ($P = 0.403$). In the MDS of incidental fauna on macrophytes in New Zealand, no separation could be discerned between invertebrate communities on *Vallisneria* and Sword plants, though *Elodea* samples were distributed on the left of the ordination (Fig. 2). One-way ANOSIM again indicated that there was no difference in communities between *Vallisneria* and Sword plants ($P = 0.279$), though differences were evident between *Elodea* and Sword ($P = 0.002$) and between *Elodea* and *Vallisneria* ($P = 0.001$). Native species such as the rotifer *Cephalodella gibba*, the cladocerans *Alona gutatta* and *Chydorus* sp., and caddisfly larvae, were found to occur frequently (> 25% of samples) on *Elodea* but were not observed on the other two macrophyte species in New Zealand stores (Table 1).

Discussion

Non-indigenous invertebrates on aquarium plants

We recorded a variety of non-indigenous species on macrophytes bought from both Canadian and New Zealand aquarium stores. Of greatest concern are the harpacticoid copepods *Nitokra pietshmanni* (both countries), *Elaphoidella sewelli* (New Zealand only) and a *Schizopera* species (Canada only). Both of the copepod species found on New Zealand macrophytes

Table 1 List of invertebrate species identified from aquarium plants (Sword plants, *Vallisneria* and *Elodea*) bought from Canadian and New Zealand stores

	Sword Canada (%)	*Vallisneria* Canada (%)	Sword New Zeal. (%)	*Vallisneria* New Zeal. (%)	*Elodea* New Zeal. (%)
Rotifera					
Bdelloids	**	**	**	**	**
Cephalodella forficula			*	*	*
Cephalodella gibba	*	*			**
Cephalodella hoodi		*			
Colurella uncinata		*	*		*
Cupelopagis vorax	**	**	*	**	
Dicranophorus epicharis	*	*	*	*	
Lecane arcuata			*	*	*
Lecane acuelata			*		
Lecane bulla	*	**	**	**	**
Lecane closterocerca	**	*	**		**
Lecane flexilis	*	*			
Lecane hamata	*	**	**	*	**
Lecane hornemanni			*		
Lecane ludwigii ludwigii			**	*	*
Lecane ludwigii ohioensis			*		
Lecane lunaris			*	*	
Lecane monostyla		*	*	*	
Lecane pyriformis		*	*	**	*
Lecane shieli				**	
Lecane signifera			*	**	
Lecane stichea	*	**		*	
Lecane quadridentata			**	**	*
Lepadella ovalis	**	*	**	*	**
Lepadella patella	*				
Mytilina ventralis				*	
Notommata tripus		*			
Proales sp.	*				
Ptygura sp.		*	*	**	
Trichocerca sp.		*			
Cladocera					
Alona gutatta	*	*			**
Chydorus sp.					**
Dunhevedia crassa	*				
Graptolebris testudinaria			*		*
Copepoda					
Elaphoidella sewelli gp.			*		
Eucyclops serrulatus	**	*	**		**
Microcyclops rubellus	*				
Microcyclops varicans			*		
Nitokra pietshmanni	**	**	*	**	
Phyllognathopus vigueri	*				

Table 1 continued

	Sword Canada (%)	*Vallisneria* Canada (%)	Sword New Zeal. (%)	*Vallisneria* New Zeal. (%)	*Elodea* New Zeal. (%)
Schizopera sp.	*	*			
Ostracoda	**	**		**	*
Mollusca					
Gyraulus spp.			*		
Lymnaeidae	*				
Melanoides tuberculata	*				
Physa acuta	**	*	**	*	*
Planorbidae	*	*	**	**	**
Potamopyrgus anitipodarum			*	*	**
Indeterminate 'limpets' (Tribe Ancylini)		*	*	*	
Cnidaria					
Craspedacusta sowerbii polyps		*			
Hydra sp.	**	*		**	*
Insecta					
Trichoptera (Caddisflies)					**
Chironomidae (Chironomids)			*		**
Diptera (True Flies)			*		
Other taxa					
Annelida: Hirudinea				*	
Annelida: Oligochaeta	**	**	**	**	**
Bryozoa: *Plumatella* sp.	*				
Mites	**	**	**	**	*
Nematoda	**	**	*	**	
Tardigrada		*			
Platyhelminthes	*		**		**

A single asterisk (*) indicates a species was present, but in less than 25% of samples. Two asterisks (**) indicates a species was present in greater than 25% of samples

have also been found in the bottom sediments of home aquaria in that country by Duggan (2010), demonstrating a clear link between fauna found in stores and homes. *Nitokra pietshmanni*, recorded widely on aquarium plants in both countries (nine stores in Canada; three in New Zealand), is known from Asia (Japan, China, South Korea) and the Hawaiian Islands (Chang and Yoon 2008), but no non-indigenous records are known outside of home aquaria. *Elaphoidella sewelli*, found only in one New Zealand store, is known from Asia, Africa, and India (Lang 1948). The shared Asian native distribution of *N. pietschmanni* and *E. sewelli* is consistent with the predominant global regions for aquarium fish culture facilities and exports (Cheong 1996). One *Schizopera* species was recorded, only in Canada (three stores); *Schizopera borutzkyi* has been found previously in North America (Horvath et al. 2001). Nevertheless, our specimens did not match any known species, including *S. borutzkyi*; that species has been found commonly in ballast tanks (e.g., Duggan et al. 2005), and the aquarium trade was thus unlikely to have contributed to its introduction. *Schizopera* is a species-rich and predominantly marine genus, although it has two significant inland water diversity hotspots, including in Africa's Lake Tanganyika (Karanovic et al. 2015), which itself is a

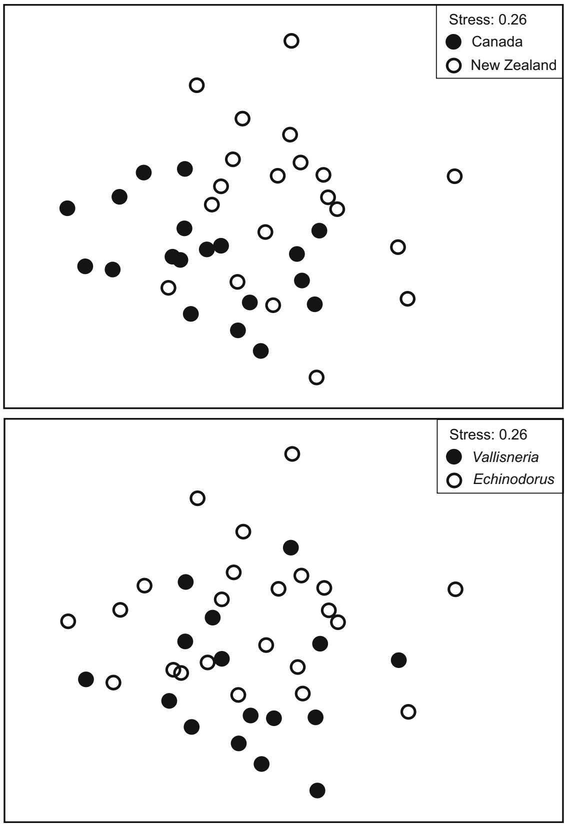

Fig. 1 Non-metric multidimensional scaling (MDS) plot representing invertebrate assemblages collected from *Vallisneria* and Sword plants bought from stores in Canada and New Zealand. The MDS is overlaid with symbols representing country (top panel) and macrophyte species (botton panel). Two factor PERMANOVA indicated that community composition differed significantly by country ($P = 0.001$), but not by plant type ($P = 0.081$)

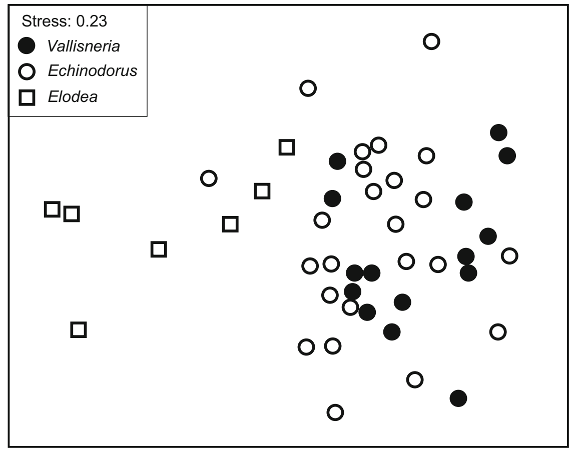

Fig. 2 Non-metric multidimensional scaling (MDS) plot representing invertebrate assemblages collected from *Vallisneria*, Sword plants and *Elodea* bought from stores in New Zealand. The MDS is overlaid with symbols representing different macrophyte species. One-way ANOSIM indicated that there was no difference in communities between *Vallisneria* and Sword plants ($P = 0.279$), but differences in community composition were evident between Elodea and Sword ($P = 0.002$) and *Elodea* and *Vallisneria* ($P = 0.001$)

source of many cichlids in the aquarium trade. In a survey of invertebrates imported into the Czech Republic in a shipment of water hyacinth (for garden ponds), numerous cyclopoid and harpacticoid copepods were recorded by Patoka et al. (2016a); as such, the harpacticoid copepods we recorded were likely imported into each respective country in a similar manner.

A number of the rotifer species we identified have not previously been found in New Zealand or the Great Lakes region. Some may potentially be native to each respective region, however, as several of the species found in this study have broad distributions and only limited research has been undertaken of the plant-associated littoral rotifer fauna in each region. For example, while *Dicranophorus epicharis* has apparently not been recorded from the Great Lakes, the type locality for this species is Maine, USA, and it is considered to have a cosmopolitan distribution (De Smet 1997). Similarly, *Cephalodella hoodi* is considered by Nogrady and Pourriot (1995) to be cosmopolitan but rare. Of the New Zealand specimens, *Lecane quadridentata* is described by Segers (1995) as eurytopic, cosmopolitan, and relatively common. Nevertheless, other species that we recorded—including *Lecane monostyla* (both countries), *L. signifera* and *L. aculeata* (New Zealand only)—are noted by Segers (1995) to be more common in "tropical and subtropical waters". This suggests that these species may not be native to New Zealand or to the Great Lakes region, though they may not be able to survive there. An interesting record in New Zealand was that of *L. shieli*, recorded to date from only a limited number of sites globally, including Thailand (Segers 1995), Australia (Kobayashi et al. 2007) and Poland (Ejsmont-Karabin 2011). In Poland, this species is considered non-indigenous, and associated with *Vallisneria* in water heated by a power station (Ejsmont-Karabin 2011); this species was also strongly associated with *Vallisneria* in our New Zealand aquarium plant survey. In the Patoka et al. (2016a) survey of invertebrates imported into the Czech Republic with water hyacinth, rotifers were among the most abundant animal group found, with *Lecane* species dominating.

Because of their small size and immaturity, confident identification of many gastropods was not possible. It is presumably in the retailers' best interests not to sell invertebrates that are visible that are also generally considered contaminants and pests in home aquaria. Nevertheless, *Melanoides tuberculata* could confidently be identified from some macrophytes in Canada and has been observed in aquarium stores in that region previously (Rixon et al. 2005). This species was predicted by MacIsaac (1999) to be a potential invader of Lake Erie, though Rixon et al. (2005) believed it would fail to establish due to intolerance to cold temperatures. Large, identifiable individuals of this species were common in New Zealand home aquaria (Duggan 2010), and it has established in one geothermal spring in that country, with the aquarium trade inferred as the vector for introduction (Duggan 2002). To accurately assess gastropods sold with macrophytes from aquarium stores, these should either be grown to adulthood in the laboratory, or identified genetically (e.g., Ng et al. 2016).

We observed a large number of *Craspedacusta sowerbii* polyps (205 individuals) on a single sample of *Vallisneria* bought from one Canadian store. Medusa of *C. sowerbii* have been noted in aquaria previously (Fish 1971; Rahat and Campbell 1974). In the Great Lakes, this species was first recorded in Lake Erie in 1933 (Grigorovich et al. 2003). No attempt was made to identify oligochaetes during this study, due to most specimens consisting of juveniles, but these were present in most samples. However, many small *Pristina* species were observed, of which three species are believed to have invaded the North American Great Lakes (Grigorovich et al. 2003); the aquarium trade provides a possible vector for these species.

While we collected a variety of non-indigenous invertebrate species, our sampling was undertaken over restricted time periods. Nothing is currently known of the seasonality of invertebrates in aquaria, and as such it is not inconceivable that a greater variety of nonindigenous species might be found with greater temporal sampling effort.

Differences in incidental fauna on macrophytes between New Zealand and Canada

Overall, the general groups of taxa identified in this study are very similar to those found by Duggan (2010) from home aquarium sediments and water in New Zealand, and by Patoka et al. (2016a) from water hyacinth imported into the Czech Republic from Indonesia. These included copepods, rotifers, gastropods, cladocerans, ostracods, annelids, bryozoans, mites, nematodes, tardigrades and flatworms. Nevertheless, using a systematic approach, we found statistically significant differences between the incidental fauna carried between two distinct geographical regions, New Zealand and Canada. Rotifers such as *Cephalodella forficula*, *Lecane arcuata*, *L. ludwigii ludwigii* and *L. quadridentata* were each recorded on all three macrophyte species in New Zealand, but none of these species were found in Canada. In contrast, *Lecane flexilis* and *Schizopera* sp. were recorded from both macrophyte taxa sampled in Canada, but not from New Zealand plants. Incidental fauna likely differed between countries due to variability in the donor regions from which plants and fish were sourced. In the Canadian survey, some of the plants were sold with labels identifying that they were cultured in Florida, USA. In turn, aquarium plants are imported into Florida in great number each year (Maki and Galatowitsch 2004). In New Zealand, aquarium macrophytes are now almost exclusively domestically cultured, and any importation is likely to be minimal; aquatic plant importers regard the current importation regulations as impractical and expensive when compared with the value of domestically cultured stock (Champion and Clayton 2001). Nevertheless, despite domestic cultivation over many years, macrophytes still harboured a variety of non-indigenous species, indicating populations of these species are persistent over long time periods in culture facilities and/or stores. Despite differences, there was an overall cosmopolitan nature to the aquarium fauna. Eighteen taxa identified to at least genus level were shared between the two countries (including 14 to species level). This included *Lecane monostyla* and *Nitokra pietshmanni*, which are non-indigenous to both regions. Overall, however, our analysis indicates that the nature of the risk may vary among countries; while commonalities do exist, analyses should be undertaken of incidental fauna transported in other countries to determine specific risks to each region.

Differences in incidental faunas between macrophyte species

Elodea in New Zealand carried different incidental fauna than that associated with *Vallisneria* and Sword plants. Macrophytes with more complex architecture commonly hold greater species richness and abundances of invertebrates than less complex plants (e.g., Lucena-Moya and Duggan 2011; Ferreiro et al. 2014), which might provide some explanation for the different communities observed of the densely leaved *Elodea*. Despite differences in the architecture of *Vallisneria*, with flat narrow leaves, and Sword plants, with larger, denser lanceolate leaves, we observed no significant differences between these species in New Zealand or Canada. For *Elodea* in New Zealand, the rotifer *Cephalodella gibba*, the cladocerans *Alona gutatta* and *Chydorus* sp., and caddisfly larvae—all native species—occurred frequently (> 25% of samples), but were not observed on the other two macrophyte species in New Zealand stores. As such, a more parsimonious explanation for differences in *Elodea* is that this species, unlike other macrophyte species sold in the New Zealand aquarium trade, is wild-collected rather than cultivated (PDC, personal observation). Prior to the declaration of *Vallisneria australis* as an unwanted organism under the Biosecurity Act 1993, this species was also wild-harvested for trade from non-indigenous populations in New Zealand (Champion 2012). This practice of wild collection for sale to the aquarium trade is still commonplace in Australia (Petroeschevsky and Champion 2008).

Management of risks and behaviours from aquarium plants in stores

It is likely that the behaviours of aquarium store operators, which could lead to the release of non-indigenous incidental species to waterways, are different to that by home aquarium owners. For example, Duggan (2010) found 37% of home aquarium owners disposed of their aquarium wastes exclusively in the garden and deemed this a low risk method of cleaning. Due to the locations of stores, in commercial settings, operators are far more likely to pour washings down drainage systems; this was the method undertaken by 44% of home aquarium owners. This may provide a risk of establishment, albeit generally low, and is dependant on the degree of sewage treatment utilised. Further, aquarium store operators are unlikely to pour washings from aquaria into external ponds or into storm-water drains, as was also observed from aquarium owners. The greatest risk for release into natural waters from stores is from invertebrates firstly being introduced to homes with macrophytes, establishing populations in home aquaria, and subsequently being released into outdoor habitats through the risky behaviours identified by Duggan (2010), or with release of macrophytes into natural waterways (Rixon et al. 2005). Of the rotifers, cladocerans and copepods recorded to species level on plants from New Zealand aquarium stores in the current study, 64% (18 of 28) were also found in the sediments or water column of New Zealand home aquaria by Duggan (2010). This indicates that there is likely a large movement of incidental species from stores to homes with aquarium inhabitants. Store owners could thus provide a role in managing this risk, by reducing this movement. Most aquarium stores held macrophytes out to dry for some period before placing them in plastic bags (and for significantly longer time periods in Canada), though this did not appear to lead to significant declines in the abundances or richness of incidental fauna being transported; large numbers of invertebrates were still found despite this practice being commonplace. Other potentially risky behaviours, such as adding water to the bags with macrophytes, were also not associated with greater abundances or richness of incidental invertebrates. Nevertheless, any patterns may have been masked by other independent, unmeasured behaviours, such as the frequency of cleaning of the aquaria containing macrophytes or the temperature of the tanks they were held in.

Aquarium plants for sale were commonly kept separate from any fish, and as such these tanks could be treated chemically to remove invertebrates from tanks, reducing the richness abundances of fauna moved. Alternatively, the onus should be on the horticulture industry to treat plants prior to transportation to stores, such as at culture facilities, to remove non-native species from their premises. Any new macrophytes introduced to the facilities from elsewhere would also require treatment, as should plants leaving the facilities. Greater emphasis should also be made to treat macrophytes pre-border, in situations where plants are imported from elsewhere, as is the case in Canada.

Acknowledgements We thank Jiri Patoka and an anonymous reviewer for providing comments that improved our manuscript. HJM was supported by an NSERC Discovery Grant and Canada Research Chair.

References

Abe H, Tanaka M, Ueno Y (2017) First report of the non-native freshwater nereidid polychaete *Namalycastis hawaiiensis* (Johnson, 1903) from a private goldfish aquarium in eastern Japan. BioInvasions Rec 6:217–223

Brunel S (2009) Pathway analysis: aquatic plants imported in 10 EPPO countries. EPPO Bull 39:201–213

Buczyński P, Bielak-Bielecki P (2012) *Crocothemis servilia* (Drury, 1773) (Odonata: Libellulidae) introduced with aquarium plants to Lublin (Poland). Ann Univ Mar Curie Skłodowska Sect C 67:21–26

Carlton JT (1996) Pattern, process, and prediction in marine invasion ecology. Biol Conserv 78:97–106

Champion PD (2012) The identity of *Vallisneria* in New Zealand. Unpublished report to Ministry for Primary Industries, 4 p

Champion PD, Clayton JS (2001) Border control for potential aquatic weeds: stage 2. Weed risk assessment. Sci Conserv 185:1–30

Chang CY, Yoon HJ (2008) *Nitokra* copepods (Harpacticoida: Ameieridae) from Korea. Korean J Syst Zool 24:115–127

Cheong L (1996) Overview of the current international trade in ornamental fish, with special reference to Singapore. Rev Sci Tech 15:445–481

Clarke KR, Warwick RM (1994) Change in Marine Communities: an Approach to Statistical Analysis and Interpretation, 2nd edn. Plymouth Marine Laboratory, National Research Council, Plymouth

Cohen J, Mirotchnick N, Leung B (2007) Thousands introduced annually: the aquarium pathway for non-indigenous plants to the St Lawrence Seaway. Front Ecol Environ 5:528–532

Connolly JK, Watkins JM, Hinchey EK, Rudstam LG, Reid JW (2017) New cyclopoid copepod (*Thermocyclops crassus*) reported in the Laurentian Great Lakes. J Great Lakes Res 43:198–203

De Smet WH (1997) The Dicranophoridae. In: De Smet WH, Pourriot R, Nogrady T (eds) Volume 5: The Dicranophoridae and the Ituridae (Monogononta). Guides to the identification of the microinvertebrates of the continental waters of the World, vol 12. SPB Academic Publishing Bv, Hague, pp 1–325

Duggan IC (2002) First record of a wild population of the tropical snail *Melanoides tuberculata* in New Zealand. N Z J Mar Freshw Res 36:825–829

Duggan IC (2010) The freshwater aquarium trade as a vector for incidental invertebrate fauna. Biol Invasions 12:3757–3770

Duggan IC (2011) Aquaria. In: Simberloff D, Rejmanek M (eds) Encyclopedia of biological invasions. University of California Press, California, pp 32–35

Duggan IC, Collier KJ (2018) Management of non-indigenous lacustrine animals. In: Howard-Williams C, Quinn J, Collier K, Hamilton D (eds) Lake restoration handbook: a New Zealand perspective. Springer, Berlin

Duggan IC, van Overdijk CDA, Bailey SA, Jenkins PT, Limén H, MacIsaac HJ (2005) Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. Can J Fish Aquat Sci 62:2463–2474

Duggan IC, Rixon CAM, MacIsaac HJ (2006) Popularity and propagule pressure: determinants of invasion success in aquarium fish. Biol Invasions 8:393–398

Ejsmont-Karabin J (2011) Does invasions of *Vallisneria spiralis* L. promote appearance of rare and new rotifer (Rotifera) species in littoral of the lakes heated by power station (Konin Lakes, W. Poland)? Pol J Ecol 59:201–207

Ferreiro N, Feijoó C, Giorgi A, Rosso J (2014) Macroinvertebrates select complex macrophytes independently of their body size and fish predation risk in a Pampean stream. Hydrobiologia 740:191–205

Fish GR (1971) *Craspedacusta sowerbyi* Lankester (Coelenterata: Limnomedusae) in New Zealand lakes. N Z J Mar Fresh Res 5:66–69

Grigorovich IA, Korniushin AV, Gray DK, Duggan IC, Colautti RI, MacIsaac HJ (2003) Lake superior: An invasion coldspot? Hydrobiologia 499:191–210

Horvath TG, Whitman RL, Last LL (2001) Establishment of two invasive crustaceans (Copepoda: Harpacticoida) in the nearshore sands of Lake Michigan. Can J Fish Aquat Sci 58:1261–1264

Karanovic T, Kim K, Grygier MJ (2015) A new species of *Schizopera* (Copepoda: Harpacticoida) from Japan, its phylogeny based on the mtCOI gene and comments on the genus *Schizoperopsis*. J Nat Hist 49:41–42

Kobayashi T, Shiel RJ, Segers H (2007) First record of the rotifer *Lecane shieli* Segers & Sanoamuang, 1994 from Australia. Aust Zool 34:181–183

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

Lang K (1948) Monographie der Harpacticiden. Hakan Ohlsson, Lund, p 1682

Lucena-Moya P, Duggan IC (2011) Macrophyte architecture affects the abundance and diversity of littoral microfauna. Aquat Ecol 45:279–287

MacIsaac HJ (1999) Biological invasion in Lake Erie: past, present and future. In: Munawar M, Edsall T, Munawar IF (eds) State of Lake Eri: past, present and future. Backhuys Publishers, Leiden, pp 305–322

Maki K, Galatowitsch S (2004) Movement of invasive aquatic plants into Minnesota (USA) through horticultural trade. Biol Conserv 118:389–396

National Research Council (NRC) (2011) Assessing the relationship between propagule pressure and invasion risk in ballast water. National Academy of Sciences, Washington, D.C.

Ng TH, Tan SK, Wong WH, Meier R, Chan S-Y, Tan HH, Yeo DCJ (2016) Molluscs for sale: assessment of freshwater gastropods and bivalves in the ornamental pet trade. PLoS

ONE 11:e0161130. https://doi.org/10.1371/journal.pone.0161130

Nogrady T, Pourriot R (1995) Rotifera 3: The Notommatidae. In: Nogrady T, Dumont HJ (eds) Guides to the identification of the microinvertebrates of the continental waters of the world. SPB Academic Publishing, Amsterdam

Patoka J, Bláha M, Kalous L, Vrabec V, Buřič M, Kouba A (2016a) Potential pest transfer mediated by international ornamental plant trade. Sci Rep 6:25896. https://doi.org/10.1038/srep25896

Patoka J, Bláha M, Devetter M, Rylková K, Čadková Z, Kalous L (2016b) Aquarium hitchhikers: attached commensals imported with freshwater shrimps via the pet trade. Biol Invasions 18:457–461

Patoka J, Kopecký O, Vrabec V, Kalous L (2017) Aquarium molluscs as a case study in risk assessment of incidental freshwater fauna. Biol Invasions 19:2039–2046

Petroeschevsky A, Champion PD (2008) Preventing further introduction and spread of aquatic weeds through the ornamental plant trade. In: van Klinken RD, Osten VA, Panetta FD, Scanlan JC (eds) Hot topics in the tropics, proceedings of the 16th Australian weed conference, 18–22 May 2008. Cairns Convention Centre, Cairns, Queensland, Australia, pp 399–402

Rahat M, Campbell RD (1974) Three forms of the tentacles and non-tentacled fresh-water coelenterate polyp genera *Craspedacusta* and *Calpasoma*. Trans Am Microsc Soc 93:235–241

Rixon CAM, Duggan IC, Bergeron NMN, Ricciardi A, MacIsaac HJ (2005) Invasion risks posed by the aquarium trade and live fish markets to the Laurentian Great Lakes. Biodivers Conserv 14:1365–1381

Segers H (1995) Rotifera 2: the Lecanidae (Monogononta). In: Nogrady T, Dumont HJ (eds) Guides to the identification of the microinvertebrates of the continental waters of the world 6. SPB Academic Publishing Bv, Hague, p 226

Strecker AL, Campbell PM, Olden JD (2011) The aquarium trade as an invasion pathway in the Pacific Northwest. Fisheries 36:74–85

Yanai Z, Dayan T, Mienis HK, Gasith A (2017) The pet and horticultural trades as introduction and dispersal agents of non-indigenous freshwater molluscs. Manag Biol Invasion 8:523–532