FEATURE:

The aquarium trade as an invasion pathway in the Pacific Northwest

ABSTRACT: The aquarium trade moves thousands of species around the globe, and unwanted organisms may be released into freshwaters, with adverse ecological and economic effects. We report on the first investigation of the ornamental pet trade as an invasion pathway in the Pacific Northwest region of the United States, where a moderate climate and a large human population present ample opportunities for the introduction and establishment of aquarium trade species. Results from a regional survey of pet stores found that the number of fish (n=400) and plant (n=124) species currently in the aquarium trade is vast. Pet stores import thousands of fish every month, the majority of which (58%) are considered to pose an ecological threat to native ecosystems. Our propagule pressure model suggests that approximately 2,500 fish (maximum ~ 21,000 individuals) are likely released annually to the Puget Sound region by aquarists, and that water temperatures in many parts of Washington are suitable for establishment of populations. In conclusion, the aquarium trade may be a significant source of past and future invasions in the Pacific Northwest, and we recommend enhanced public education programs, greater regulation of the aquarium industry, and improved legislation of nonnative species in the ornamental trade.

Tratado de Comercio en Acuarios como una vía de invasión en el Pacífico noroeste

RESUMEN: El Tratado de Comercio en Acuarios mueve miles de especies en todo el mundo y organismos no deseados pueden ser liberados en aguas continentales, lo que provoca efectos ecológicos adversos. En la presente contribución se reporta la primera investigación sobre el tratado de especies ornamentales como una vía de invasión en la región del Pacífico noroeste de los Estados Unidos de Norteamérica, donde tanto el clima como la enorme población humana representan amplias oportunidades para la introducción y establecimiento de especies comerciales de acuario. Los resultados de un sondeo realizado a nivel regional a los negocios de mascotas muestran que el número de especies de peces (n=400) y plantas (n=124) que actualmente contiene el Tratado de Comercio en Acuarios es vasto. Los negocios de mascotas importan miles de peces cada mes, la mayor parte de los cuales (58%) se considera que representan una amenaza ecológica a los ecosistemas nativos. Se utilizó un modelo de presión de propágulo y los resultados sugieren que aproximadamente 2,500 peces (máximo ~ 21,000 individuos) pueden estar liberándose anualmente a la región de Sonda Puget por parte de los acuaristas y que la temperatura del agua en muchas partes del estado de Washington son adecuadas para el establecimiento de las poblaciones. Concluyendo, el Tratado de Comercio de Acuarios puede ser una fuente importante de invasiones en el Pacífico noroeste tanto en el pasado como en el futuro; se recomienda perfeccionar los programas de educación pública, aumentar la regulación de la industria del acuarismo y mejorar la legislación en lo tocante a especies foráneas dentro del tratado.

Angela L. Strecker
Philip M. Campbell, and
Julian D. Olden*

School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA, 98105. *Olden, 206-616-3112, olden@uw.edu.

INTRODUCTION

Human activities have greatly increased the number and geographical extent of aquatic invasive species (AIS) throughout the United States and globally. Prevention of species introductions is considered the cornerstone of invasive species management (Vander Zanden and Olden 2008), yet integrated approaches to managing invasion vectors (sensu Ruiz and Carlton 2003) are difficult to develop and implement because pathways to aquatic species introductions are diverse, dynamic over time, and vary both taxonomically and geographically (e.g., Moyle and Marchetti 2006, Ricciardi 2006). An understanding of the full complement of invasion pathways is critical to improve policy actions, guide integrated management strategies, and enhance educational campaigns aimed at reducing the threat of future invasions (Lodge et al. 2006).

To date, considerable research activity and management attention has focused on unintentional pathways to AIS introductions through ballast-water transfer in ships (e.g., Carlton and Geller 1993, Ruiz et al. 1997), transport via trailered boats (e.g., Leung et al. 2006, Rothlisberger et al. 2010), bait-bucket releases by recreational anglers (e.g., Litvak and Mandrak 1993, DiStefano et al. 2009), and escapes associated with aquaculture (e.g., Naylor et al. 2001, De Silva et al. 2009). By contrast, the ornamental pet and aquarium trade has only recently been recognized as a major pathway for freshwater fish and plant introductions (Copp et al. 2010). This is despite the fact that the ornamental pet trade represents a multi-billion dollar industry that includes thousands of foreign species and has grown by 14% annually since the 1970s (Padilla and Williams 2004, Cohen et al. 2007). Although the import of some nonnative species common to the pet trade are regulated by certain countries (e.g., reptiles in Australia and New Zealand), ornamental fish generally have not received attention from regulatory agencies (Thomas et al. 2009, Secretariat of the Convention on Biological Diversity 2010). Additionally, reliable record keeping of the type and number of organisms currently in the trade is lacking (Schlaepfer et al. 2005, Smith et al. 2008, Chang et al. 2009). Given the present uncertainty in the taxonomy of many ornamental fish and plant species within the aquarium trade, as well as the widespread contamination of many aquarium plants with unidentified organisms (e.g., molluscs: Keller and Lodge 2007), our ability to assess invasion risk associated with this pathway is limited.

Aquarium trade species are introduced when owners release unwanted organisms into natural waterbodies for various reasons, including large size, humane treatment, aggressiveness, and high reproductive rates (Padilla and Williams 2004, Gertzen et al. 2008). The most popular fish sold in the aquarium trade are also the most likely to be introduced and establish in freshwater habitats (Duggan et al. 2006). Although the aquarium trade and its associated vectors have been increasingly recognized as a primary pathway of biological invasions in the Laurentian Great Lakes region (Rixon et al. 2005, Cohen et al. 2007, Gertzen et al. 2008) and the San Francisco Bay-Delta region (Chang et al. 2009), surprisingly little is known regarding the scope of the issue in the Pacific Northwest of the United States. In a region where invasive species are considered a significant threat to native biodiversity, ecosystem function, and culturally- and economically-important Pacific salmon (Sanderson et al. 2009), it is imperative that scientific research is available to quantify the strength of the aquarium trade as a pathway of new invasions. In Washington and Oregon, there are a number of plant and animal species that have likely been introduced into the wild via the aquarium trade, including oriental weatherfish (*Misgurnus anguillicaudatus)*, Amur goby (*Rhinogobius brunneus*), red-bellied pacu (*Piaractus brachypomus),* goldfish (*Carassius auratus*), red swamp crayfish (*Procambarus clarkii*), Chinese mystery snail (*Cipangopaludina chinensis malleata*), Eurasian watermilfoil (*Myriophyllum spicatum*), and parrot feather (*Myriophyllum aquaticum*)[1]. Thus, the scope of this problem is significant. Furthermore, many aquarium species may become more successful at establishing in higher latitudes with warmer temperatures projected to occur under climate change scenarios (Rahel and Olden 2008, Chang et al. 2009).

Our paper is the first to examine the ornamental pet trade as an invasion pathway in the Pacific Northwest region of the United States, where a moderate climate—in combination with a large and growing human population—presents ample opportunities for the introduction and establishment of aquarium trade species. We combine data, gathered over time, from a regional survey of aquarium pet stores with a detailed investigation of fish and plant sales to quantify the type and number of organisms in the ornamental trade. From this we examined selected common aquarium fish species with high invasion potential according to previous invasion history and thermal suitability for establishment in Washington State waters. Next, we report on the results from a survey of live organism used by aquarists, which is used to parameterize a model of propagule pressure to estimate the number of aquarium fish likely to be introduced annually to the Puget Sound region of western Washington.

METHODS

Store Inventory Surveys and Aquarist Questionnaires

We conducted an intensive (temporal trends in a single store) and extensive (spatial trends from multiple stores) survey of pet stores in the Puget Sound area of Washington to document the numbers and types of fish and plant taxa in the ornamental pet trade (Figure 1). The intensive survey analyzed monthly sale invoices from a single (large and independent) pet store in 2007. All fish and plant species were identified and individuals counted. Fish were separated into ornamental (i.e., fish of primary interest for viewing) and feeder (i.e., to feed to other fish) species. When there was a disparity between a store label scientific name and common name, we used the scientific name provided in FishBase (Froese and Pauly 2009). Additionally, 30 pet stores in Snohomish, King, and Pierce counties were surveyed over a two-week period in February 2008 to regionally characterize the ornamental pet trade (Figure 1). Two different national pet store chains were chosen for the survey (chain A, n = 14; chain B, n = 8), as well as eight independently owned stores. There is some evidence that independent retailers differ in the numbers and types of species sold compared to chain stores (Chang et al. 2009); understanding differences between store types can help direct educational efforts. Preliminary analyses indicated that the two sets of chain stores differed in the numbers and types of species sold, therefore, we analyzed the chains separately. Stores were visited a day after receiving their fish and plant shipment (determined by contacting the store managers) to enumerate organisms before they were purchased, but after any had died from travel stress. This ensured that our survey accurately reflected the current inventory of store. Time constraints precluded the enumeration of individual plants, resulting in species being reported as present or absent.

Fish and plant taxa observed in our regional survey were compared to the USGS Nonindigenous Aquatic Species list[1] and the Washington State Aquatic Nuisance Species list[2] to determine whether the species have a demonstrated history of invasion in other regions of the United States. The USGS defines nonindigenous species as species that are outside of their historic or native range, whereas Washington State defines invasive species as species that are not historically native to the state. Previous invasion history is one of the best predictors of invasion potential (Ricciardi and Rasmussen 1998). For the purposes of our study these taxa were designated as "invasive." Next, optimal and lethal temperature requirements for selected fish species were obtained from FishBase (Froese and Pauly 2009): when lethal limits were unavailable, values were obtained from primary literature (white cloud mountain minnow, *Tanichthys albonubes*: Cheverie and Lynn (1963); goldfish, *Carassius auratus:* Ford and Beitinger (2005); molly, *Poecilia sphenops:* Hernández and Bückle (2002); koi carp, *Cyprinus carpio carpio:* Opuszyński et al. (1989)). These species were chosen because they represent a cross-section of species common to aquarium and pet stores; they have been identified previously

1 http://nas.er.usgs.gov/

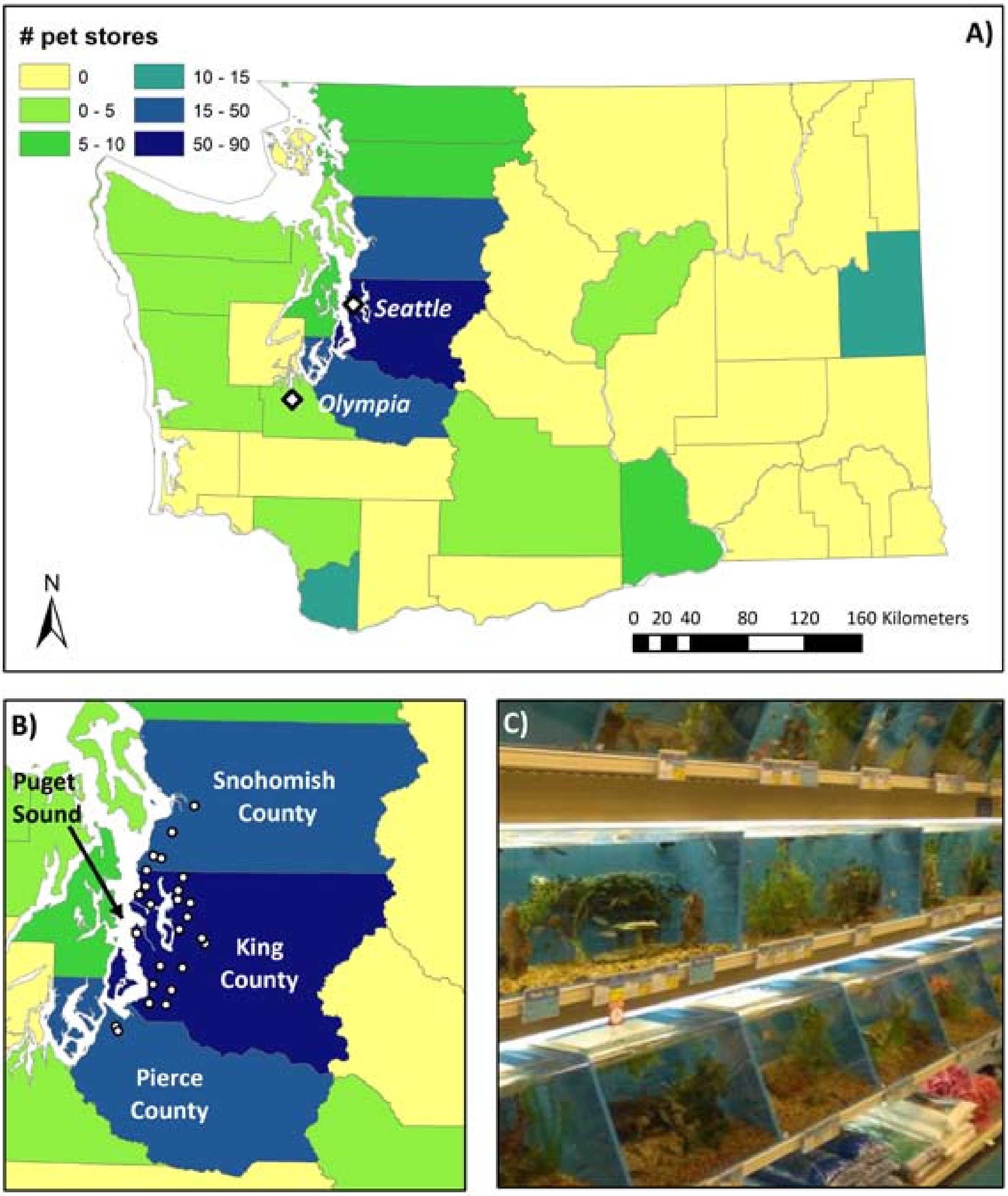
2 http://wdfw.wa.gov/ais/; http://www.ecy.wa.gov/programs/wq/plants/weeds/exotic.html

as species with potential to establish in temperate North America and Washington State (Tabor et al. 2001, Rixon et al. 2005, Gertzen et al. 2008); and there was optimal and lethal temperature range data available in the literature. Optimum temperature was defined as the range of temperatures in which fish species typically habituate in the wild, whereas lethal temperatures indicate absolute minimum and maximum temperatures that fish can survive in under experimental settings (Brett 1956). Additionally, data layers for stream water temperatures from 2000-2008 were obtained from 236 monthly monitoring sites in the Environmental Protection Agency's STORET Database[3]. Water temperature data were summarized as mean annual temperature because values from winter months (i.e., minimum values) were not consistently available. We use stream temperatures as our proxy of water temperatures across the state because data from a suitable number of lakes was not available.

A survey of 92 aquarists was conducted at an independent pet store (same store as the intensive survey) in June 2008 to assess the numbers of pet fish owned, and to estimate the proportion of aquarists releasing fish or plants into local waterways. Questions asked included: 1) total number of freshwater fish species typically owned each year; 2) whether or not the owner had released live fish or plants into the wild in their lifetime; 3) where live fish and/or plants were released; and 4) methods of deposing of fish and plants. Questionnaires were randomly given to aquarists in the store, and responses were anonymous.

3 http://www.epa.gov/storet/dbtop.html

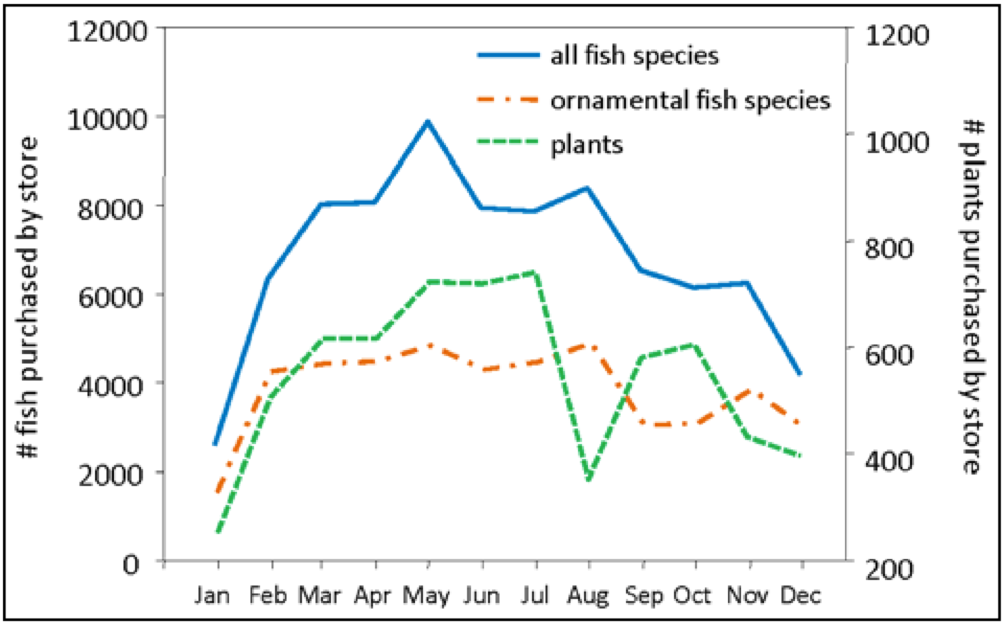
Figure 1: A) Number of pet stores in Washington state counties (US Economic Census 2007: http://www.census.gov/econ/census07/), B) location of stores included in the regional survey of the Puget Sound area (n = 30), and C) a typical aquarium showroom.



Analysis of Taxa Currently in the Pet and Aquarium Trade

The number of fish and plant species per store, and the number of fish individuals per store recorded during the spatial survey of pet stores were averaged within store category (chain A, chain B, and independent). Fish abundance and number of fish species were ln-transformed, and the number of plant species was square-root transformed prior to analysis to normalize data. We used analysis of variance (ANOVA) tests, followed by a Tukey HSD post hoc test, to examine differences among store types in fish abundance, and fish and plant species richness. Additionally, we performed a multivariate analysis to examine similarities and differences in the abundances of each fish species sold across the different store types. Fish species that occurred in <10% of stores were excluded from the analysis, and counts were standardized to z-scores to reduce the influence of rare and/or abundant taxa. We used non-metric multidimensional scaling (NMDS) to summarize store differences, as NMDS is effective with non-normal data and can use any distance measure (Legendre and Legendre 1998). We used the Bray-Curtis dissimilarity index, and tested for significance of the ordination with randomization tests. According to multivariate stress values we found that the optimal ordination utilized three dimensions. Ordination analyses were performed using the MASS library in R (R Development Core Team 2010).

Figure 2: Total monthly number of fish (i.e., feeders and ornamentals), ornamental fish, and plant individuals purchased by the store (reflecting monthly sales). Note that plants are represented on the right-hand axis.



Propagule Pressure Model

One of our objectives was to estimate the number of fish likely to be introduced annually to the Puget Sound region of Washington (King, Snohomish, and Pierce counties), which was the location of our regional survey, and held the most populated counties in the state. We used the results of our aquarist survey to parameterize a propagule pressure model by modifying the approach of Gertzen et al. (2008). The model structure is:

$$\text{propagule pressure} = M \cdot P(I) \cdot N \cdot P(R|I)$$

where M is the number of households that own aquarium fishes, $P(I)$ is the probability that an owner is a releaser, N is average number of fish owned annually, and $P(R|I)$ is the probability fish are released given that an owner is a releaser (Gertzen et al. 2008). We based the parameters $P(I)$ and N on data from our aquarist survey, whereas $P(R|I)$ was derived from Gertzen et al. (2008). We used a Bayesian approach to incorporate uncertainty about the representativeness of our values in the model. Bayesian statistics consider prior information in the determination of parameters from a data set. Thus, we created probability distributions that reflected our data: we multiplied these distributions by each other to generate a joint probability distribution.

The number of households that own aquarium fishes, M, was determined by multiplying the number of households in King, Snohomish, and Pierce counties (1,196,568; US Census 2000[4]) by the percentage of U.S. households that own fishes (10.6%), and by the percentage of fish in the aquarium trade that are freshwater (96%) (values from Chapman et al. 1997). $P(I)$, the probability of being a releaser, was a binary variable determined from survey data and modeled with a binomial distribution (i.e., heads or tails), which reflects the uncertainty from our random survey of 92 people (Bolker 2008). N, the number of fish owned annually, was determined from our survey and was modeled with a negative binomial distribution. In a negative binomial distribution, the variance is larger than the mean (Bolker 2008), which reflects the large number of aquarists who own a small number of fish (e.g., ≤5), but that a small number of aquarists own large numbers of fish. $P(R|I)$, the probability that fish are released given that the owner is a releaser, was based on the value of Gertzen et al. (2008) (5.1%) and modeled with a beta distribution that is bound by 0 and 1 (Bolker 2008). Each probability distribution ($P(I)$, N, and $P(R|I)$) was then combined with a uniform flat prior to generate posterior distributions; we used uniform priors as we had no prior expectations about model parameters (Bolker 2008, Gertzen et al. 2008). Finally, all combinations of the posterior distributions and the constant, M, were multiplied together to create a joint probability distribution that reflects the inherent uncertainty in our survey data. All propagule pressure model steps were performed in R (R Development Core Team 2010).

RESULTS

A year-long intensive survey of a pet store in the Puget Sound area revealed a distinct peak in the number of ornamental fish, the number of total fish (ornamentals + feeder fish), and number of plants purchased starting in late spring (February and March) and extending through the summer to September (Figure 2). Fish sales peaked in May, with >9,700 fish purchased in the store, half of which were ornamental fish, whereas sales for plants peaked in July, at >700 plants (Figure 2).

Our regional survey of 30 pet stores identified 400 fish species and 124 plant taxa currently in the ornamental trade, a number that represents the minimal species pool for the Pacific Northwest region. None of the fish species are native to Washington State and only 8 plant species are natives. Of the 400 fish species, 29 occurred in greater than 75% of stores, including two taxa, tiger barb (*Puntius tetrazona*) and three spot gourami (*Trichogaster trichopterus*), which occurred in all of the stores surveyed (Table 1). Other commonly encountered fish species included goldfish, Siamese fighting fish (*Betta splendens*), several different tetras, mollies, and guppies (*Poecilia* spp.) (Table 1). Additionally, a

4 http://www.census.gov/main/www/cen2000.html

number of species that have previously been detected in the wild in Washington State were found in a lower proportion of stores in the survey: koi carp = 60%, oriental weatherfish = 33%, and Amazon sailfin pleco (*Pterygoplichthys pardalis*) = 3%. Plant species occurred with lower frequency: the top species, Amazon sword (*Echinodorus amazonicus*), occurred in 77% of stores, and an additional 19 taxa occurred in >25% of stores (Table 2). On average, 58% of fish individuals, 43% of fish species, and 5% of plant species found in pet stores were considered invasive according to the USGS Nonindigenous Aquatic Species list and the Washington State Aquatic Nuisance Species list. However, the maximum number of invasive taxa encountered in a single store in our survey indicated that invaders comprised up to 72% of fish individuals, 61% of fish species, and 17% of plant species. Further, a number of fish and plant species in our survey are within the same genera as species that are considered invasive (Table 1 and 2). Additionally, in one store we found an aquatic plant species, the water chestnut (*Trapa natans*), which is banned for sale by the Washington State Department of Agriculture.

As many aquarium species are tropical in origin, their minimum optimal temperatures typically exceed winter water temperatures observed in temperate waterbodies (Table 1, Figure 3). However, several aquarium species that are prevalent in the ornamental trade have lower-lethal and minimum optimal temperatures that are found well within the range of mean annual stream water temperatures in Washington State (Figure 3). This includes several taxa, such as the white cloud mountain minnow and the oriental weatherfish, which may have high invasion potential. Oriental weatherfish have established a population in Washington (Tabor et al. 2001), and the white cloud mountain minnow are considered a high risk invader in the United States as a result of broad thermal tolerance (Rixon et al. 2005). Additionally, koi carp and goldfish (both present in Washington) have very broad thermal tolerance ranges, suggesting that these species may have elevated establishment potential. Our estimates of concordance between fish species thermal tolerance and water temperatures in Washington State may be conservative, as lakes may exhibit greater thermal heterogeneity compared to streams.

We found differences between fish and plant inventories from our regional survey of chain stores and a set of independent stores. Chain A had significantly lower numbers of fish individuals per store compared to chain B (ANOVA: $F_{2,27} = 9.56$, $p < 0.01$; Tukey HSD $p < 0.05$), but neither chain store was different from the set of independent stores (Tukey HSD $p > 0.05$) (Figure 4A). Chain A also had significantly fewer fish species compared to both chain B and independent stores (ANOVA: $F_{2,27} = 13.00$, $p < 0.01$; Tukey HSD $p < 0.05$), but there was no difference between chain

Table 1. Frequency of occurrence of aquarium fish species in pet stores (>75%) and minimum optimum temperature (°C).

Scientific name	Common name	Frequency of occurrence (%)	Minimum optimum temperature (°C)
Puntius tetrazona[a]	tiger barb	100.0	20.0
Trichogaster trichopterus[a]	three spot gourami	100.0	22.0
Betta splendens[a]	Siamese fighting fish	96.7	24.0
Carassius auratus[a,b]	goldfish	96.7	0.0
Danio rerio[c]	zebra danio	96.7	18.0
Gymnocorymbus ternetzi[a]	black tetra	96.7	20.0
Hemigrammus erythrozonus[c]	glowlight tetra	96.7	24.0
Paracheirodon innesi[a]	neon tetra	96.7	20.0
Poecilia latipinna[a]	sailfin molly	96.7	20.0
Poecilia reticulata[a]	guppy	96.7	18.0
Poecilia sphenops[a]	molly	96.7	18.0
Puntius titteya[c]	cherry barb	96.7	23.0
Xiphophorus maculatus[a]	southern platyfish	96.7	18.0
Colisa lalia[a]	dwarf gourami	90.0	25.0
Pristella maxillaris	x-ray tetra	90.0	24.0
Puntius conchonius[a]	rosy barb	90.0	18.0
Astronotus ocellatus[a]	oscar	83.3	22.0
Epalzeorhynchos frenatum	rainbow sharkminnow	83.3	24.0
Gyrinocheilus aymonieri[a]	Chinese algae-eater	83.3	25.0
Moenkhausia sanctaefilomenae[a]	redeye tetra	83.3	22.0
Trigonostigma heteromorpha	harlequin rasbora	83.3	22.0
Balantiocheilos melanopterus[a]	tricolor sharkminnow	80.0	22.0
Corydoras paleatus[a]	peppered corydoras	80.0	18.0
Devario aequipinnatus	giant danio	80.0	22.0
Xiphophorus helleri[a]	green swordtail	80.0	22.0
Hyphessobrycon eques[c]	jewel tetra	76.7	22.0
Labidochromis caeruleus	blue streak hap	76.7	23.0
Metynnis hypsauchen[a]	silver dollar	76.7	24.0
Tanichthys albonubes[a]	white cloud mountain minnow	76.7	18.0

[a] listed as USGS Nonindigenous Species
[b] listed as Washington State Aquatic Nuisance Species
[c] species within same genus listed as USGS Nonindigenous Species

Table 2. Frequency of occurrence of aquarium plant species in pet stores (>25%).

Scientific name	Common name	Frequency of occurrence (%)
Echinodorus amazonicus	Amazon sword	76.7
Microsorium pteropus	java fern	73.3
Hygrophila difformis	wisteria	70.0
Cryptocoryne wendtii[a]	*Cryptocoryne wendtii*	63.3
Ceratophyllum demersum	hornwort	56.7
Echinodorus tennellus	narrow leaf chain sword	56.7
Acorus gramineus	Japanese rush	50.0
Dracena sanderiana	green sandriana	50.0
Nymphoides aquatica	banana	50.0
Ophiopogon japonicus	mondo grass	50.0
Trichomanes javanicum	*Trichomanes javanicum*	46.7
Echinodorus paniculatus	bleheri sword	43.3
Echinodorus argentinensis	Argentine sword	40.0
Anubias barteri	*Anubias barteri*	36.7
Vesicularia dubyana	java moss	36.7
Crinum thaianum	crinum bulb	33.3
Echinodorus osiris	melon sword	33.3
Bacopa monnieri	moneywort	26.7
Sagittaria subulata[b]	dwarf sagittaria	26.7
Spathiphyllum wallisii	peace lily	26.7

[a] listed as USGS Nonindigenous Species
[b] species within same genus listed as Washington State Noxious Aquatic Weed

B and independents (Tukey HSD $p > 0.05$) (Figure 4B). We found no differences in the number of plant species between store types (ANOVA: $F_{2,27} = 0.75$, $p = 0.48$) (Figure 4C). Results from the multivariate analysis on fish abundance revealed strong clustering of store types in ordination space (Figure 5). Little overlap of stores in multivariate space was observed, suggesting that store types have fairly distinctive inventories of ornamental fishes (although clear similarities exist in that all stores have a core suite of species in their inventories). Most notably, stores that are independently owned occupied the greatest area in ordination space, suggesting that they carry the highest diversity of fish species (Figure 5, supported by Figure 4).

The results from our questionnaire indicated that, on average, aquarists owned ~ 9 fish (median = 5) and that 6.4% of aquarists had released live fish in the past. The majority of introductions were into lakes or streams. Using a Bayesian statistical approach, we estimated that the most likely number of fish introduced annually into the Puget Sound area was 2,536; however, the 95% confidence interval suggests that 20,869 fish could be introduced in a year (Figure 6).

Figure 3: Mean annual stream water temperatures (°C) in Washington streams (left panel), thermal preferenda (°C) of certain common fish species found in the aquarium trade (right panel). Water temperatures are represented by a box plot, where the centre line is the median, the lower and upper box boundaries are the 25th and 75th percentiles, the whiskers are the 10th and 90th percentiles, and outliers are represented by circles. The shaded boxes in the background correspond to the 10th and 90th percentiles of stream temperatures (dark grey) and the most extreme outliers (light grey). Thermal preferenda for the fish species are represented by ranges, where the box represents the optimal temperature range and the whiskers represent the upper and lower lethal limits. Fish species are arranged by increasing thermal range. Note that water temperature data was not available for lakes, although we would expect that introductions occur in both lakes and streams.

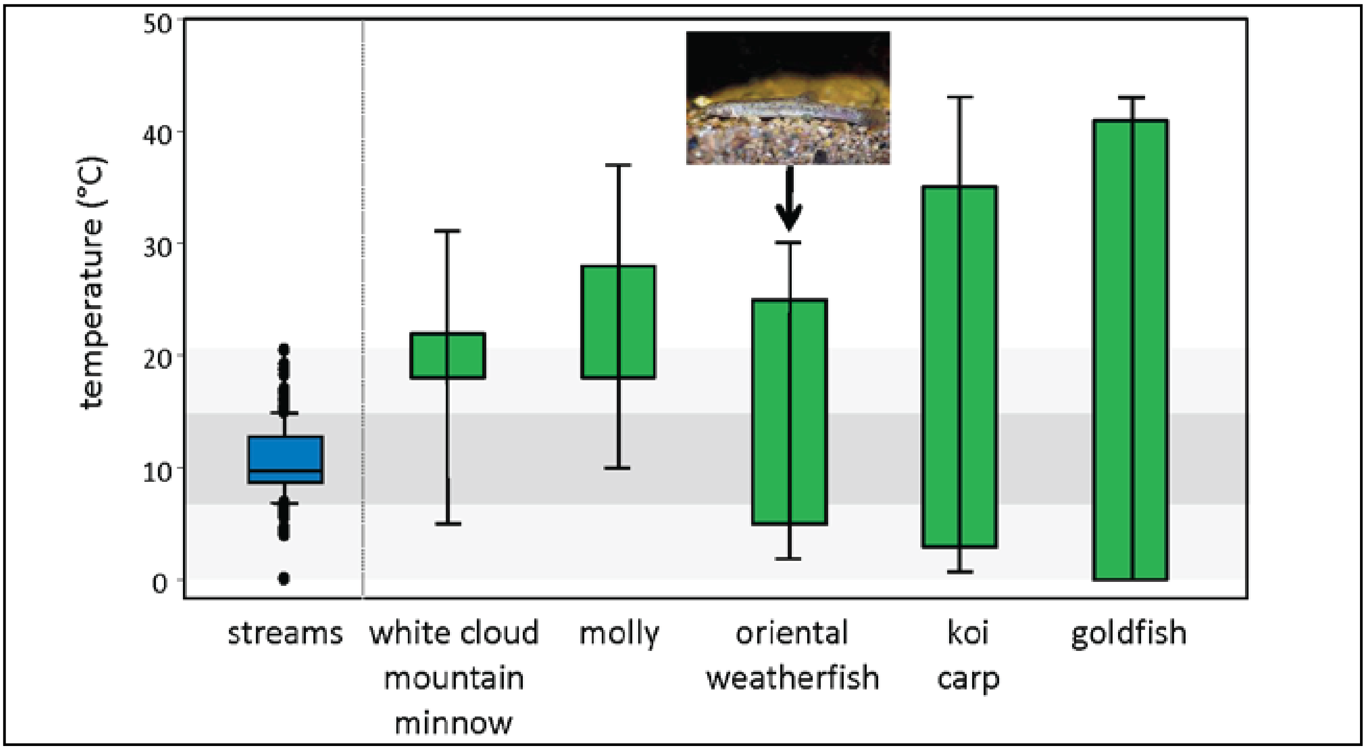


Figure 4: Comparisons of chain and independent pet stores for A) number of fish individuals per store, B) number of fish species per store, and C) number of plant species per store. Center line in box plots represents median, lower and upper box boundaries are the 25th and 75th percentiles, and whiskers are the 10th and 90th percentiles. Outliers are represented by circles. Letters above the bars represent the results of Tukey HSD post hoc tests, where different letters indicate significant differences between store categories ($p < 0.05$).

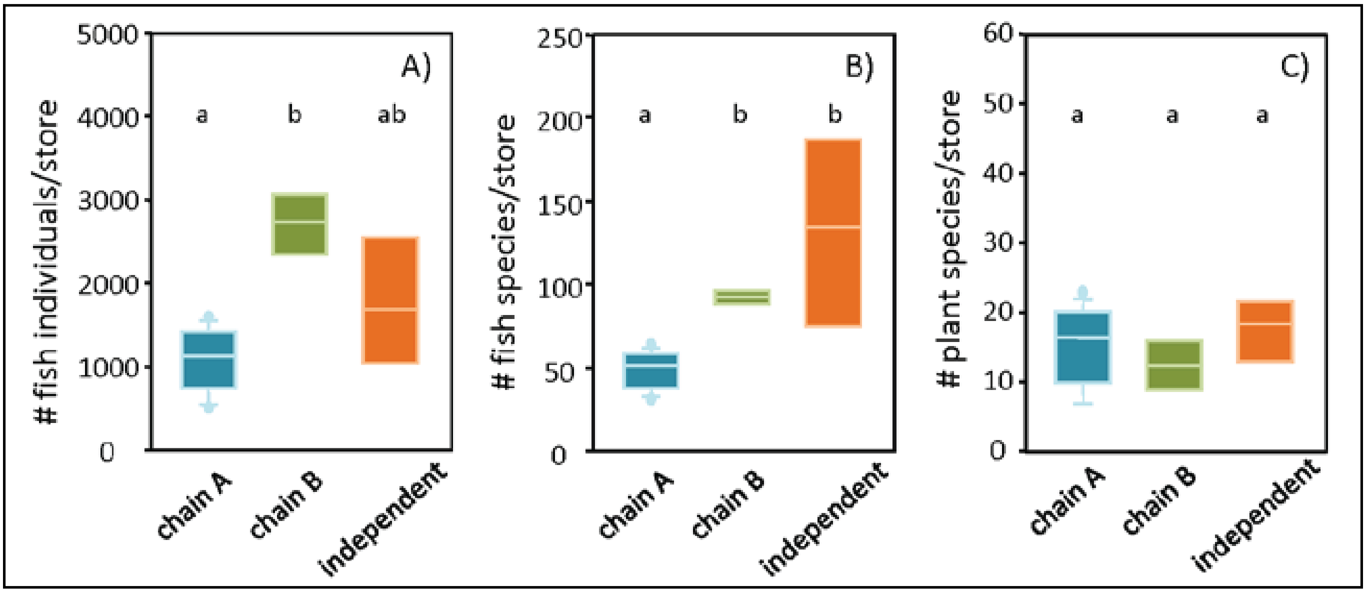


Figure 5: Non-metric multidimensional scaling of relative abundance of aquarium fishes in pet stores in the Puget Sound area using Bray-Curtis dissimilarity. Chain stores (triangles, n = 8; squares, n = 14) are contrasted with a set of independent stores (circles, n = 8). Common names of fishes highly correlated with NMDS axes are indicated on the outer edges of the graph. NMDS stress = 10.2, p = 0.02 on three dimensions. Ellipses drawn around the outer edges of groups of stores are simply for illustration. Star indicates the species score of oriental weatherfish (Misgurnus anguillicaudatus), one of several aquarium fishes with an established population in Washington.

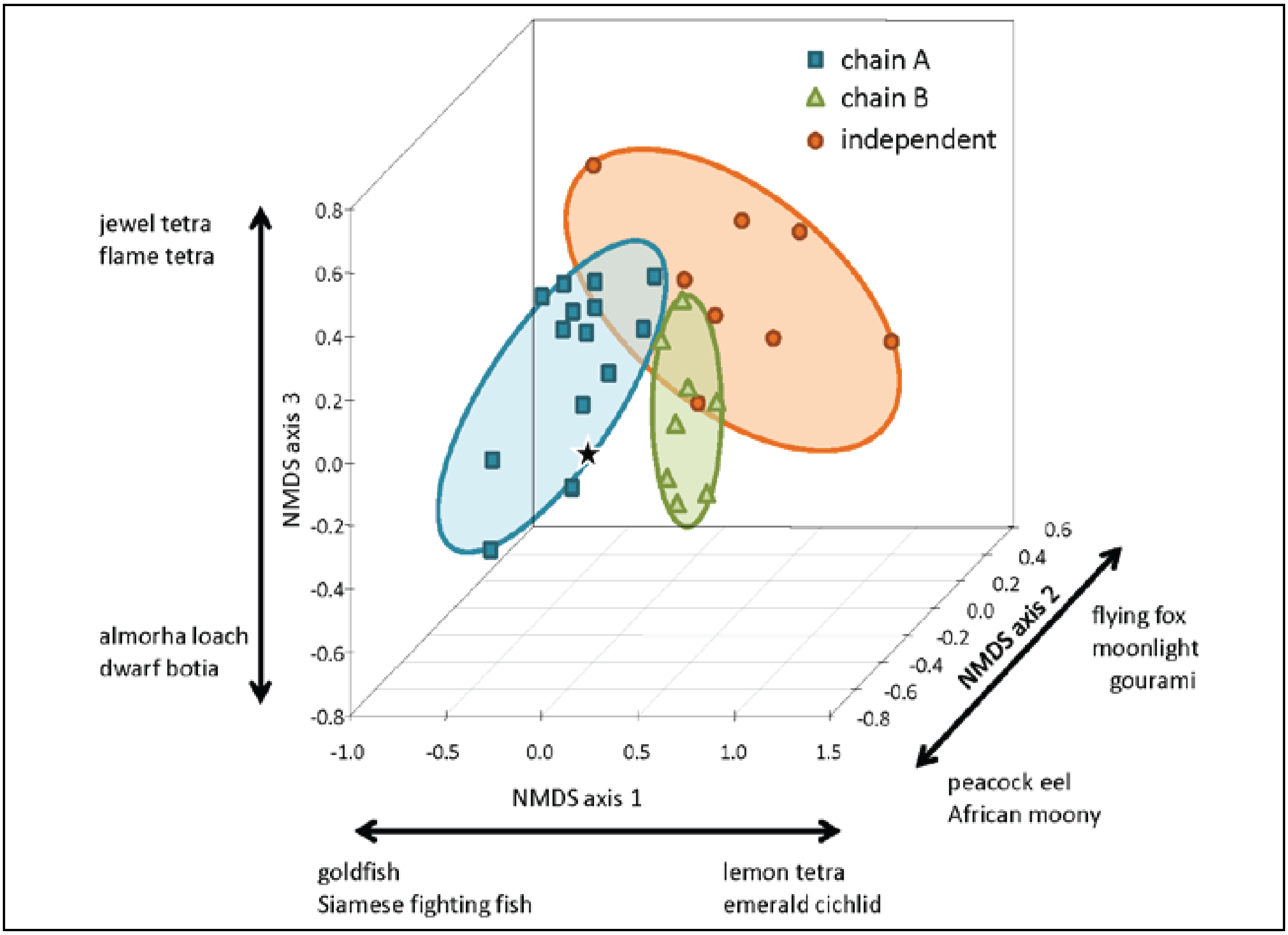
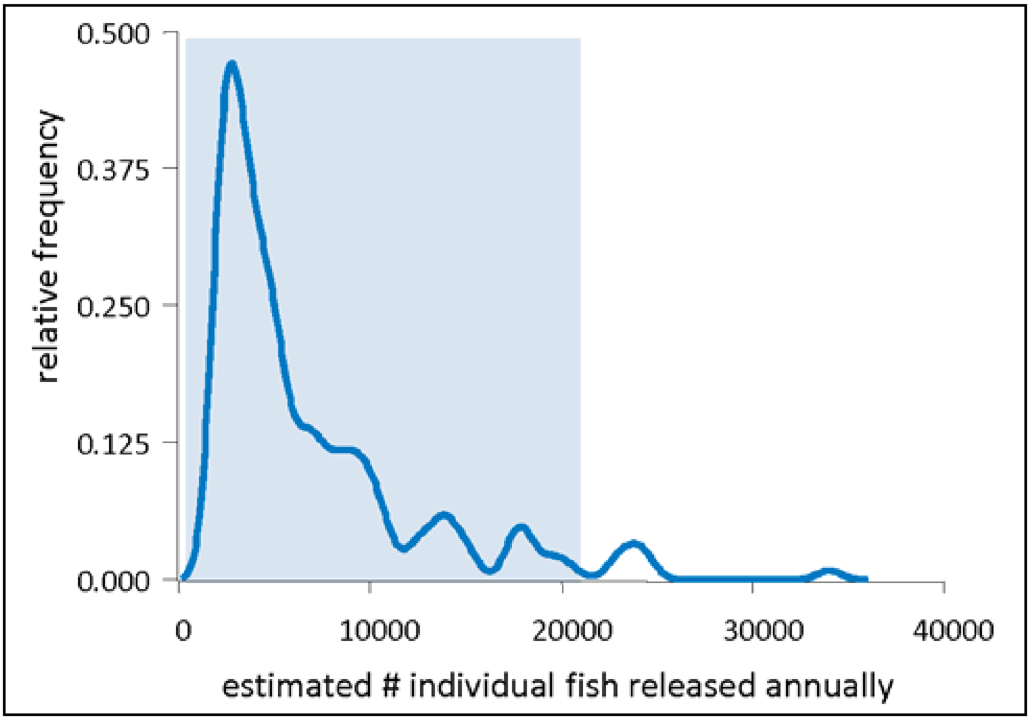


Figure 6: Histogram of estimated relative frequency of number of individual fish released annually in King County. The median value of fish released each year is 2,536, the mean is 4,707, and the upper 95th confidence interval (indicated by grey box) is 20,869.



DISCUSSION

Using a combination of regional store surveys, aquarist questionnaires, and statistical models, we have demonstrated that the number of fish (n=400) and plant (n=124) species currently in the aquarium trade is vast, the majority of species in the trade are not native to the region, and that this introduction pathway deserves greater research and regulation in the Pacific Northwest. Pet and aquarium stores import thousands of fish every month, the majority of which (58%) are considered to pose an ecological threat to native ecosystems. Our model suggests that up to 21,000 fish (average of 2,500 individuals) are likely released into the wild each year in the Puget Sound area by aquarists, and that water temperatures in many parts of Washington State are suitable to allow establishment of populations. The predictions of our model suggest that the pet trade is a significant pathway of AIS introductions, particularly around populated urban centers, yet far greater research effort and funding for prevention have been directed towards boater movement as an invasion pathway (e.g., Leung et al. 2006, Rothlisberger et al. 2010). This is particular true in Washington State where management efforts continue to focus on preventing invasions via trailered boats (State of Washington Joint Legislative Audit & Review Committee 2010). To illustrate the potential importance of the ornamental pet trade, we compared several different features of the aquarium and boater pathways (Table 3). Our comparison suggests that the number of aquarists is similar to the number of registered boats in Washington State (i.e., vector strength), and that propagule pressure from the aquarium trade is high relative to boats for some taxonomic groups (e.g., fish, invertebrates: Duggan (2010)), but low for others (e.g., plants). Management and educational challenges are likely very different between the pathways; the distribution of aquarists (i.e., reflecting the location of potential introductions) is spatially-diffuse, whereas the distribution of boat launches is well defined. This comparison underscores the importance of this understudied pathway, and highlights the significant management and educational challenges that the aquarium pathway represents.

The regional survey of 30 pet and aquarium stores indicated that independently owned stores tend to carry a greater number of —and a more unique variety of—fish species compared to some chain stores (e.g., chain A: Figures 4,5). Despite the lower diversity, chain store B had a larger inventory available for sale; presumably, related to faster turnover of stocks. Our results concur with the study of Chang et al. (2009) in the San Francisco Bay-Delta region, where independent retailers generally sold greater numbers of fish species compared to chain stores. Goldfish, Siamese fighting fish, neon tetras (*Paracheirodon innesi*), and guppies and/or mollies all occur frequently in our study, as well as those of Gertzen et al. (2008) and Rixon et al. (2005) conducted in the Laurentian Great Lakes region. In our survey, the number of aquatic plants was similar between store types, but the composition tended to be relatively different, as only six species occurred in more than half of the stores surveyed. In contrast to aquarium fish, the most common aquatic plants differed from a similar study conducted in another region: only two taxa, Amazon sword and hornwort (*Ceratophyllum demersum*), were frequently encountered in our study and the study conducted by Rixon et al. (2005). Overall, the moderate climate of the Pacific Northwest, as well as large population centers in the Puget Sound basin, suggests that freshwater ecosystems are threatened by the establishment of nonnative species from the aquarium trade. We expect that other large urban centers in the Pacific Northwest, such as Portland, Oregon and Vancouver, British Columbia, would be similarly at risk of nonnative species introductions via the aquarium trade pathway, and thus, should be targets for educational campaigns. Further, climate change will certainly increase establishment of nonnative aquarium and pet trade species in the Pacific Northwest, where temperatures are predicted to increase by > 3°C by the end of the 21st century (Mote and Salathé Jr. 2010). Additionally, nonnative species introductions via the aquarium trade in milder tropical and sub-tropical habitats will have substantially greater establishment success because of greater thermal suitability. Indeed, established populations of aquarium trade species have been increasingly detected in the southern United States (e.g., Florida: Padilla and Williams 2004).

Our study identified several fish species that may be of particular concern for establishment of populations via the aquarium pathway. The oriental weatherfish currently has an established, but limited, distribution in Washington State (i.e., Lake Washington basin in Seattle: Tabor et al. 2001), and further invasions seem likely without successful intervention and management of this pathway. According to our regional survey of pet stores, oriental weatherfish are found in chain and independent stores, but are currently more common in the inventories of chain stores compared to independent stores (Figure 5). The invasion of oriental weatherfish may have serious consequences for fisheries in the Pacific Northwest. Perhaps most notably, the virus birnavirus LV1 was isolated from invasive oriental weatherfish in Australia (Lintermans et al. 1990). Birnavirus LV1 is related to the infectious pancreatic necrosis virus, a disease of salmonid fishes (Wolf 1988). Additionally invasive parasites have been found in oriental weatherfish (Dove and Ernst 1998). Further, it has been shown that oriental weatherfish can reduce the abundance and biomass of macroinvertebrates (Keller and Lake 2007) and prey on fish larvae (Logan et al. 1996). Altogether, these factors suggest that oriental weatherfish may have significant effects on native fish populations and should be a target for invasion vector management.

Table 3. Comparison of the aquarium trade and trailered boats as pathways of nonnative species invasions. This illustrative comparison indicates that the threat posed by the aquarium trade may be comparable to that of boat trailers. For establishment success, categories of low, moderate, and high are simply qualitative characterizations based on relative comparisons of potential establishment between taxonomic groups.

Characteristic	Aquarium trade	Trailered boats
Taxonomy	fish, aquatic invertebrates, and plants[1,2,3]	aquatic invertebrates and plants[4]
Propagule pressure	*fish*: <1 fish released per aquarist per year[1] *invertebrates*: >4,000 released per aquarist per year[3] *plants*: <1 released per aquarist per year[2]	*invertebrates* + *plants*: ~37 organisms per boat[4]
Vector strength	estimated number of households in Washington State with an aquarium = 227,140[5]	estimated number of recreational boats in Washington State = 264,000[6]
Establishment success	*fish*: low, Allee effects *invertebrates*: moderate, some asexual reproduction *plants*: high, vegetative reproduction	*invertebrates*: moderate, some asexual reproduction *plants*: high, vegetative reproduction
Prevention compliance	5.0-6.4% of aquarists release live fish[1,7]	13% of boaters never remove aquatic plants[4]
Management and educational challenges	spatially-diffuse: target pet/aquarium stores and groups	spatially-specific: target high traffic boat launches

[1] this study
[2] Cohen et al. (2007)
[3] Duggan (2010)
[4] Rothlisberger et al. (2010)
[5] 2000 US Census: http://www.census.gov/main/www/cen2000.html and based on the percentage of U.S. households that own fishes (10.6%) according to values from Chapman et al. (1997).
[6] State of Washington Joint Legislative Audit and Review Committee (2010). Recreational vessels include sailboats, yachts, and motorized boats that were registered in 2008; only a fraction of these boats are trailered.
[7] Gertzen et al. (2008)

A number of additional fish and plant species are currently in the ornamental pet trade and are regulated or prohibited in Washington State. Strikingly, we found the water chestnut (*Trapa natans*) for sale in one store; a species that is banned for sale by the Washington Department of Agriculture. Although we did not report on invertebrates, we also found a single crayfish species of the Family Cambaridae in a pet store: taxa from this family are prohibited by the Washington Department of Fish and Wildlife. Additionally, goldfish and koi carp are considered regulated fishes (e.g., species may not be released into state waters) by the Washington Department of Fish and Wildlife: goldfish were found in almost all surveyed pet stores (97%), and koi carp were found in 60% of the stores. The federal USGS Nonindigenous Aquatic Species list has designated oriental weatherfish and Amazon sailfin pleco as invasive species in Washington State: both species were found with a much lower frequency in pet store inventories compared to goldfish and koi carp (oriental weatherfish: 33%; Amazon sailfin pleco: 3%). We recommend that research and management efforts target species that have been identified by the state and federal governments as threats to native organisms.

Recommendations

Our study represents the first scientific investigation of the ornamental pet trade in the Pacific Northwest, thereby enhancing the scientific basis for improving policy and management intended to reduce the threat of this pathway. Based on our findings we have three primary recommendations to slow the introduction of AIS from the pet trade.

First, we believe that public education programs targeted at the interface of aquarium owners and retailers will likely have the greatest success. One such program, Habitattitude™, is a partnership of the Pet Industry Joint Advisory Council, the U.S. Fish and Wildlife Service, and NOAA National Sea Grant College Program, with the mandate, "…to eliminate the transfer and survival of any species outside of [an] enclosed, artificial system, which has the potential to cause the loss or decline of native plants and animals." The Habitattitude™ program supplies educational materials (e.g., pamphlets and stickers) to pet stores, as well as plastic bags with the message "Do not release fish and aquatic plants." This is an important step towards educating aquarium owners about the harm of releasing live organisms into the wild; however, our study found that these materials were only present in chain stores and absent from independent retailers. We have demonstrated that independent stores tend to carry a larger variety of fish species compared to chain stores, therefore, we recommend that independent retailers should be the next focal point of the Habitattitude™ campaign and other private and government funded education programs (Table 4). Efforts to educate aquarists on the repercussions of releasing aquarium fish and plants to the wild will only be successful if the distribution of educational materials reaches the broadest possible audience, including the vast and under-appreciated Internet trade in ornamental species (Secretariat of the Convention on Biological Diversity 2010). However, these efforts should be coupled with more directed educational campaigns that target pet enthusiasts that belong to the hundreds of aquarium societies across the United States (e.g., Greater Seattle Aquarium Society), national and international aquarium associations (e.g., Heart of America Aquarium Society, Canadian Association of Aquarium Clubs, Federation of British Aquatic Societies), and online aquarium forums and websites in which thousands of people exchange information daily. Finally, similar to how boat inspection and cleaning campaigns target focal "hub" lakes that receive greater amounts of boat traffic (Rothlisberger et al. 2010), we suggest that particular pet stores that sell large numbers of cosmopolitan taxa should be approached (perhaps with financial incentives) to participate in the distribution of educational materials.

Our second recommendation is that the responsibility of identifying and regulating species that are at great risk to invade native habitats should be shifted to the aquarium industry. This approach can take a number of routes. Padilla and Williams (2004) recommended that businesses post bonds equal to the cost of repairing damage resulting from the invasion and establishment of aquarium species. We fear that this policy may be costly and difficult to establish, particularly without the strong support of the aquarium industry (including importers, manufacturers, wholesalers, retailers), and conflicts with the "precautionary principle," which would prohibit the entry of any species that could become invasive (McDowall 2004). Peters and Lodge (2009) suggested that the industry be held responsible for demonstrating that a species will not cause "economic or ecological harm" via the creation of lists of allowed and banned species. However, the approach of creating lists of permitted species is not always successful. In Australia, >40% of established invasive aquarium species are on a list of species that are permitted for importation (McNee 2002). Additionally, blacklists of banned species can be difficult to enforce, particularly given the lack of knowledge about the ecological effects of most aquarium trade species (Lintermans 2004, Padilla and Williams 2004). The lack of information about most aquarium fishes is a more general problem and should be considered a research priority. For example, lethal temperatures for most of the fish species in the aquarium trade are unknown, despite the importance of temperature to invasion success.

A third possible strategy would involve the aquarium industry, but would shift the responsibility of disposing of unwanted fish to the aquarists; we call this the 'cash for critters' approach. The strategy involves providing a financial incentive to aquarists for returning unwanted live organisms to a pet store, which then can be re-sold (although concern regarding disease transmission may limit this option) or euthanized in a humane manner. The store benefits from re-selling the organism, and from the likelihood that the aquarist will buy more fish, whereas the aquarist could benefit by receiving a store voucher or discount. Notably, over a quarter of aquarists in our survey indicated that they had taken organisms to a store that has a return program.

Our final recommendation is to improve legislation on the importation and distribution of nonnative species in the ornamental trade, as well as response guidelines for local, state and federal jurisdictions. The aquarium trade pathway has been noted as having particularly weak regulatory oversight compared to other invasion pathways for fish (Thomas et al. 2009). Legislation that allows for a rapid management response to the detection of nonnative species can be a significant deterrent to their successful establishment: the marine alga, *Caulerpa taxifolia*, is a prominent aquarium species that invaded and was subsequently contained in California coastal waters by the enactment of a rapid response legislation (Anderson 2005). However, this type of legislation is rare in North America, especially in jurisdictions that have shared international waters, such as the Pacific Northwest (Thomas et al. 2009). The challenge of having different regulations across jurisdictions, i.e., "multiple

Table 4. List of educational resources on the release of aquarium organisms.

Source	Website
California Sea Grant	http://www-csgc.ucsd.edu/extension/
Convention on Biological Diversity	http://www.cbd.int/invasive/
Don't Release a Pest! University of Southern California - Sea Grant	http://www.usc.edu/org/seagrant/caulerpa/index.html
Global Invasive Species Program	http://www.gisp.org
Habitattitude™	http://www.habitattitude.net/
Oregon Sea Grant	http://seagrant.oregonstate.edu/themes/invasives/index.html
Ornamental Aquatic Trade Association	http://www.ornamentalfish.org/
Pet Industry Joint Advisory Council	http://www.pijac.org/aquatic
United States Geological Survey	http://nas.er.usgs.gov/taxgroup/fish/docs/dont_rel.asp

weak links," has been identified as a significant barrier to preventing the establishment and spread of nonnative species (Peters and Lodge 2009, Thomas et al. 2009). Greater legislative and regulatory control of nonnative aquatic species currently in the ornamental pet trade is needed, but requires coordinated action across state, provincial, federal, and international jurisdictions. International trade regulations on economically-valuable species can be successfully implemented (e.g., CITES: Ginsberg 2002). Although the US Fish and Wildlife Service's Lacey Act has successfully regulated the trade and prevented secondary spread of a handful of species (e.g., Java sparrow, brown tree snake), the Act is generally considered inefficient at preventing species invasions (Fowler et al. 2007). New federal policy is needed to support the necessary legal tools to better prevent further introduction of potentially and already harmful nonnative animals. One possibility to meet this objective is the recently introduced Nonnative Wildlife Invasion Prevention Act (H.R. 669); a bill that requires the Secretary of the Interior to promulgate regulations establishing a process for assessing the risk of all nonnative wildlife species proposed for importation into the United States, other than those included in a list of approved species issued under this Act. Thus far, Bill H.R. 669 has garnered a mixed reaction: the bill is supported by the National Wildlife Federation and Humane Society of the United States (among other organizations), but is adamantly opposed by the Pet Industry Joint Advisory Commission and a number of other sectors of the aquarium industry including importers and manufacturers.

In conclusion, the aquarium and ornamental trade represent a significant invasion pathway for fish and aquatic plants in the Pacific Northwest. Although the introduction pathways associated with ballast water and transport by trailered boats continue to receive the greatest attention with respect to research, management and policy, we cite the need for a greater appreciation of the ornamental pet trade as a source of nonnative species introductions. The greatest risk of nonnative fish species introductions via the aquarium trade likely lies in regions of higher human population sizes, and by association, higher numbers of aquarists and aquarium stores. These regions should be targeted for educational and legislative efforts. However, given the widespread availability of invasive species through mail-order and e-commerce, even rural areas are susceptible to species invasions via the aquarium trade (Kay and Hoyle 2001). Thus, there is a need for a comprehensive plan of action. Greater attention to educational programs involving the aquarium industry and new legislative action may help to reduce the importance of the aquarium trade as a pathway of freshwater species invasions in the Pacific Northwest.

ACKNOWLEDGMENTS

We would like to thank local pet stores for access to their invoices, and the questionnaire respondents. Tim Essington, Brian Leung, Kristin Jaeger, Eric Larson, David Lawrence, Thomas Pool, Mariana Tamayo, and two anonymous reviewers provided constructive feedback. Funding support for ALS and JDO was provided by the U.S. Geological Survey Gap Analysis Program.

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