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THE AQUARIUM TRADE AS AN INVASION PATHWAY IN THE PACIFIC NORTHWEST

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Running head: Aquarium trade and aquatic invasions

26 **ABSTRACT**

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

41

42 **INTRODUCTION**

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

52 To date, considerable research activity and management attention has focused on
53 unintentional pathways to AIS introductions through ballast-water transfer in ships (e.g., Carlton
54 and Geller 1993, Ruiz et al. 1997), transport via trailered boats (e.g., Leung et al. 2006,
55 Rothlisberger et al. 2010), bait-bucket releases by recreational anglers (e.g., Litvak and Mandrak
56 1993, DiStefano et al. 2009), and escapes associated with aquaculture (e.g., Naylor et al. 2001,
57 De Silva et al. 2009). By contrast, the ornamental pet and aquarium trade has only recently been
58 recognized as a major pathway for freshwater fish and plant introductions (Copp et al. 2010).
59 This is despite the fact that the ornamental pet trade represents a multi-billion dollar industry that
60 includes thousands of foreign species and has grown by 14% annually since the 1970s (Padilla
61 and Williams 2004, Cohen et al. 2007). Although the import of some nonnative species common
62 to the pet trade are regulated by certain countries (e.g., reptiles in Australia and New Zealand),
63 ornamental fish generally have not received attention from regulatory agencies (Thomas et al.
64 2009, Secretariat of the Convention on Biological Diversity 2010). Additionally, reliable record

65 keeping of the type and number of organisms currently in the trade is lacking (Schlaepfer et al.
66 2005, Smith et al. 2008, Chang et al. 2009). Given the present uncertainty in the taxonomy of
67 many ornamental fish and plant species within the aquarium trade, as well as the widespread
68 contamination of many aquarium plants with unidentified organisms (e.g., molluscs: Keller and
69 Lodge 2007), our ability to assess invasion risk associated with this pathway is limited.

70 Aquarium trade species are introduced when owners release unwanted organisms into
71 natural waterbodies for various reasons, including large size, humane treatment, aggressiveness,
72 and high reproductive rates (Padilla and Williams 2004, Gertzen et al. 2008). The most popular
73 fish sold in the aquarium trade are also the most likely to be introduced and establish in
74 freshwater habitats (Duggan et al. 2006). Although the aquarium trade and its associated vectors
75 have been increasingly recognized as a primary pathway of biological invasions in the
76 Laurentian Great Lakes region (Rixon et al. 2005, Cohen et al. 2007, Gertzen et al. 2008) and the
77 San Francisco Bay-Delta region (Chang et al. 2009), surprisingly little is known regarding the
78 scope of the issue in the Pacific Northwest of the United States. In a region where invasive
79 species are considered a significant threat to native biodiversity, ecosystem function, and
80 culturally- and economically-important Pacific salmon (Sanderson et al. 2009), it is imperative
81 that scientific research is available to quantify the strength of the aquarium trade as a pathway of
82 new invasions. In Washington and Oregon, there are a number of plant and animal species that
83 have likely been introduced into the wild via the aquarium trade, including oriental weatherfish
84 (*Misgurnus anguillicaudatus*), Amur goby (*Rhinogobius brunneus*), red-bellied pacu (*Piaractus*
85 *brachypomus*), goldfish (*Carassius auratus*), red swamp crayfish (*Procambarus clarkii*), Chinese
86 mystery snail (*Cipangopaludina chinensis malleata*), Eurasian watermilfoil (*Myriophyllum*

87 *spicatum*), and parrot feather (*Myriophyllum aquaticum*)¹. Thus, the scope of this problem is
88 significant. Furthermore, many aquarium species may become more successful at establishing in
89 higher latitudes with warmer temperatures projected to occur under climate change scenarios
90 (Rahel and Olden 2008, Chang et al. 2009).

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

102

103 **METHODS**

104 *Store Inventory Surveys and Aquarist Questionnaires*

105 We conducted an intensive (temporal trends in a single store) and extensive (spatial trends from
106 multiple stores) survey of pet stores in the Puget Sound area of Washington to document the
107 numbers and types of fish and plant taxa in the ornamental pet trade (Figure 1). The intensive
108 survey analyzed monthly sale invoices from a single (large and independent) pet store in 2007.
109 All fish and plant species were identified and individuals counted. Fish were separated into

¹ <http://nas.er.usgs.gov/>

110 ornamental (i.e., fish of primary interest for viewing) and feeder (i.e., to feed to other fish)
111 species. When there was a disparity between a store label scientific name and common name, we
112 used the scientific name provided in FishBase (Froese and Pauly 2009). Additionally, 30 pet
113 stores in Snohomish, King, and Pierce counties were surveyed over a two-week period in
114 February 2008 to regionally characterize the ornamental pet trade (Figure 1). Two different
115 national pet store chains were chosen for the survey (chain A, n = 14; chain B, n = 8), as well as
116 eight independently owned stores. There is some evidence that independent retailers differ in the
117 numbers and types of species sold compared to chain stores (Chang et al. 2009); understanding
118 differences between store types can help direct educational efforts. Preliminary analyses
119 indicated that the two sets of chain stores differed in the numbers and types of species sold,
120 therefore, we analyzed the chains separately. Stores were visited a day after receiving their fish
121 and plant shipment (determined by contacting the store managers) to enumerate organisms
122 before they were purchased, but after any had died from travel stress. This ensured that our
123 survey accurately reflected the current inventory of store. Time constraints precluded the
124 enumeration of individual plants, resulting in species being reported as present or absent.

125 Fish and plant taxa observed in our regional survey were compared to the USGS
126 Nonindigenous Aquatic Species list¹ and the Washington State Aquatic Nuisance Species list² to
127 determine whether the species have a demonstrated history of invasion in other regions of the
128 United States. The USGS defines nonindigenous species as species that are outside of their
129 historic or native range, whereas Washington State defines invasive species as species that are
130 not historically native to the state. Previous invasion history is one of the best predictors of
131 invasion potential (Ricciardi and Rasmussen 1998). For the purposes of our study these taxa
132 were designated as “invasive.” Next, optimal and lethal temperature requirements for selected

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

133 fish species were obtained from FishBase (Froese and Pauly 2009): when lethal limits were
134 unavailable, values were obtained from primary literature (white cloud mountain minnow,
135 *Tanichthys albonubes*: Cheverie and Lynn (1963); goldfish, *Carassius auratus*: Ford and
136 Beitinger (2005); molly, *Poecilia sphenops*: Hernández and Bückle (2002); koi carp, *Cyprinus*
137 *carpio carpio*: Opuszyński et al. (1989)). These species were chosen because they represent a
138 cross-section of species common to aquarium and pet stores; they have been identified
139 previously as species with potential to establish in temperate North America and Washington
140 State (Tabor et al. 2001, Rixon et al. 2005, Gertzen et al. 2008); and there was optimal and lethal
141 temperature range data available in the literature. Optimum temperature was defined as the range
142 of temperatures in which fish species typically habituate in the wild, whereas lethal temperatures
143 indicate absolute minimum and maximum temperatures that fish can survive in under
144 experimental settings (Brett 1956). Additionally, data layers for stream water temperatures from
145 2000-2008 were obtained from 236 monthly monitoring sites in the Environmental Protection
146 Agency's STORET Database³. Water temperature data were summarized as mean annual
147 temperature because values from winter months (i.e., minimum values) were not consistently
148 available. We use stream temperatures as our proxy of water temperatures across the state
149 because data from a suitable number of lakes was not available.

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

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

155 were released; and 4) methods of depositing of fish and plants. Questionnaires were randomly
156 given to aquarists in the store, and responses were anonymous.

157

158 *Analysis of Taxa Currently in the Pet Aquarium Trade*

159 The number of fish and plant species per store, and the number of fish individuals per store
160 recorded during the spatial survey of pet stores were averaged within store category (chain A,
161 chain B, and independent). Fish abundance and number of fish species were ln-transformed, and
162 the number of plant species was square-root transformed prior to analysis to normalize data. We
163 used analysis of variance (ANOVA) tests, followed by a Tukey HSD post hoc test, to examine
164 differences among store types in fish abundance, and fish and plant species richness.

165 Additionally, we performed a multivariate analysis to examine similarities and differences in the
166 abundances of each fish species sold across the different store types. Fish species that occurred in
167 <10% of stores were excluded from the analysis, and counts were standardized to z-scores to
168 reduce the influence of rare and/or abundant taxa. We used non-metric multidimensional scaling
169 (NMDS) to summarize store differences, as NMDS is effective with non-normal data and can
170 use any distance measure (Legendre and Legendre 1998). We used the Bray-Curtis dissimilarity
171 index, and tested for significance of the ordination with randomization tests. According to
172 multivariate stress values we found that the optimal ordination utilized three dimensions.
173 Ordination analyses were performed using the *MASS* library in R (R Development Core Team
174 2010).

175

176 *Propagule Pressure Model*

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

$$182 \quad \text{propagule pressure} = M \cdot P(I) \cdot N \cdot P(R|I)$$

183 where M is the number of households that own aquarium fish, $P(I)$ is the probability that an
184 owner is a releaser, N is average number of fish owned annually, and $P(R|I)$ is the probability
185 fish are released given that an owner is a releaser (Gertzen et al. 2008). We based the parameters
186 $P(I)$ and N on data from our aquarist survey, whereas $P(R|I)$ was derived from Gertzen et al.
187 (2008). We used a Bayesian approach to incorporate uncertainty about the representativeness of
188 our values in the model. Bayesian statistics consider prior information in the determination of
189 parameters from a data set. Thus, we created probability distributions that reflected our data: we
190 multiplied these distributions by each other to generate a joint probability distribution.

191 The number of households that own aquarium fish, M , was determined by multiplying the
192 number of households in King, Snohomish, and Pierce counties (1,196,568; US Census 2000⁴)
193 by the percentage of U.S. households that own fish (10.6%), and by the percentage of fish in the
194 aquarium trade that are freshwater (96%) (values from Chapman et al. 1997). $P(I)$, the
195 probability of being a releaser, was a binary variable determined from survey data and modeled
196 with a binomial distribution (i.e., heads or tails), which reflects the uncertainty from our random
197 survey of 92 people (Bolker 2008). N , the number of fish owned annually, was determined from
198 our survey and was modeled with a negative binomial distribution. In a negative binomial
199 distribution, the variance is larger than the mean (Bolker 2008), which reflects the large number

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

200 of aquarists who own a small number of fish (e.g., ≤ 5), but that a small number of aquarists own
201 large numbers of fish. $P(R|I)$, the probability that fish are released given that the owner is a
202 releaser, was based on the value of Gertzen et al. (2008) (5.1%) and modeled with a beta
203 distribution that is bound by 0 and 1 (Bolker 2008). Each probability distribution ($P(I)$, N , and
204 $P(R|I)$) was then combined with a uniform flat prior to generate posterior distributions; we used
205 uniform priors as we had no prior expectations about model parameters (Bolker 2008, Gertzen et
206 al. 2008). Finally, all combinations of the posterior distributions and the constant, M , were
207 multiplied together to create a joint probability distribution that reflects the inherent uncertainty
208 in our survey data. All propagule pressure model steps were performed in R (R Development
209 Core Team 2010).

210

211 **RESULTS**

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

217 Our regional survey of 30 pet stores identified 400 fish species and 124 plant taxa
218 currently in the ornamental trade, a number that represents the minimal species pool for the
219 Pacific Northwest region. None of the fish species are native to Washington State and only 8
220 plant species are natives. Of the 400 fish species, 29 occurred in greater than 75% of stores,
221 including two taxa, tiger barb (*Puntius tetrazona*) and three spot gourami (*Trichogaster*
222 *trichopterus*), which occurred in all of the stores surveyed (Table 1). Other commonly

223 encountered fish species included goldfish, Siamese fighting fish (*Betta splendens*), several
224 different tetras, mollies, and guppies (*Poecilia* spp.) (Table 1). Additionally, a number of species
225 that have previously been detected in the wild in Washington State were found in a lower
226 proportion of stores in the survey: koi carp = 60%, oriental weatherfish = 33%, and Amazon
227 sailfin pleco (*Pterygoplichthys pardalis*) = 3%. Plant species occurred with lower frequency: the
228 top species, Amazon sword (*Echinodorus amazonicus*), occurred in 77% of stores, and an
229 additional 19 taxa occurred in >25% of stores (Table 2). On average, 58% of fish individuals,
230 43% of fish species, and 5% of plant species found in pet stores were considered invasive
231 according to the USGS Nonindigenous Aquatic Species list and the Washington State Aquatic
232 Nuisance Species list. However, the maximum number of invasive taxa encountered in a single
233 store in our survey indicated that invaders comprised up to 72% of fish individuals, 61% of fish
234 species, and 17% plant species in a store. Further, a number of fish and plant species in our
235 survey are within the same genera as species that are considered invasive (Table 1 and 2).
236 Additionally, in one store we found an aquatic plant species, the water chestnut (*Trapa natans*),
237 which is banned for sale by the Washington State Department of Agriculture.

238 As many aquarium species are tropical in origin, their minimum optimal temperatures
239 typically exceed winter water temperatures observed in temperate waterbodies (Table 1, Figure
240 3). However, several aquarium species that are prevalent in the ornamental trade have lower-
241 lethal and minimum optimal temperatures that are found well within the range of mean annual
242 stream water temperatures in Washington State (Figure 3). This includes several taxa, such as the
243 white cloud mountain minnow and the oriental weatherfish, which may have high invasion
244 potential. Oriental weatherfish have established a population in Washington (Tabor et al. 2001),
245 and the white cloud mountain minnow are considered a high risk invader in the United States as

246 a result of broad thermal tolerance (Rixon et al. 2005). Additionally, koi carp and goldfish (both
247 present in Washington) have very broad thermal tolerance ranges, suggesting that these species
248 may have elevated establishment potential. Our estimates of concordance between fish species
249 thermal tolerance and water temperatures in Washington State may be conservative, as lakes may
250 exhibit greater thermal heterogeneity compared to streams.

251 We found differences between fish and plant inventories from our regional survey of
252 chain stores and a set of independent stores. Chain A had significantly lower numbers of fish
253 individuals per store compared to chain B (ANOVA: $F_{2,27} = 9.56$, $p < 0.01$; Tukey HSD $p <$
254 0.05), but neither chain store was different from the set of independent stores (Tukey HSD $p >$
255 0.05) (Figure 4A). Chain A also had significantly fewer fish species compared to both chain B
256 and independent stores (ANOVA: $F_{2,27} = 13.00$, $p < 0.01$; Tukey HSD $p < 0.05$), but there was
257 no difference between chain B and independents (Tukey HSD $p > 0.05$) (Figure 4B). We found
258 no differences in the number of plant species between store types (ANOVA: $F_{2,27} = 0.75$, $p =$
259 0.48) (Figure 4C). Results from the multivariate analysis on fish abundance revealed strong
260 clustering of store types in ordination space (Figure 5). Little overlap of stores in multivariate
261 space was observed, suggesting that store types have fairly distinctive inventories of ornamental
262 fish (although clear similarities exist in that all stores have a core suite of species in their
263 inventories). Most notably, stores that are independently owned occupied the greatest area in
264 ordination space, suggesting that they carry the highest diversity of fish species (Figure 5,
265 supported by Figure 4).

266 The results from our questionnaire indicated that, on average, aquarists owned ~ 9 fish
267 (median = 5) and that 6.4% of aquarists had released live fish in the past. The majority of
268 introductions were into lakes or streams. Using a Bayesian statistical approach, we estimated that

269 the most likely number of fish introduced annually into the Puget Sound area was 2,536;
270 however, the 95% confidence interval suggests that 20,869 fish could be introduced in a year
271 (Figure 6).

272

273 **DISCUSSION**

274 Using a combination of regional store surveys, aquarist questionnaires, and statistical models, we
275 have demonstrated that the number of fish (n=400) and plant (n=124) species currently in the
276 aquarium trade is vast, the majority of species in the trade are not native to the region, and that
277 this introduction pathway deserves greater research and regulation in the Pacific Northwest. Pet
278 and aquarium stores import thousands of fish every month, the majority of which (58%) are
279 considered to pose an ecological threat to native ecosystems. Our model suggests that up to
280 21,000 fish (average of 2,500 individuals) are likely released into the wild each year in the Puget
281 Sound area by aquarists, and that water temperatures in many parts of Washington State are
282 suitable to allow establishment of populations. The predictions of our model suggest that the pet
283 trade is a significant pathway of AIS introductions, particularly around populated urban centers,
284 yet far greater research effort and funding for prevention have been directed towards boater
285 movement as an invasion pathway (e.g., Leung et al. 2006, Rothlisberger et al. 2010). This is
286 particular true in Washington State where management efforts continue to focus on preventing
287 invasions via trailered boats (State of Washington Joint Legislative Audit & Review Committee
288 2010). To illustrate the potential importance of the ornamental pet trade, we compared several
289 different features of the aquarium and boater pathways (Table 3). Our comparison suggests that
290 the number of aquarists is similar to the number of registered boats in Washington State (i.e.,
291 vector strength), and that propagule pressure from the aquarium trade is high relative to boats for

292 some taxonomic groups (e.g., fish, invertebrates: Duggan (2010)), but low for others (e.g.,
293 plants). Management and educational challenges are likely very different between the pathways;
294 the distribution of aquarists (i.e., reflecting the location of potential introductions) is spatially-
295 diffuse, whereas the distribution of boat launches is well defined. This comparison underscores
296 the importance of this understudied pathway, and highlights the significant management and
297 educational challenges that the aquarium pathway represents.

298 The regional survey of 30 pet and aquarium stores indicated that independently owned
299 stores tend to carry a greater number of—and a more unique variety of—fish species compared
300 to some chain stores (e.g., chain A: Figures 4,5). Despite the lower diversity, chain store B had a
301 larger inventory available for sale; presumably, related to faster turnover of stocks. Our results
302 concur with the study of Chang et al. (2009) in the San Francisco Bay-Delta region, where
303 independent retailers generally sold greater numbers of fish species compared to chain stores.
304 Goldfish, Siamese fighting fish, neon tetras (*Paracheirodon innesi*), and guppies and/or mollies
305 all occur frequently in our study, as well as those of Gertzen et al. (2008) and Rixon et al. (2005)
306 conducted in the Laurentian Great Lakes region. In our survey, the number of aquatic plants was
307 similar between store types, but the composition tended to be relatively different, as only six
308 species occurred in more than half of the stores surveyed. In contrast to aquarium fish, the most
309 common aquatic plants differed from a similar study conducted in another region: only two taxa,
310 Amazon sword and hornwort (*Ceratophyllum demersum*), were frequently encountered in our
311 study and the study conducted by Rixon et al. (2005). Overall, the moderate climate of the
312 Pacific Northwest, as well as large population centers in the Puget Sound basin, suggests that
313 freshwater ecosystems are threatened by the establishment of nonnative species from the
314 aquarium trade. We expect that other large urban centers in the Pacific Northwest, such as

315 Portland, Oregon and Vancouver, British Columbia, would be similarly at risk of nonnative
316 species introductions via the aquarium trade pathway, and thus, should be targets for educational
317 campaigns. Further, climate change will certainly increase establishment of nonnative aquarium
318 and pet trade species in the Pacific Northwest, where temperatures are predicted to increase by >
319 3°C by the end of the 21st century (Mote and Salathé Jr. 2010). Additionally, nonnative species
320 introductions via the aquarium trade in milder tropical and sub-tropical habitats will have
321 substantially greater establishment success because of greater thermal suitability. Indeed,
322 established populations of aquarium trade species have been increasingly detected in the
323 southern United States (e.g., Florida: Padilla and Williams 2004).

324 Our study identified several fish species that may be of particular concern for
325 establishment of populations via the aquarium pathway. The oriental weatherfish currently has
326 an established, but limited, distribution in Washington State (i.e., Lake Washington basin in
327 Seattle: Tabor et al. 2001), and further invasions seem likely without successful intervention and
328 management of this pathway. According to our regional survey of pet stores, oriental weatherfish
329 are found in chain and independent stores, but are currently more common in the inventories of
330 chain stores compared to independent stores (Figure 5). The invasion of oriental weatherfish may
331 have serious consequences for fisheries in the Pacific Northwest. Perhaps most notably, the virus
332 birnavirus LV1 was isolated from invasive oriental weatherfish in Australia (Lintermans et al.
333 1990). Birnavirus LV1 is related to the infectious pancreatic necrosis virus, a disease of salmonid
334 fish (Wolf 1988). Additionally invasive parasites have been found in oriental weatherfish (Dove
335 and Ernst 1998). Further, it has been shown that oriental weatherfish can reduce the abundance
336 and biomass of macroinvertebrates (Keller and Lake 2007) and prey on fish larvae (Logan et al.

337 1996). Altogether, these factors suggest that oriental weatherfish may have significant effects on
338 native fish populations and should be a target for invasion vector management.

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

353

354 *Recommendations*

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

359 First, we believe that public education programs targeted at the interface of aquarium
360 owners and retailers will likely have the greatest success. One such program, Habitattitude™, is
361 a partnership of the Pet Industry Joint Advisory Council, the U.S. Fish and Wildlife Service, and
362 NOAA National Sea Grant College Program, with the mandate, "...to eliminate the transfer and
363 survival of any species outside of [an] enclosed, artificial system, which has the potential to
364 cause the loss or decline of native plants and animals." The Habitattitude™ program supplies
365 educational materials (e.g., pamphlets and stickers) to pet stores, as well as plastic bags with the
366 message "Do not release fish and aquatic plants." This is an important step towards educating
367 aquarium owners about the harm of releasing live organisms into the wild; however, our study
368 found that these materials were only present in chain stores and absent from independent
369 retailers. We have demonstrated that independent stores tend to carry a larger variety of fish
370 species compared to chain stores, therefore, we recommend that independent retailers should be
371 the next focal point of the Habitattitude™ campaign and other private and government funded
372 education programs (Table 4). Efforts to educate aquarists on the repercussions of releasing
373 aquarium fish and plants to the wild will only be successful if the distribution of educational
374 materials reaches the broadest possible audience, including the vast and under-appreciated
375 Internet trade in ornamental species (Secretariat of the Convention on Biological Diversity
376 2010). However, these efforts should be coupled with more directed educational campaigns that
377 target pet enthusiasts that belong to the hundreds of aquarium societies across the United States
378 (e.g., Greater Seattle Aquarium Society), national and international aquarium associations (e.g.,
379 Heart of America Aquarium Society, Canadian Association of Aquarium Clubs, Federation of
380 British Aquatic Societies), and online aquarium forums and websites in which thousands of
381 people exchange information daily. Finally, similar to how boat inspection and cleaning

382 campaigns target focal “hub” lakes that receive greater amounts of boat traffic (Rothlisberger et
383 al. 2010), we suggest that particular pet stores that sell large numbers of cosmopolitan taxa
384 should be approached (perhaps with financial incentives) to participate in the distribution of
385 educational materials.

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

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

413 Our final recommendation is to improve legislation on the importation and distribution of
414 nonnative species in the ornamental trade, as well as response guidelines for local, state and
415 federal jurisdictions. The aquarium trade pathway has been noted as having particularly weak
416 regulatory oversight compared to other invasion pathways for fish (Thomas et al. 2009).
417 Legislation that allows for a rapid management response to the detection of nonnative species
418 can be a significant deterrent to their successful establishment: the marine alga, *Caulerpa*
419 *taxifolia*, is a prominent aquarium species that invaded and was subsequently contained in
420 California coastal waters by the enactment of a rapid response legislation (Anderson 2005).
421 However, this type of legislation is rare in North America, especially in jurisdictions that have
422 shared international waters, such as the Pacific Northwest (Thomas et al. 2009). The challenge of
423 having different regulations across jurisdictions, i.e., “multiple weak links,” has been identified
424 as a significant barrier to preventing the establishment and spread of nonnative species (Peters
425 and Lodge 2009, Thomas et al. 2009). Greater legislative and regulatory control of nonnative
426 aquatic species currently in the ornamental pet trade is needed, but requires coordinated action

427 across state, provincial, federal, and international jurisdictions. International trade regulations on
428 economically-valuable species can be successfully implemented (e.g., CITES: Ginsberg 2002).
429 Although the US Fish and Wildlife Service's Lacey Act has successfully regulated the trade and
430 prevented secondary spread of a handful of species (e.g., Java sparrow, brown tree snake), the
431 Act is generally considered inefficient at preventing species invasions (Fowler et al. 2007). New
432 federal policy is needed to support the necessary legal tools to better prevent further introduction
433 of potentially and already harmful nonnative animals. One possibility to meet this objective is
434 the recently introduced Nonnative Wildlife Invasion Prevention Act (H.R. 669); a bill that
435 requires the Secretary of the Interior to promulgate regulations establishing a process for
436 assessing the risk of all nonnative wildlife species proposed for importation into the United
437 States, other than those included in a list of approved species issued under this Act. Thus far, Bill
438 H.R. 669 has garnered a mixed reaction: the bill is supported by the National Wildlife Federation
439 and Humane Society of the United States (among other organizations), but is adamantly opposed
440 by the Pet Industry Joint Advisory Commission and a number of other sectors of the aquarium
441 industry including importers and manufacturers.

442 In conclusion, the aquarium and ornamental trade represent a significant invasion
443 pathway for fish and aquatic plants in the Pacific Northwest. Although the introduction pathways
444 associated with ballast water and transport by trailered boats continue to receive the greatest
445 attention with respect to research, management and policy, we cite the need for a greater
446 appreciation of the ornamental pet trade as a source of nonnative species introductions. The
447 greatest risk of nonnative fish species introductions via the aquarium trade likely lies in regions
448 of higher human population sizes, and by association, higher numbers of aquarists and aquarium
449 stores. These regions should be targeted for educational and legislative efforts. However, given

450 the widespread availability of invasive species through mail-order and e-commerce, even rural
451 areas are susceptible to species invasions via the aquarium trade (Kay and Hoyle 2001). Thus,
452 there is a need for a comprehensive plan of action. Greater attention to educational programs
453 involving the aquarium industry and new legislative action may help to reduce the importance of
454 the aquarium trade as a pathway of freshwater species invasions in the Pacific Northwest.

455

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461

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599
600
601

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

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

604 ^a listed as USGS Nonindigenous Species

605 ^b listed as Washington State Aquatic Nuisance Species

606 ^c species within same genus listed as USGS Nonindigenous Species

607 Table 2. Frequency of occurrence of aquarium plant species in pet stores (>25%).
 608

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

609 ^a listed as USGS Nonindigenous Species

610 ^b species within same genus listed as Washington State Noxious Aquatic Weed

611

612 Table 3. Comparison of the aquarium trade and trailered boats as pathways of nonnative species
 613 invasions. This illustrative comparison indicates that the threat posed by the aquarium trade may
 614 be comparable to that of boat trailers. For establishment success, categories of low, moderate,
 615 and high are simply qualitative characterizations based on relative comparisons of potential
 616 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	<i>fish</i> : <1 fish released per aquarist per year ¹ <i>invertebrates</i> : >4,000 released per aquarist per year ³ <i>plants</i> : <1 released per aquarist per year ²	<i>invertebrates + plants</i> : ~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	<i>fish</i> : low, Allee effects <i>invertebrates</i> : moderate, some asexual reproduction <i>plants</i> : high, vegetative reproduction	<i>invertebrates</i> : moderate, some asexual reproduction <i>plants</i> : 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

617 ¹ this study

618 ² Cohen et al. (2007)

619 ³ Duggan (2010)

620 ⁴ Rothlisberger et al. (2010)

621 ⁵ 2000 US Census: <http://www.census.gov/main/www/cen2000.html> and based on the percentage
 622 of U.S. households that own fishes (10.6%) according to values from Chapman et al. (1997).

623 ⁶ State of Washington Joint Legislative Audit and Review Committee (2010). Recreational
 624 vessels include sailboats, yachts, and motorized boats that were registered in 2008; only a
 625 fraction of these boats are trailered.

626 ⁷ Gertzen et al. (2008)

627

628

629 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

630

631

632 **FIGURE CAPTIONS**

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

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

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

651 Figure 4: Comparisons of chain and independent pet stores for A) number of fish individuals per
652 store, B) number of fish species per store, and C) number of plant species per store.
653 Center line in box plots represents median, lower and upper box boundaries are the 25th
654 and 75th percentiles, and whiskers are the 10th and 90th percentiles. Outliers are

655 represented by circles. Letters above the bars represent the results of Tukey HSD post
656 hoc tests, where different letters indicate significant differences between store categories
657 ($p < 0.05$).

658 Figure 5: Non-metric multidimensional scaling of relative abundance of aquarium fishes in pet
659 stores in the Puget Sound area using Bray-Curtis dissimilarity. Chain stores (triangles, n
660 = 8; squares, $n = 14$) are contrasted with a set of independent stores (circles, $n = 8$).
661 Common names of fishes highly correlated with NMDS axes are indicated on the outer
662 edges of the graph. NMDS stress = 10.2, $p = 0.02$ on three dimensions. Ellipses drawn
663 around the outer edges of groups of stores are simply for illustration. Star indicates the
664 species score of oriental weatherfish (*Misgurnus anguillicaudatus*), one of several
665 aquarium fishes with an established population in Washington.

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

669

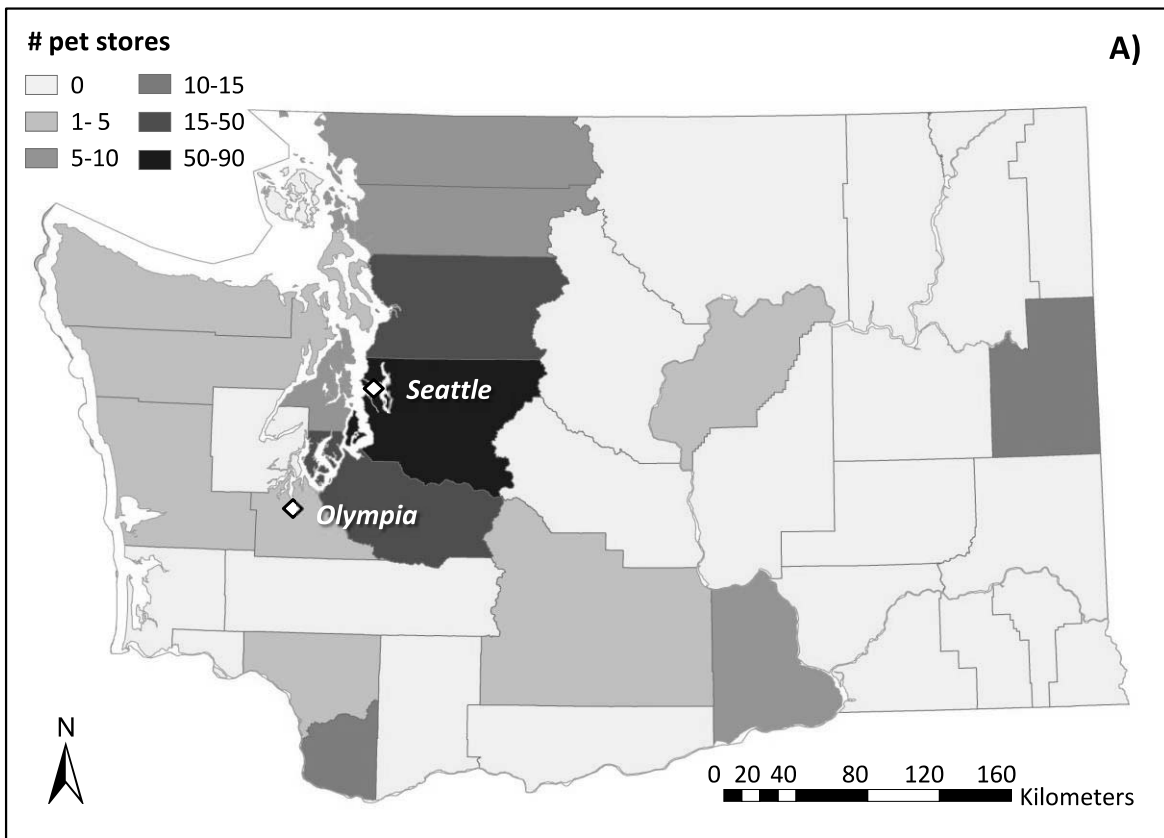


Figure 1

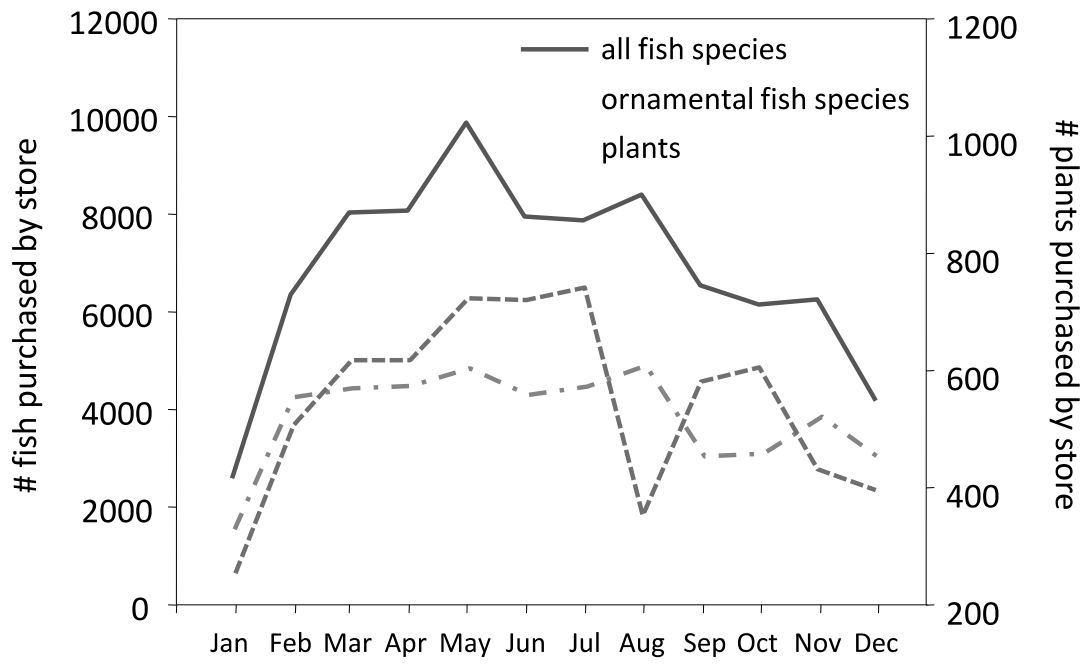


Figure 2

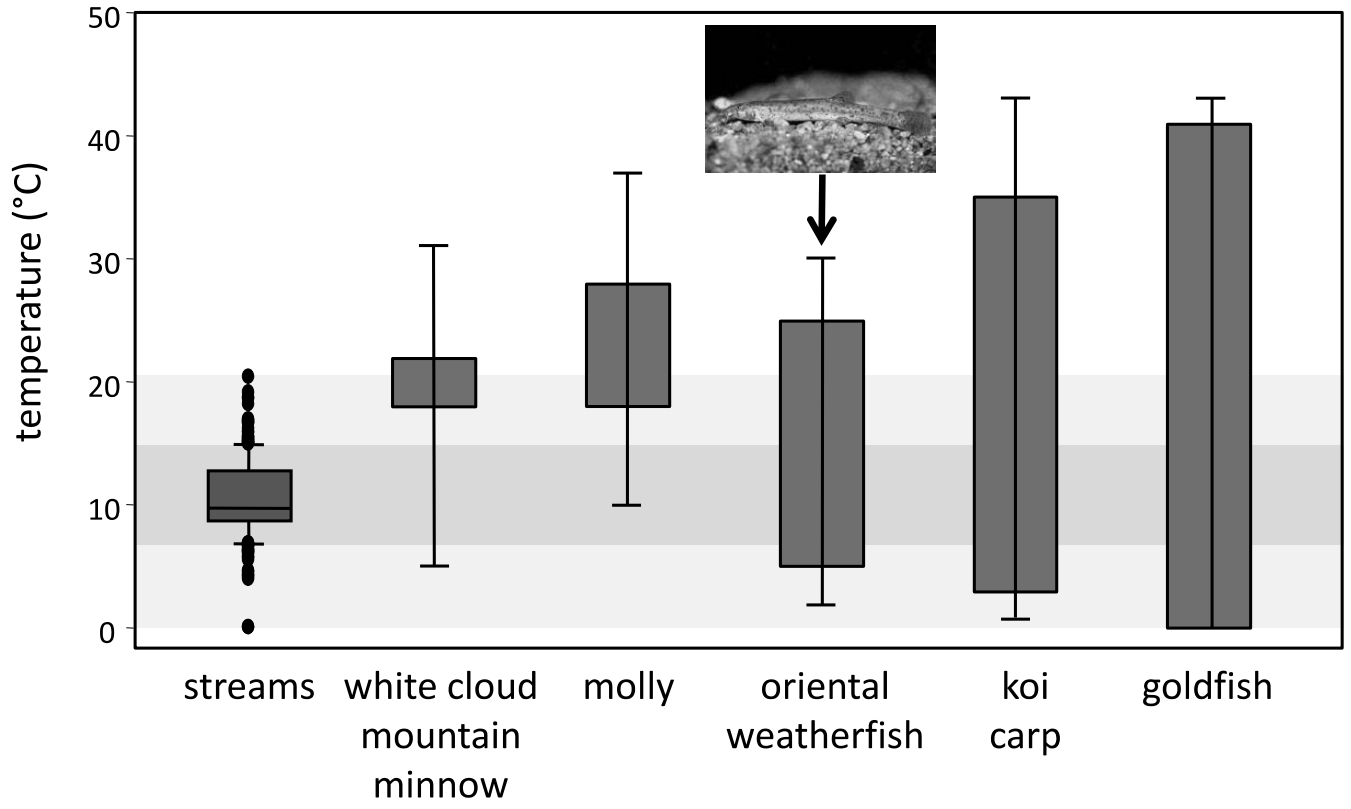


Figure 3

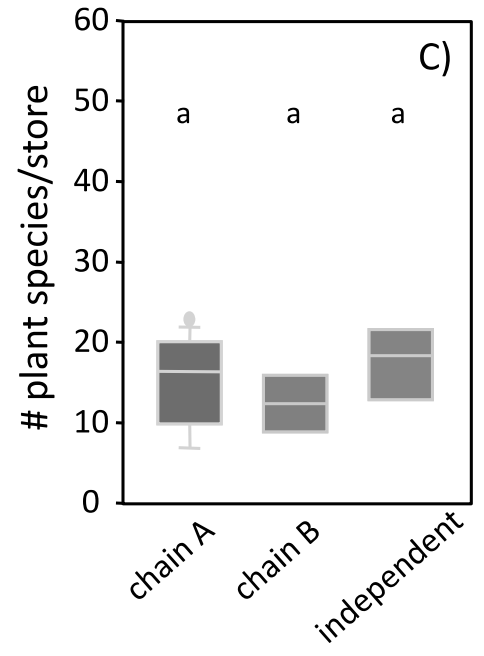
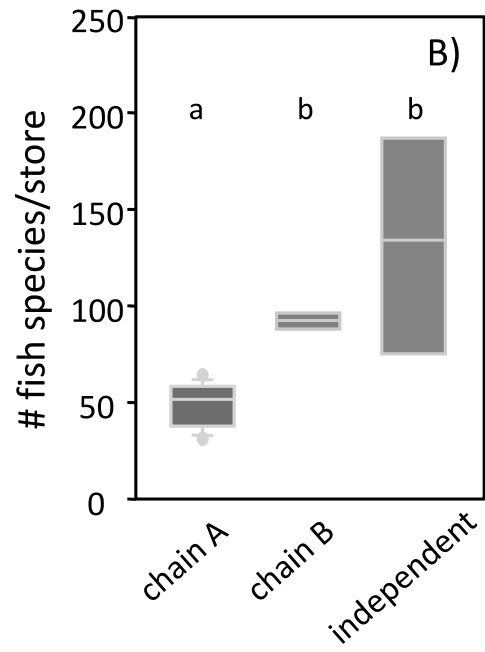
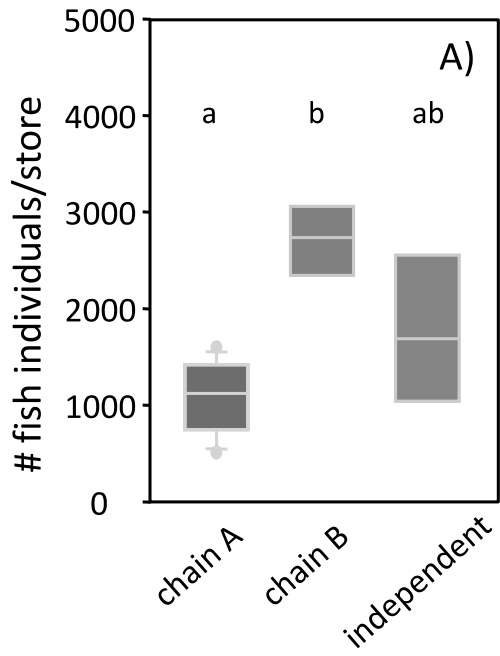


Figure 4

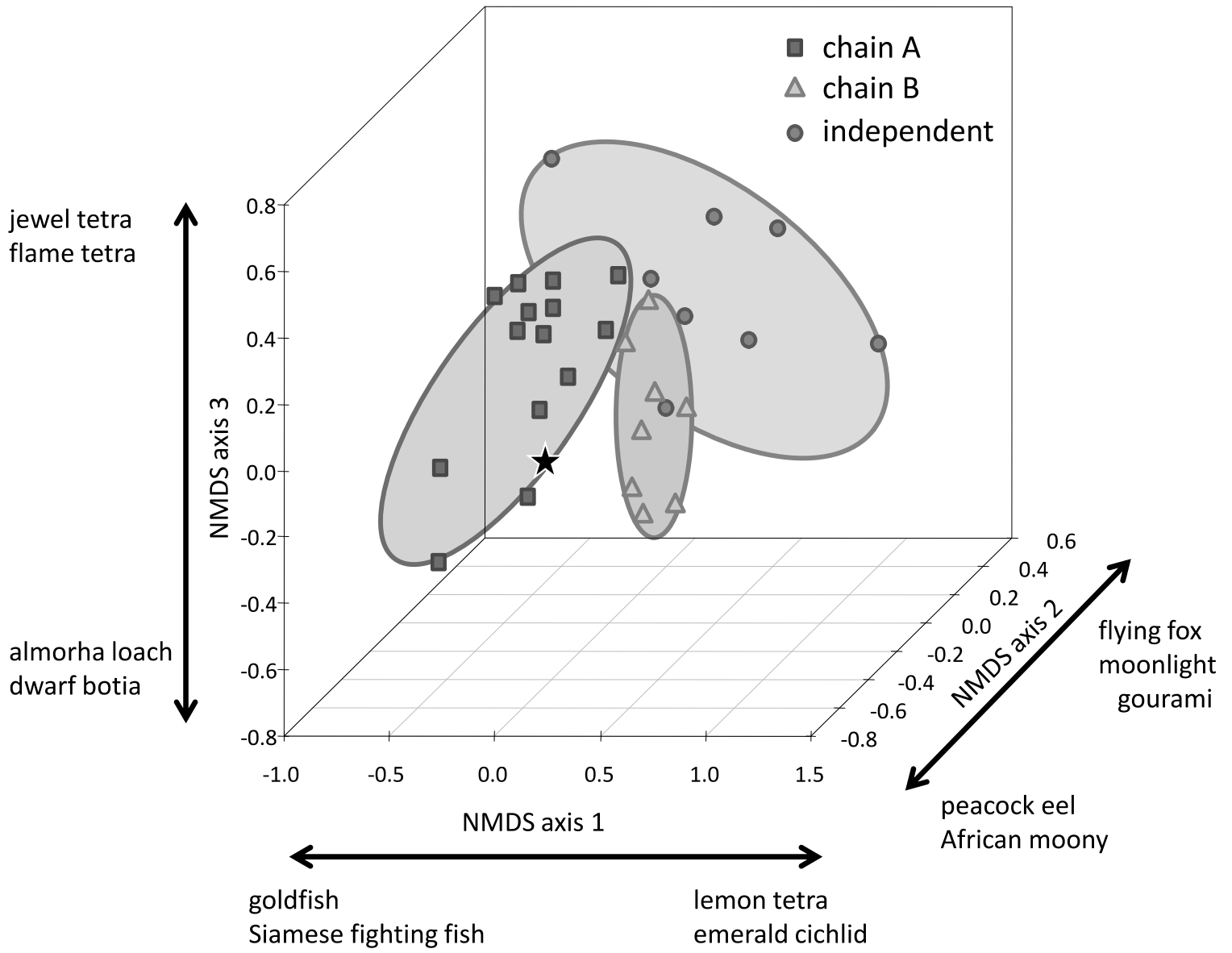


Figure 5

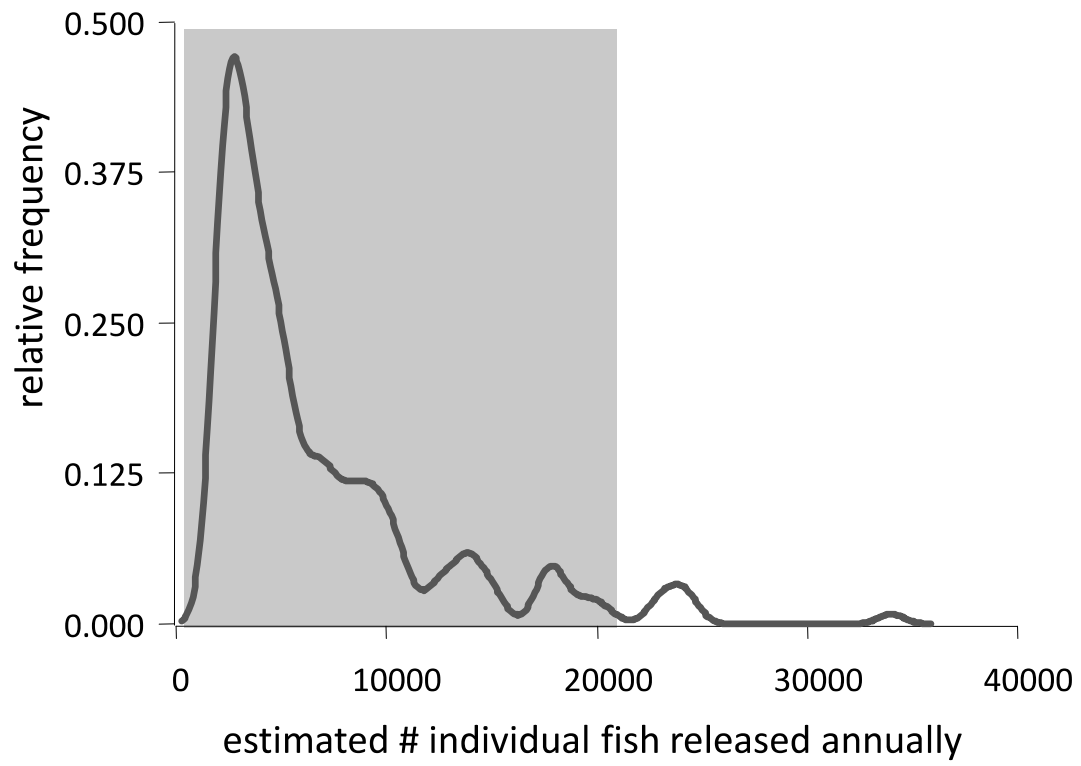


Figure 6