Urban development modifies lake food webs in the Pacific Northwest

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Abstract

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Residential shoreline and watershed development by humans are leading drivers of biodiversity loss in lake ecosystems that reduce abundances and diversity littoral invertebrates. Invertebrate biological and life history traits provide good indicators of environmental quality and ecosystem functioning, yet surprisingly few studies have utilized trait-based approaches to assess impacts of human development to lake littoral communities. My thesis addresses how human development modifies lake food webs by restructuring littoral macroinvertebrate communities and altering the flow of energy to lake consumers. In Ch. III, I used a traits-based approach to assess the impacts of development to littoral macroinvertebrate community structure, and I discuss environmental mechanisms and implications to ecosystem functioning. Multiple linear regressions revealed that functional diversity declined with increasing watershed development, concentrations of total phosphorus, and littoral macrophyte cover. Results from multivariate constrained ordination and

fourth corner analysis indicated that high phosphorus concentrations and macrophyte cover filtered taxa with semivoltine life cycles and filter feeders from lake communities, and that both regional and local characteristics of watershed development were important determinants of invertebrate community structure. Urban development had particularly pronounced effects on invertebrate communities in deep littoral zones, for which overall community abundances declined as a result of removals of woody debris and increased phosphorus concentrations. My study indicates that lake shoreline development and nutrient loading favor assemblages of short-lived organisms and herbivores and act as environmental filters of other functional feeding groups. These changes to invertebrate community structure may have important implications for energy flow between terrestrial, littoral, and pelagic food webs.

In Ch. II, I examined whether a non-native species provides a prey resource to consumers in lakes across gradients of urban development and native prey availability. I used stable isotopes of carbon, nitrogen, and hydrogen to assess resource use by consumers in undeveloped and developed lakes and determine whether non-native Chinese Mystery snail maintains the integration of benthic resources in food webs of developed lakes by providing an abundant prey resource. I found that consumers in undeveloped lakes were supported primarily by benthic resources, and lakeshore development dramatically reduced consumer reliance on these resources. This was at least partly due to a reduction in the availability of native snails, a high quality prey item, to the dominant littoral consumer, molluscivorous pumpkinseed sunfish (*Lepomis gibbosus*). In developed lakes with non-native *Bellamya*, generalist yellow perch (*Perca flavescens*) and piscivorous largemouth bass (*Micropterus salmoides*) consumed benthic resources in proportions similar to undeveloped lakes, and pumpkinseed sunfish consumed *Bellamya* in higher proportions than in undeveloped lakes.

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Executive Summary

Freshwater food webs are particularly susceptible to human development because they are highly connected to adjacent terrestrial habitats, which provide inputs of physical habitat structure, nutrients, and organic matter that increase freshwater food web production (Vannote et al. 1980, Nakano and Murakami 2001; Baxter et al. 2005; Dudgeon et al. 2006). For example, coarse woody debris that enters lakes from adjacent riparian habitat serves as substrate for colonization by aquatic macroinvertebrates and provides fish with refuge from predation, thus increasing production in the shallow littoral zone (Everett and Ruiz 1993; Schindler and Scheurell 2002, Roth et al. 2007). Thus, freshwater food webs are impacted both by within-system modifications, such as flow regulation in rivers (Ward and Stanford 1995), and degradation of the surrounding terrestrial environment that reduce inputs of terrestrial organic matter (Brauns et al. 2011).

Lakes are well suited for examining the impacts of human development to freshwater food webs. Lakes in the Pacific Northwest have undergone extensive shoreline development characterized by removals of riparian and aquatic vegetation and coarse woody debris, which together reduce the retention of organic matter and densities of macrophytes that provide food and habitat for littoral-zone invertebrates and fish (Francis et al. 2007; Larson et al. 2011). Lake watershed development for human land-use has resulted in runoff and eutrophication that erode the ecological integrity of invertebrate communities in lake littoral zones (Brauns et al. 2007; Donohue et al. 2009; McGoff et al. 2013a). Littoral invertebrates contribute to essential ecosystem processes by recycling nutrients and converting organic matter into energy for other organisms in littoral, pelagic, and riparian food webs (Covich et al. 1999; Schindler and Scheuerell 2002; Vadeboncoeur et al. 2002). Therefore, by reducing or altering invertebrate

abundances and diversity, human development can negatively impact lake-ecosystem functioning and shift consumer diets from reliance on terrestrial and littoral to pelagic resources (Schindler and Scheuerell 2002; Francis et al. 2007; Brauns et al. 2011).

Introduced species may provide habitat or food resources for consumers in degraded ecosystems, thus supporting food web structure and functions (Schlaepfer et al. 2011). For example, Martin and Valentine (2011) demonstrated that rainwater Killifish of the Mobile-Tensaw Delta, AL, USA, which are strong interactors in macrophyte-associated food webs, use introduced Eurasian milfoil as habitat, and invertebrate and fish communities were similar between stands of Eurasian milfoil and native water stargrass. Similarly, Johnston and Lipcius (2012) found that a non-native macroalga provides predation refuge for juvenile native blue crabs in the Chesapeake Bay, where much of the historic nursery habitat has been destroyed. In addition to replacing native habitat that has been removed, non-native species may substitute for extirpated native prey to support higher trophic level consumers. An observational study of dietary items consumed by native Cooper's Hawks in urban Victoria, BC, Canada, identified that more than half of hawk prey items were non-native species (Cava et al. 2012). This recent evidence suggests that non-native species can contribute to ecosystem functions, and that potential benefits of non-native species may be anticipated in environments that have undergone habitat loss or extirpations of native species. Given that many ecologists anticipate future declines in environmental quality, accompanied by species introductions (Sala et al. 2000), there is a need for more research that examines the bidirectional ecological roles of non-native species in degraded ecosystems.

Chinese mystery snail (*Bellamya chinensis*; hereafter CMS) was introduced into Washington by aquarium hobbyists over 40 years ago, is now distributed in hundreds of lakes, and can achieve very high densities in lake littoral zones (Olden et al. 2009). CMS is native to Asia and is the second largest freshwater snail in North America. Because CMS are large and abundant, they may be an important prey resource in Washington lake ecosystems where native invertebrate communities are in decline due to urban development (Francis et al. 2007). Alternatively, their thick shell and operculum may make them inaccessible to consumers, and they would thus represent a trophic cul-de-sac (*sensu* Bishop et al. 2007) or trophic dead-end, by channeling energy and organic matter away from higher trophic levels.

My thesis research addresses the impacts of urban development to lake littoral macroinverrtebrate communities. Further, I examine the role of non-native CMS in lake food webs across gradients of urban development and native prey availability. I address three objectives.

(1) Identify community-level differences in benthic invertebrates and fishes in lakes with and without CMS that span an urbanization gradient.
(2) Determine if mobile predators consume CMS in developed and undeveloped lakes.
(3) Examine how CMS and urban development influence the relative contributions of food resources from lake littoral and pelagic habitats to consumers.

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Ch. I. A global meta-analysis of the ecological impacts of non-native crayfish

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Abstract

Non-native crayfish have been widely introduced and are a major threat to freshwater biodiversity and ecosystem functioning. Despite documentation of the ecological impacts of non-native crayfish from over three decades of case studies, no comprehensive synthesis has quantitatively tested for their general and/or species-specific effects on recipient ecosystems. Here, we provide the first global meta-analysis of the ecological impacts of non-native crayfish under experimental settings to compare effects among species and across levels of ecological organization. Our meta-analysis revealed strong, but variable, negative ecological effects of non-native crayfish with strikingly consistent effects among introduced species. We found that in experimental settings non-native crayfish generally affect all levels of freshwater food webs, ranging from reducing the abundance of basal resources like aquatic macrophytes, to preying on invertebrates like snails and mayflies, to reducing abundances and growth of amphibians and fish. Contrary to our predictions, we did not find that non-native crayfish consistently increase algal biomass. Furthermore, when compared directly to native crayfish, non-native crayfish showed a slight tendency to exhibit larger positive effects on growth of algae and larger negative effects on invertebrates and fish, although there was considerable variability among effect sizes. In conclusion, our study supports the assessment of crayfish as strong interactors in food webs that, by way of their polytrophic, generalist feeding habits, have significant impacts across native taxa. We suggest that species identity may be less important than extrinsic attributes of the recipient ecosystems in determining impacts of non-native crayfish. We identify some under-studied and emerging non-native crayfish that would benefit from further studies and suggest expanding research to encompass more comparisons of native vs. non-native crayfish and different geographic regions, but we note that the consistent and general negative effects of non-native crayfish warrant efforts to discourage their introduction beyond native ranges.

Key words: *Orconectes rusticus, Orconectes virilis, Pacifastacus leniusculus, Procambarus clarkii*, taxonomic effect, manipulative experiment

Introduction

Humans have a penchant for introducing species to areas beyond their native geographic distributions, giving the potential for these non-native species to become invaders (Elton 1958). It is now recognized that the ecological consequences of non-native species can range from beneficial (Schlaepfer et al. 2011) to detrimental; in the latter case often leading to significant ecological damage ranging from the extinction of native species to alteration of ecosystem processes (e.g., Vitousek et al. 1996, Mack et al. 2000, Vilà et al. 2009). Ecologists have long been challenged to identify, quantify, and predict the ecological impacts of invasive species (Parker et al. 1999), and meta-analytical approaches have emerged as a powerful tool to shed insight into the general ecological effects of and traits possessed by invasive species. Recent applications for meta-analyses in invasion ecology have developed frameworks to organize invader impacts based on attributes of invasive species (Thomsen et al. 2011). Meta-analyses have also addressed such fundamental topics in invasion ecology as exploring the influence of taxonomic identity on invasion impact (Ricciardi et al. 2004), understanding the environmental determinants of establishment success (Cassey et al. 2005), and assessing the differential vulnerability of native vs. non-native species to climate change (Sorte et al. 2012). Limited only by the taxonomy and geography of available data on non-native species (Pyšek et al. 2008), meta-analyses will continue to be an important tool in invasion biology.

Through a variety of pathways, including bait bucket releases, intentional introduction to support fisheries, and release after use for education, crayfish have become one of the most widely introduced freshwater taxa (Hobbs et al. 1989, Gherardi 2010). The ecological impacts of non-native crayfish are well-documented from over three decades of case-studies (Hobbs et al. 1989, Snyder and Evans 2006, Lodge et al. 2012), but have yet to be quantitatively synthesized to test for generalized effects on recipient ecosystems. Population declines, extirpations, and extinctions of native crayfishes are among the most alarming effects of widespread crayfish introductions (Lodge et al. 2000, Perry et al. 2001). However, the ecological impacts are much more far-reaching. Crayfish are generalist omnivores, and thus, have large effects on both primary and secondary producers (Lodge et al. 1994, Perry et al. 2000). Crayfish are ecosystem engineers that increase rates of leaf litter breakdown and nutrient cycling in streams (Charlebois and Lamberti 1996, Bobeldyk and Lamberti 2008), and their grazing and burrowing can reduce benthic algae and macrophyte cover, producing a state change in lakes and wetlands from clear to phytoplankton-dominated turbid-water systems (Feminella and Resh 1989, Matsuzaki et al. 2009). Coupled with habitat modification, crayfish predation drives declines in diversity and abundances of native invertebrates (McCarthy et al. 2006, Correia and Anastacio 2008), and reduces amphibian populations through predation on eggs and larvae (Gamradt and Kats 1996, Gamradt et al. 1997). Finally, crayfish invasions have resulted in fish declines through predation, shelter competition, and indirect competition for prey (reviewed in Reynolds 2011). Because of their potential impacts across all levels of ecological organization, non-native crayfish are a major threat to freshwater biodiversity and ecosystem functioning.

Difficulties in evaluating and predicting impacts of invasive species are particularly relevant to crayfish, because past research suggests that the ecological effects of crayfish may differ according to both the crayfish species and resident species in the environment where it is introduced (Larson and Olden 2010, Lodge et al. 2012). Case studies of red swamp crayfish *Procambarus clarkii* invasions have documented dramatic declines in macrophytes (Matsuzaki et al. 2009), whereas studies of rusty crayfish *Orconectes rusticus* invasions often reveal the largest impacts on benthic invertebrate communities (McCarthy et al. 2006). These examples suggest that the species of crayfish, by way of preferential feeding habits, may influence the type and magnitude of their ecological effects on resident native species. Factors extrinsic to the crayfish species, including competitors and prey in the receiving environment, may also play an important role in determining the impacts of crayfish invasions.

Caution should always be practiced when making generalizations about effects of invasive species, and previous reviews on the ecological impacts of non-native crayfish have been largely narrative and lacked quantitative synthesis across species. Case studies examining the ecological effects of specific crayfish species on recipient ecosystems are beneficial, but individually they provide little insight into generalized patterns of crayfish impacts. However, taken together, the rich body of existing literature lends itself to a systematic overview of the ecological effects of non-native crayfish. A literature synthesis that compares impacts across non-native crayfish species (and where possible in relation to native crayfish effects) will help identify generalities in effects that cannot be addressed by individual studies alone.

Here, we provide the first systematic review and global meta-analysis of the ecological impacts of non-native crayfish. Our aim is to test current evidence for the ecological effects of non-native crayfish across different levels of ecological organization by addressing the following questions: What is the empirical evidence for the effects of non-native crayfish? Do non-native crayfish differ from native crayfish in their ecological effects? Does species identity of non-native crayfish determine the magnitude and direction of their ecological effects? We expect that non-native crayfish will have larger effects compared to native crayfish at a given location. We also predict that all non-native crayfish species will have direct and indirect negative effects on most levels of ecological organization including macrophytes, benthic invertebrates such as insects and snails, crayfish, amphibians and fish; and positive effects on algal biomass. Finally, we predict that non-native crayfish will be more successful than native crayfish in agonistic interactions for territory and shelter. By comparing and contrasting the ecological effects of different non-native and native crayfish species across levels of ecological organization, we aim to inform future risk assessments and control strategies for non-native crayfish.

Methods

Systematic search

Our protocols for search and selection followed those outlined by Pullin and Stewart (2006) for systematic review, which included formation of search protocol and data inclusion, data extraction, and analysis. By searching the online database ISI Web of Science we identified peer-reviewed papers published through the end of 2012 that used experimental manipulations to quantify the effects of non-native crayfish on recipient ecosystems. We also included studies referenced within articles obtained from this search. Only manipulative experimental studies were included to avoid unstandardized treatment levels (crayfish densities) and the confounding effects of other predators on response organisms. Our search terms included key word combinations of 'experiment* or manipulation*' and 'non-native* or nonnative * or invasive* or alien* or exotic* or non-indigenous* or nonindigenous* or introduced*' and 'crayfish' or 'crawfish' or '*Procambarus clarkii** or *Pacifastacus leniusculus** or *Orconectes rusticus** or *Orconectes virilis** or *Orconectes propinquus**' in the article. The latter species list has the longest and most widespread history of invasion studies (Hobbs et al. 1989, Lodge et al. 2012), and additional searching revealed only limited sample sizes for other non-native crayfish (e.g., *Orconectes neglectus*; Rabalais and Magoulick 2006). Although we included *O. propinquus* in our search terms, we did not include this species in our meta-analysis because of the continued uncertainty of whether *O. propinquus* is non-native to the regions where it has been studied in Wisconsin (Hobbs and Jass 1988). Search results were further filtered to include journals that publish articles in English within the categories of ecology, marine or freshwater biology, fisheries, limnology, zoology, behavioral sciences, environmental sciences, or biodiversity conservation.

Study selection

We read the abstract for each article returned from the Web of Science search and further filtered results to include studies that fell into one of three categories: those that tested responses in biomass or abundance of native taxa to 1) native and non-native crayfish, 2) non-native crayfish only, or 3) experiments that paired a native and non-native crayfish in agonistic or competitive interactions. To compare the ecological effects of native and non-native crayfish we included only those studies that simultaneously examined the response of groups exposed to a native crayfish versus non-native crayfish versus a control with no crayfish.

We determined effects of non-native crayfish across levels of ecological organization by selecting studies that examined the effects of exposure to non-native crayfish. Studies were included in these analyses if they utilized groups of organisms exposed to non-native crayfish to groups without crayfish. All of the articles included in these analyses manipulated species in the laboratory, outdoor ponds, or in situ using crayfish enclosures or exclusions.

For examination of crayfish-crayfish agonism, we assessed whether non-native crayfish ‘win’ more agonistic interactions for shelter or territorial dominance than native crayfish. All of the studies included in this analysis utilized laboratory tanks to observe agonistic behaviors. Studies defined the ‘winner’ in shelter dominance as the crayfish occupying the shelter, and studies reported the number of wins for each species. The ‘winner’ in territorial dominance interactions was determined from a set of behaviors, including approaches, threats, and strikes, and each study reported the number of each behavior displayed by each crayfish species or reported the number of wins as determined by the experimenters. Because size is often a determinant of the success of an aggressor, we used replicates with large non-native crayfish and small native crayfish as our treatment group and replicates with large native crayfish and small non-native crayfish as our controls when calculating the effect size for each study.

Data extraction

Articles were categorized according to the focal non-native crayfish species examined and response organisms were grouped taxonomically as benthic algae, macrophytes, benthic invertebrates (further divided by family or order), crayfish, fish, or amphibians (Table 1). Herein, we refer to these as ‘ecological responses’. Furthermore, we coded each study for experimental venue, i.e., whether the experimenters utilized laboratory tanks, outdoor mesocosms, or cages in situ, which permitted analyses of how experimental design potentially influenced the responses.

We extracted statistics for control and treatment groups, including sample sizes, means, proportions, and standard deviations or standard errors, from tables and results in the articles. For articles that did not report those statistics, we requested data from authors and, when necessary, extracted data from figures using the data-extraction software, Data Thief (*B. Tummers, DataThief III. 2006* http://datathief.org/*)*. Some articles reported data in a way that did not permit extraction of sample size, standard deviation, or standard error measurements; these studies were excluded from our analysis. Of the 96 articles that fit our search criteria, we were able to obtain the required data from 52 studies representing 61 experiments (Appendix A) from 8 countries for 6 non-native crayfish species (Fig. 1).

Analysis

To examine whether non-native crayfish have larger ecological effects than native crayfish, we determined directional effect sizes for ecological responses to non-native crayfish relative to native crayfish. We tested for effects of crayfish on biomass and abundances of macrophytes, invertebrates, and fish, and effects on algal biomass.

We tested whether mean effect sizes of ecological responses differed among non-native crayfish species (Table 1). Responses examined for benthic algae were direct consumptive, and indirect, via trophic cascades, changes in biomass or concentrations of chlorophyll-a. For floating, submerged, and emergent macrophytes, we tested the effects of consumption by non-native crayfish on abundances and biomass of seeds, seedlings, and plants. We also tested the direct effects of predation by non-native crayfish on abundances and biomass of benthic invertebrates. We examined changes in rates of native crayfish survival as both direct (predation) and indirect (behavioral) effects of exposure to non-native crayfish, or number of victories in agonistic interactions. Furthermore, we examined predatory effects of non-native crayfish on abundances of fish and amphibians and changes in growth mediated by behavioral responses to predation risk. Finally, adequate sample sizes allowed us to test for differences in mean effect sizes among experimental venues for macrophytes and invertebrates, but no other ecological response.

All meta-analytic and statistical calculations were implemented using the software MetaWin, version 2.0 (Rosenberg et al. 1999). We used Hedges' d (Hedges 1981) as the effect size metric for ecological responses. Hedges' d is a commonly used measure of effect size in ecological studies (Møller and Jennions 2002) and is appropriate for use in traditional meta-analyses because it has a low Type I error rate (Lajeunesse and Forbes 2003). Hedges' d computes the effect size as the standardized mean difference between treatment and control groups, and includes a weighting factor to correct for small sample sizes (Rosenberg et al. 1999). We calculated Hedges' d as,

$$\mathrm{d} = \frac{(\bar{X}_T - \bar{X}_C)}{S} J\ , \tag{1}$$

where $\bar{X}_T$ is the mean of the treatment group, $\bar{X}_C$ is the mean for the control group, and S is the pooled standard deviation. We calculated S as:

$$S = \sqrt{\frac{(N^T\text{-}1)(S^T)^2 + (N^C\text{-}1)(S^C)^2}{N^T + N^C\text{-}2}}\ . \tag{2}$$

Here, S^T and S^C are the standard deviations for the treatment and control groups, and J is the weighting factor based on the sample sizes for the treatment (N^T) and control (N^C) groups, respectively. J is calculated as,

$$J = 1 - \frac{3}{4(N^c + N^T - 2) - 1}. \tag{3}$$

We computed the variance of Hedges' d as:

$$V_d = \frac{N^c + N^T}{N^C N^T} + \frac{d^2}{2(N^c + N^T)}. \tag{4}$$

Negative values for Hedges' d indicate a negative effect of non-native crayfish on the measured response variable (biomass or abundance) compared to controls of native crayfish or no crayfish.

We calculated the log odds ratio for the effect size estimate of agonistic interactions between non-native and native crayfish. The log odds ratio computes the mean effect size for studies where a 2x2 contingency table with categorical responses is appropriate. We compared the probability of the native species winning an agonistic interaction in control groups, in which the native species is larger than the non-native, to treatment groups with non-native crayfish larger than native. Following Rosenberg et al. (1999) we computed the log odds ratio (OR) as:

$$\ln OR = \frac{\sum O_i - \hat{O}_i}{\sum v_i}. \qquad (5)$$

Here O_i equals the observed responses from the treatment group (# times the native wins), $\hat{O}_i$ is the expected number of responses assuming no treatment effect, and v_i is the variance. A negative effect size for the log odds ratio indicates that the native species wins agonistic interactions fewer times than expected by chance alone.

All control and treatment groups used in our analyses were exposed to experimenter-defined crayfish densities; therefore, we excluded in situ experiments that considered natural crayfish densities (e.g. un-caged stream plots). When calculating effect sizes for ecological responses to native versus non-native crayfish, we used groups exposed to non-native crayfish as treatments and groups exposed to native crayfish as controls in our effect size calculations. In this way, we compared directly the effects of non-native crayfish to effects of native crayfish. In our analyses of non-crayfish ecological responses to non-native crayfish, groups exposed to non-native crayfish served as treatments and were compared to groups without crayfish (controls). When different crayfish densities were used in a study we selected only the highest density treatment group for our analysis. Furthermore, in many experiments the effects of crayfish on treatment groups were not manifested immediately after the experiment began. In order to capture the delayed response to crayfish treatments, we used only data from the last sampling date when multiple post-treatment samples were collected.

Some studies reported more than one response measure for the final sampling date, e.g., multiple biomass and/or abundance measures, on the same individuals or taxonomic groups within an experiment. We calculated separate effect sizes and variances for each response measure in each crayfish-response pairing. To avoid pseudo-replication in our analysis we pooled effect sizes and variances from repeated response measures to obtain a mean effect size for every non-native crayfish-response pairing in each experiment and used these effect sizes in our final analysis (see Van Kleuenen et al. 2010 for further description and an example of this method). To retain as much information as possible from each study we did not pool effect sizes across different experiments from the same publication (Gurevitch et al. 1992).

For all analyses, we used random-effects and mixed-effects models (random-effects models with a grouping variable). Random-effects models assume that there are random sources of variation in effect sizes between studies and that sampling error accounts for heterogeneity within studies. Mixed-effects models incorporate two sources of variation that are important to ecological studies, the study-specific sampling error and between-study differences in true effect-sizes (Gurevitch and Hedges 1999). Grouping variables in mixed-effects models allow for tests of significance on the cumulative effect size (e.g., across crayfish species) and between effect sizes (e.g., between crayfish species) for groups of studies (Rosenberg et al. 1999). For comparisons of effects among non-native crayfish we used crayfish species as the grouping variable, and in tests for effects of experimental venue we used venue (laboratory, outdoor mesocosm, or cage) as the grouping variable.

We calculated bias-corrected 95% bootstrap-confidence intervals (9,999 permutations) to test whether mean effect sizes were significantly different from zero (Adams et al. 1997). A confidence interval that does not overlap with zero indicates a statistically significant effect size (Gurevitch et al. 1992). Pearson's chi-squared tests were employed to examine whether variance among effect sizes for each experiment (Q_{Total}) was significantly larger than would be expected from sampling error alone. For mixed-effects models, we also used a chi-squared test to evaluate differences in variance between study groups (Q_b) and within groups (Q_w). A significant Q_{Total} indicates that effect sizes are not equal across studies; whereas a significant Q_b or Q_w indicates that effect sizes vary significantly between study groups (e.g., between non-native crayfish species) or within study groups, respectively.

For the overall ecological response to non-native crayfish and the effects of experimental venue, we present data from mixed-effects models. Otherwise, we present data from random-effects models for two reasons: (1) low sample sizes did not permit inclusion of all crayfish species and experiment types into mixed-effects models, resulting in reduced statistical power for some analyses; (2) results from mixed-effects models indicated that total and between-species heterogeneity were not statistically significant for any response (Appendix B, C), and therefore, random-effects models are appropriate for our analyses (Rosenberg et al. 1999). To incorporate more experiments into our estimates of the cumulative effects on each ecological response, we ran a separate random-effects model for each species of crayfish and experimental venue with a sample size of $n \geq 2$. We also obtained an estimate of the cumulative effect size variance, and confidence interval for crayfish on each ecological response.

Meta-analyses may be influenced by the publication bias associated with journals tending to publish studies showing significant results, also termed “the file drawer problem” (Rosenthal 1979). To address potential publication bias, we calculated a fail-safe number for each mixed-effects and random-effects model (Appendix B, C). The fail-safe number reflects the number of additional non-significant studies that, if included in the analysis, could change the model output from significant to non-significant (Rosenberg 2005). Thus, the larger the fail-safe number, the more confidence we have in the validity of a significant result.

Results

In comparison to native crayfish, our meta-analysis indicates that non-native crayfish exhibit larger positive effects on algae, slightly smaller negative effects on macrophytes, and somewhat larger negative effects on aquatic insects, snails, and fish (Fig. 2). However, there was considerable variability in these patterns, resulting in large differences for only four studies that examined responses in algal biomass and snails. Given the limited number of studies, a direct comparison of the ecological effects of native and non-native crayfish remains a challenge; consequently, differences observed here may not exist or may vary as a function of species identity and type of ecological response.

Our meta-analyses supported predicted effects of non-native crayfish on the suite of ecological responses considered, with the exception of impacts on algae (Table 1). The negative impacts of non-native crayfish across the food web are substantial (Fig. 3). With the exception of *O. virilis*, the mean effect sizes for all species were statistically significant and greatly exceeded the absolute value of 0.8, which is considered a "strong" effect according to Gurevitch et al. (1992). Mean effect sizes among crayfish species were not significantly different (Q_b = 5.25, P = 0.15), with *P. clarkii* and *P. leniusculus* demonstrating the largest overall effects across ecological responses. However, there was significant heterogeneity in effect sizes across studies within each group (i.e., crayfish species; Q_w = 81.4, P = 0.02), indicating that differences in effect sizes for each species are, in part, explained by other independent variables not considered in this analysis. According to Rosenthal's fail-safe number an additional 1,127 studies with non-significant results would be required to reverse our findings (i.e., support the null hypothesis that crayfish have no ecological impacts).

Effects on primary producers

Non-native crayfish showed little effect on algae under experimental conditions (Fig. 3A). Effect sizes for *P. leniusculus* and *O. rusticus* were only slightly positive, but not statistically significant (Fig. 3A). Total heterogeneity was significantly larger than the expected sampling error (Q_{Total} = 22.6, P < 0.01; Appendix C), suggesting that factors such as experimental design, geographical, or ecological factors other than crayfish identity may explain the heterogeneity among effect sizes. By contrast, the effects of non-native crayfish on macrophytes were consistently negative across all species individually and combined (Fig. 3B), and the total heterogeneity in effect sizes for the random effects model was not significant (Q_{Total} = 26.0, P = 0.17), indicating broad similarities across species in their effects on macrophytes. Rosenthal's fail-safe number indicated that adding 278 additional studies with non-significant results to the analysis could reverse our finding that crayfish have significant, negative effects on macrophytes.

Effects on invertebrates

Non-native crayfish were responsible for large changes in the biomass and abundance of all benthic invertebrates (Fig. 4A; Q_{total} = 27.8, P = 0.15) and snails specifically (Fig. 4B; Q_{total} = 13.6, P = 0.32), and total heterogeneity across crayfish species was not significant. Amphipoda, Chironomidae, and Ephemeroptera responded negatively in non-native crayfish treatments, although the effects were significant only for Ephemeroptera (Fig. 4C; Appendix C). By contrast, the response of Trichoptera was slightly positive, although this effect also was highly variable and not significant (Fig. 4C). Rosenthal's fail-safe number lends substantial support to our results; 284 and 110 additional studies with non-significant results are required to reverse our findings of significant negative impacts on all benthic invertebrates and snails, respectively.

Effects on crayfish

Non-native crayfish were more aggressive (i.e., more victorious) in agonistic interactions compared to native crayfish, although results were highly variable (Fig. 5A). Overall, non-native crayfish did not significantly influence survival of native crayfish (Fig. 5B). Total heterogeneity was not significantly different from the expected sampling error for random effects analyses of agonistic interactions ($Q_{Total} = 2.78$, $P = 0.60$) or survival ($Q_{Total} = 3.96$, $P = 0.41$).

Effects on vertebrates

Non-native crayfish had dramatic ecological effects on the growth and abundances of fish (Fig. 6A) and amphibians (Fig. 6B) in experimental settings. Experiments using *P. clarkii* were responsible for most of the overall crayfish effect on amphibians (Fig. 6C). Total heterogeneity was not significant for total effects on fish ($Q_{Total} = 6.48$, $P = 0.37$) or amphibians ($Q_{Total} = 8.09$, $P = 0.42$), indicating that sampling error explains most of the heterogeneity among effect sizes. Rosenthal's fail-safe number indicated that 42 additional studies with non-significant effects are required to change our results for the effects of crayfish on fish and amphibians.

Effects of experimental venue

The magnitude of ecological effects was similar regardless of experiment venue. We found no significant differences among experiments testing for effects on invertebrates (Table 2;

Q_{Total} = 28.2, P = 0.13; Q_b = 4.14, P = 0.13; Q_w = 24.1, P = 0.19) and macrophytes (Table 2; Q_{Total} = 25.1, P = 0.16; Q_b = 2.33, P = 0.31; Q_w = 22.7, P = 0.16).

Discussion

Crayfish often play a pivotal role in food webs by feeding across trophic levels. Crayfish have also been regarded as ecosystem engineers because of their burrowing activities that increase sediment transport in lotic systems, their rapid detrital processing, and their ability to shift ecosystems from macrophyte to algal dominated (Creed and Reed 2004, Matsuzaki et al. 2009, Statzner 2012). Our meta-analysis supports to varying degrees the assessment of crayfish as ecosystem engineers and strong interactors that affect all levels of food webs, contributing to the ecological impacts of crayfish as invasive species.

Ecological effects of native and non-native crayfish

Our comparison of the effects of native and non-native crayfish found that non-native crayfish often had larger, but variable, effects on ecological responses compared to native crayfish. Non-native crayfish had more positive effects than native crayfish on algae and weakly negative effects on other organisms. There were too few studies that tested for impacts of both native and non-native crayfish to calculate an overall effect size, precluding a determination of whether non-native crayfish are inherently stronger interactors in ecosystems than native crayfish. However, a recent meta-analysis that compared global impacts of native and non-native consumers found that non-native species have significantly larger ecological impacts than native consumers, and non-native invertebrate consumers have particularly strong impacts (Paolucci et al. 2013). Given these results and our analysis, we suggest that there is a high potential for non-native crayfish to exhibit larger impacts than native crayfish in some ecosystems.

Experiments using *O. rusticus* comprised the majority of the comparative literature and three of the four large effect sizes in this analysis. Thus, it is difficult to say whether the slight trends toward larger effects of non-native species are due to intrinsic behavioral traits or other ecological functions of *O. rusticus* specifically as opposed to general traits of non-native crayfish. Invader body and/or chelae size, susceptibility to predation, and behavioral traits (e.g., burrowing activity) may all contribute to differences in ecological effects of non-native and native crayfish species (reviewed in Gherardi [2006] for *P. clarkii*). Furthermore, non-native crayfish may occupy a wider range of habitats than native crayfish and thus impact a greater variety of food resources (Olsson et al. 2009). We suggest that future research explore differences in ecological traits between co-occurring native and non-native crayfish that explain the potential absence of or heightened ecological effects of non-native crayfish.

Our study highlights that direct comparisons of non-native to native crayfish are scarce and support for greater ecological effects of non-native crayfish is limited or equivocal. It is likely that the greatest effects of non-native crayfish will manifest in ecosystems that historically lacked crayfish or functionally similar organisms (e.g., freshwater shrimp, crabs) altogether. More studies of greater taxonomic breadth and geographic scope are needed to investigate the potentially more subtle ecological effects that may manifest when non-native crayfish are introduced into communities with native crayfish.

Ecological effects across trophic levels

Our meta-analytical review of the non-native crayfish literature revealed large negative, general ecological effects of non-native crayfish on recipient ecosystems with consistent impacts among crayfish species. Crayfish had significant negative effects on biomass and abundances of macrophytes, invertebrates (all benthic invertebrates and snails specifically), fish, and amphibians within experimental settings. These findings are consistent with previous meta-analyses that showed negative impacts of crayfish on invertebrates and macrophytes with select crayfish species and regions (McCarthy et al. 2006, Matsuzaki et al. 2009). Taken together, our results provide quantitative evidence that non-native crayfish invasions are associated with substantial, but variable, effects across multiple levels of freshwater food webs.

Relationships between crayfish and primary producers can be complex; non-native crayfish have often been reported to directly reduce abundances of macrophytes (Nyström et al. 1999, Matsuzaki et al. 2009) and have indirect positive effects on algal biomass via consumption of grazers and competing macrophytes (e.g. Lodge et al. 1994, Nyström et al. 1999). Across a number of experimental studies we found little evidence for directional effects of non-native crayfish on algal biomass. Only *O. rusticus* and *P. leniusculus* showed positive (albeit weak) effects on algae. This may be an expected result given that short-term experiments, which are common in the literature, are not likely to capture trophic cascades that manifest over longer time-scales. Alternatively, crayfish effects on algae may be confounded by taxa-specific responses; for example, Creed (1994) found grazing crayfish reduced abundance of the filamentous algae *Cladophera* by 10x, releasing diatoms and increasing their abundance 20x.

Non-native crayfish had strong, negative effects on macrophyte biomass and abundances. Coupled with the large negative effects of crayfish on snails, these results suggest that the activities of non-native crayfish may ultimately lead to trophic cascades and shifts from macrophyte to algal dominated systems (e.g., Lodge et al. 1994, Smart et al. 2002). Although many such studies have focused on the impacts of *P. clarkii* to macrophytes (reviewed in Matsuzaki et al. 2009), our analysis suggests that *O. rusticus* and *P. leniusculus* are similarly capable of eliminating macrophyte stands (e.g., Peters et al. 2008, Usio et al. 2009).

Our synthesis of experimental studies also revealed strongly negative, yet variable, effects of non-native crayfish on benthic invertebrates. Negative effects on snails were the largest across non-native crayfish, suggesting that these taxa may be particularly susceptible prey. These results are consistent with previous studies illustrating dramatic changes to benthic invertebrate communities (e.g., Nyström et al. 1999, McCarthy et al. 2006), especially thin-shelled species of snails, in response to crayfish over both short and long time scales (Kreps et al. 2012). Few studies in our analysis examined impacts to other invertebrate taxa including Amphipoda, Chironomidae, Ephemeroptera, and Trichoptera, pointing to a need for more research to assess crayfish impacts on common benthic invertebrates, which are important prey items in crayfish diets (Momot 1995).

Crayfish have been implicated in declines of fish and amphibians throughout the world, and these effects are mediated by predation and indirect competition for habitat and prey (Ilhéu et al. 2007, Reynolds 2011). According to our review, crayfish had significant negative effects on benthic invertebrates, which when combined with evidence that crayfish modify habitat conditions by reducing macrophyte biomass and abundances, support the notion that crayfish decrease the abundance of shared prey resources and available habitat for fish and amphibians (Dorn and Mittelbach 1999, Ilhéu et al. 2007). When we examined effects of crayfish on juvenile and adult fish and amphibian abundances and growth, these effects were significantly negative, suggesting that crayfish have substantial effects on vertebrates during early and mature life stages. Our analyses on the effects to vertebrates were based on small sample sizes; however, we obtained large Rosenthal's fail-safe numbers, suggesting that our results are relatively unbiased, and non-native crayfish should be anticipated to have negative effects on aquatic vertebrates. With both amphibians and freshwater fish exhibiting among the greatest levels of global imperilment (Dudgeon et al. 2006), we call for further research linking data on non-native crayfish invasions and the management actions aimed at minimizing their ecological impacts.

Interactions among crayfish

Non-native crayfish are widely considered the largest threat to native crayfish in regions including Japan, North America (Taylor et al. 1997), and Europe (Lodge et al. 2000), yet there is little consensus over the relative importance of aggression in displacement of native crayfish (Hobbs et al. 1989, Snyder and Evans 2006). Our analysis did not indicate that non-native crayfish influenced survival of native crayfish; however, non-native crayfish won more agonistic interactions than native crayfish. Non-native crayfish are frequently cited as more aggressive than their native counterparts (Gherardi and Cioni 2004), and we suggest that enhanced competitive ability may be a behavioral trait common to non-native crayfish (Pintor and Sih 2009). Increased aggression during encounters is hypothesized to give non-native crayfish an edge over native species in predator avoidance and the pursuit of shelter and food resources, which may explain their success in displacing native crayfishes in the invasive range (Hill and Lodge 1994, Lodge et al. 2000, Olden et al. 2011). Finally, studies of aggression between native and non-native crayfish most often use naïve individuals with no experience with invaders (e.g., Larson and Magoulick 2009). Hayes et al. (2009) suggest that native crayfish with experience with non-natives fare better in aggressive encounters than their naïve counterparts. Due to the small number of studies in our analysis that examined aggression and survival of native crayfish in the presence of non-native crayfish, we believe that this area could benefit from further exploration as a means of mitigating non-native crayfish impacts on native congeners. Given our results, agonistic encounters with non-native crayfish may be an important component of risk to native crayfish and should be considered in any management plans for threatened or endangered species.

Comparisons of ecological effects among crayfish species

Invasion success and ecological impacts vary greatly across non-native crayfish (Larson and Olden 2010, Lodge et al. 2012), yet the lack of significant heterogeneity in effect sizes among crayfish species in our study suggests that species identity may be less important than extrinsic factors in determining impacts of crayfish. Crayfish are opportunistic feeders that will consume what is available in the environment. For example, *P. clarkii* consumes macroinvertebrates in proportion to their seasonal availability in rice fields of Portugal (Correia 2002) and supplements with vegetative material when macroinvertebrates are scarce (Correia 2003). Furthermore, stable isotope analyses, in conjunction with traditional feeding studies and diet analysis, have demonstrated that *P. leniusculus* and *O. rusticus* prey preferentially on macroinvertebrates but also consume large volumes of detritus and vegetative material as they are available (Bondar et al. 2005, Roth et al. 2006). Our results are consistent with these feeding studies in illustrating that non-native crayfish, by way of their polytrophic, generalist feeding habits, have similar impacts across native taxa, from macrophytes to vertebrates.

Effects of experimental venue on ecological impacts

Experimental design can affect conclusions drawn from manipulative experiments by influencing how species interact with one-another (Skelly 2002). Specifically, experimental enclosures may over-emphasize the importance of interactions because of small venue size (Gurevitch et al. 1992). Despite evidence suggesting that experimental venue can influence experimental outcomes, we did not find evidence for these effects on the strength of crayfish impacts. Across our analyses, venue type had little influence on ecological effects of non-native crayfish, suggesting that each experimental type utilized in our study (laboratory tanks, outdoor mesocosms, and cages in situ) is appropriate relative to the others for experimentally testing the effects of crayfish without biasing results.

Recommendations for future research

Non-native crayfish have been introduced to every continent except Antarctica (Lodge et al. 2012); yet the experimental studies considered here focused on non-native crayfish effects in only three regions: North America, Europe, and Japan (Fig. 1). Geographical and taxonomic bias are common problems in invasion ecology that limit our understanding of the impacts of invaders, because data is often missing from less-studied regions like Africa and Asia and from recently introduced species (Pyšek et al. 2008). This bias is prevalent in the crayfish literature and limits our understanding of both global and regionally specific impacts of non-native crayfish. Experimental work on non-native crayfish impacts is needed from heavily invaded regions like China (Liu et al. 2011), as well as from regions like Madagascar where invasions threaten rare and imperiled native crayfish species (Jones et al. 2009). Surprisingly, we found almost no experimental studies on non-native crayfish effects in Australia, a hotspot of global crayfish diversity and conservation need (Crandall and Buhay 2008). Australian crayfish species

Cherax quadricarinatus and *Cherax destructor* have been introduced within the continent where they threaten other Australian endemic crayfishes (Elvey et al. 1996), and are increasingly found globally in locations ranging from southeast Asia (Ahyong and Yeo 2007) to Europe (Scalici et al. 2009). Experimental studies are needed to gauge whether impacts of these emerging invaders resemble those of more widespread and commonly studied non-native crayfish originating from North America.

Most experiments used in our study tested effects of four major non-native crayfish (Fig. 1), all originating from North America and confined to the families Astacidae and Cambaridae, and some responses were dominated by effects of a single species. For example, our study revealed substantial ecological impacts on amphibians, but almost all available experiments were testing for effects of *P. clarkii* (e.g., Cruz and Rebelo 2005, Cruz et al. 2006, Gamradt and Kats 2006). Our meta-analysis demonstrates that competition and predation by non-native crayfish can have major consequences for freshwater vertebrates, and the impetus is on researchers to both assess and prevent effects of other non-native crayfish species on amphibian and fish populations. Non-native crayfish in need of study include the emerging Australian invaders (above), as well as the parthenogenetic “Marmorkrebs” (Jimenez and Faulkes 2011) and the many “extralimital” species that have been introduced in regions of high crayfish diversity like the southeastern United States (Larson and Olden 2010). The abundance of studies on non-native crayfish like *P. clarkii* (e.g., Portugal - 5 studies) and O. *rusticus* (e.g., USA - 14 studies) is the product of researcher- and region- specific programs using these widespread species as model organisms in invasion biology, an invaluable service in our understanding of invasive crayfish impacts that would benefit from expansion by additional researchers to other regions and over-looked or emerging non-native species.

Conclusions

Our quantitative meta-analysis supports past qualitative reviews in concluding that invasion by non-native crayfish can have major effects throughout freshwater communities and ecosystems (Lodge et al. 2000, Snyder and Evans 2006, Gherardi 2010, Lodge et al. 2012). We found that non-native crayfish affect all levels of freshwater food webs, ranging from reducing the abundance of basal resources like aquatic macrophytes, to preying directly on invertebrates, to negatively impacting amphibians and fish. We did not find that non-native crayfish consistently increase algae, a result supporting conclusions from Usio (2000) that omnivorous crayfish can “decouple” trophic cascades by consuming both freshwater invertebrates and their preferred basal resources. Yet we caution that crayfish invasions should be anticipated to occasionally cause complex and context-dependent indirect effects in freshwater ecosystems (e.g., stable state shifts; Matsuzaki et al. 2009), owing to their potentially strong interactions with all trophic levels.

Our study revealed a number of geographic and taxonomic biases in studies of non-native crayfish effects; however, results across these species were generally, albeit not universally, consistent. We identified a number of future directions for research on the ecological effects of non-native crayfish, but emphasize that ample evidence supports preventing and discouraging the introduction of these organisms beyond their native ranges.

Table 1. Evidence for effects of non-native crayfish on different taxonomic groups. "Evidence" column indicates whether our meta-analysis provided empirical support for positive or negative effects.

Taxonomic group*	**# Experiments**	***O. rusticus***	***O. virilis***	***P. clarkii***	***P. leniusculus***	**Evidence**
Algae	9	5	0	1	3	+/-
Macrophyte	20	8	0	9	3	-
Invertebrate	23	6	1	7	9	-
Amphipoda	5	1	1	1	2	+/-
Chironomidae	6	2	1	1	2	+/-
Ephemeroptera	3	2	0	1	0	-
Gastropoda	13	4	1	4	4	-
Trichoptera	5	3	0	1	1	+/-
Crayfish*	10	0	0	4	4	-
Agonism	5	0	0	1	3	-
Survival	5	0	0	3	1	+/-
Fish	7	0	2	2	3	-
Amphibian	9	0	0	8	1	-

*We have omitted columns for the crayfish *Orconectes hylas* and *Orconectes neglectus*, but these crayfish are included in sums for crayfish-crayfish studies.

Table 2 Results of mixed-effects model analysis comparing mean effect sizes of non-native crayfish on invertebrates and macrophytes for different experimental venues.

Taxonomic group	**Experimental venue**	**# Experiments**	**Effect size**	**95% Confidence interval**
Invertebrate		22	-1.49	-2.09, -1.04
	Cage	10	-1.33	-1.99, -0.89
	Lab	7	-2.23	-3.92, -1.39
	Outdoor	5	-0.771	-2.09, 0.163
Macrophyte	mesocosm	20	-2.02	-2.84, -1.29
		12	-1.59	-2.60, -0.748
	Cage	3	-2.58	-6.74, -0.819
	Lab	5	-2.96	-4.25, -1.94
	Outdoor			
	mesocosm			

Fig. 1. Geographic distribution of publication patterns across countries (shading) and states/ provinces of the United States and Canada, respectively (circle size). Pictured are the four most studied non-native crayfish, including (A) *Orconectes rusticus*; (B) *Orconectes virilis*; (C) *Pacifastacus leniusculus*; and (D) *Procambarus clarkii.* Photographs by E.R. Larson (A); J.D. Olden (B); C. Capinha (C); and F. Tomasinelli (D).

Fig. 2. Non-native crayfish demonstrate slightly greater, but highly variable, ecological effects compared to native crayfish. Symbols represent mean effect sizes based on Hedges' d estimates (scaled to precision of estimate) from a single study comparing effects of non-native and native crayfish, and whiskers are mean variance estimates. Non-native species used in each study are listed on the left, and native species are listed to the right. References: (1) Luttenton et al. 1998, Crustaceana; (2) Nyström and Strand 1996, Freshwater Biology; (3) Arce et al. 2006, Bull. Fr. Péche Piscic.; (4) Hazlett et al. 1992, Journal of Freshwater Ecology; (5) Olsen et al. 1991, Can. J. Fish. Aquat. Sci.; (6) Olden et al. 2009, Aquatic Ecology; (7) Usio et al. 2006, Arch. Hydrobiol.; (8) Ellrott et al. 2007, J. Great Lakes Research.

Fig. 3. Non-native crayfish demonstrate strong negative effects across all ecological responses of recipient ecosystems. Symbols represent mean effect sizes based on Hedges' d estimates from mixed-effects models (scaled according to the precision of the estimate), and whiskers represent 95% bias-corrected, boot-strap confidence intervals. Number of experiments is indicated in parentheses.

Fig. 4. (A) Non-native crayfish do not significantly influence algal biomass or chlorophyll-a concentrations, but (B) demonstrate strong negative effects on macrophyte biomass and abundances. Symbols represent mean effect sizes based on Hedges' d estimates obtained using random-effects models (scaled according to the precision of the estimate), and whiskers represent 95% bias-corrected, boot-strap confidence intervals. Number of experiments is indicated in parentheses.

Fig. 5. (A) Non-native crayfish significantly reduce overall abundances and biomass of all benthic invertebrates, (B) gastropods, (C) the invertebrate sub-group Ephemeroptera but not Amphipoda, Chironomidae, or Trichoptera. Symbols represent mean effect sizes based on Hedges' d estimates obtained using random-effects models (scaled according to the precision of the estimate), and whiskers represent 95% bias-corrected, boot-strap confidence intervals. Number of experiments is indicated in parentheses.

Fig. 6. (A) Non-native crayfish win significantly more agonistic interactions than native crayfish, but (B) do not significantly reduce survival of native crayfish in competitive interactions. Symbols in (A) represent mean effect sizes based on log odds' ratio estimates, and (B) mean effect sizes based on Hedges' d estimates obtained using random-effects models (both scaled according to the precision of the estimate). Whiskers represent 95% bias-corrected, boot-strap confidence intervals. Number of experiments is indicated in parentheses.

Fig. 7. (A) Non-native crayfish demonstrate strong negative effects on fish growth and abundances in recipient ecosystems. (B) Non-native crayfish cause strong reductions in amphibian growth and abundances. Symbols represent mean effect sizes based on Hedges' d estimates obtained using random-effects models (scaled according to the precision of the estimate), and whiskers represent 95% bias-corrected, boot-strap confidence intervals. Number of experiments is indicated in parentheses.

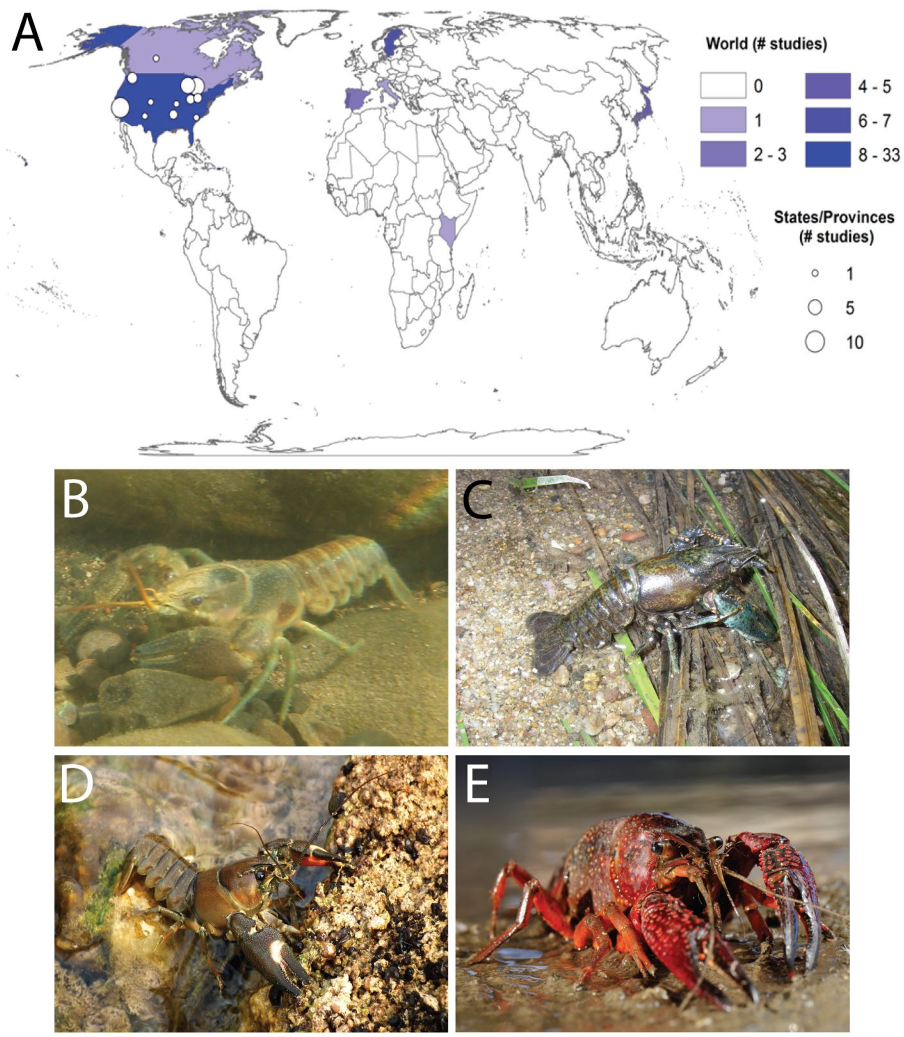
A
World (# studies)
0
1
2 - 3
4 - 5
6 - 7
8 - 33
States/Provinces
(# studies)
1
5
10
B
C
D
E

Fig. 1

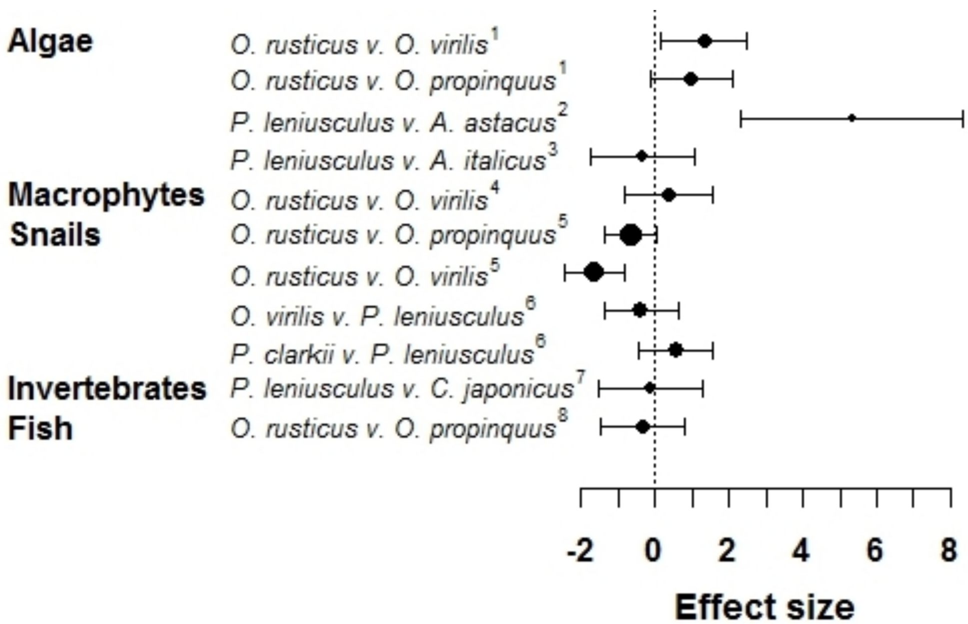
Algae
O. rusticus v. O. virilis1
O. rusticus v. O. propinquus1
P. leniusculus v. A. astacus2
P. leniusculus v. A. italicus3
Macrophytes
O. rusticus v. O. virilis4
Snails
O. rusticus v. O. propinquus5
O. rusticus v. O. virilis5
O. virilis v. P. leniusculus6
P. clarkii v. P. leniusculus6
Invertebrates
P. leniusculus v. C. japonicus7
Fish
O. rusticus v. O. propinquus8
-2 0 2 4 6 8
Effect size

Fig. 2

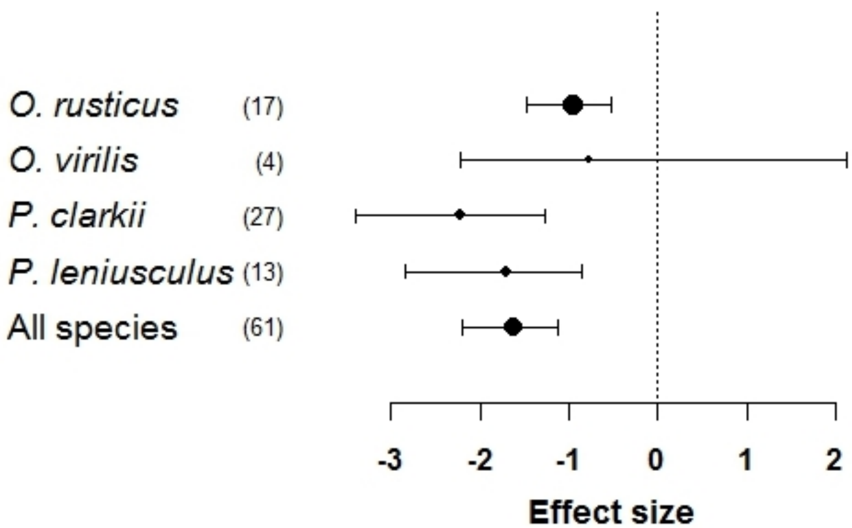
O. rusticus (17)
O. virilis (4)
P. clarkii (27)
P. leniusculus (13)
All species (61)
-3 -2 -1 0 1 2
Effect size

Fig. 3

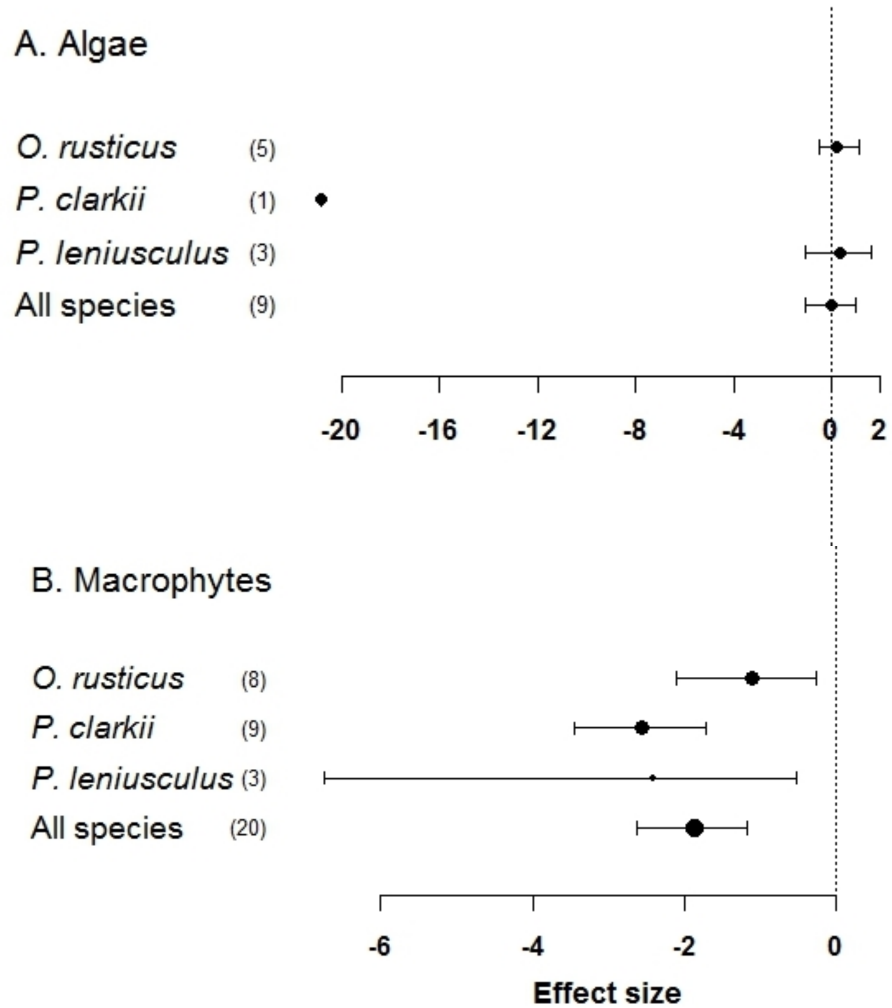
A. Algae
O. rusticus (5)
P. clarkii (1)
P. leniusculus (3)
All species (9)
-20
-16
-12
-8
-4
0
2
B. Macrophytes
O. rusticus (8)
P. clarkii (9)
P. leniusculus (3)
All species (20)
-6
-4
-2
0
Effect size

Fig. 4

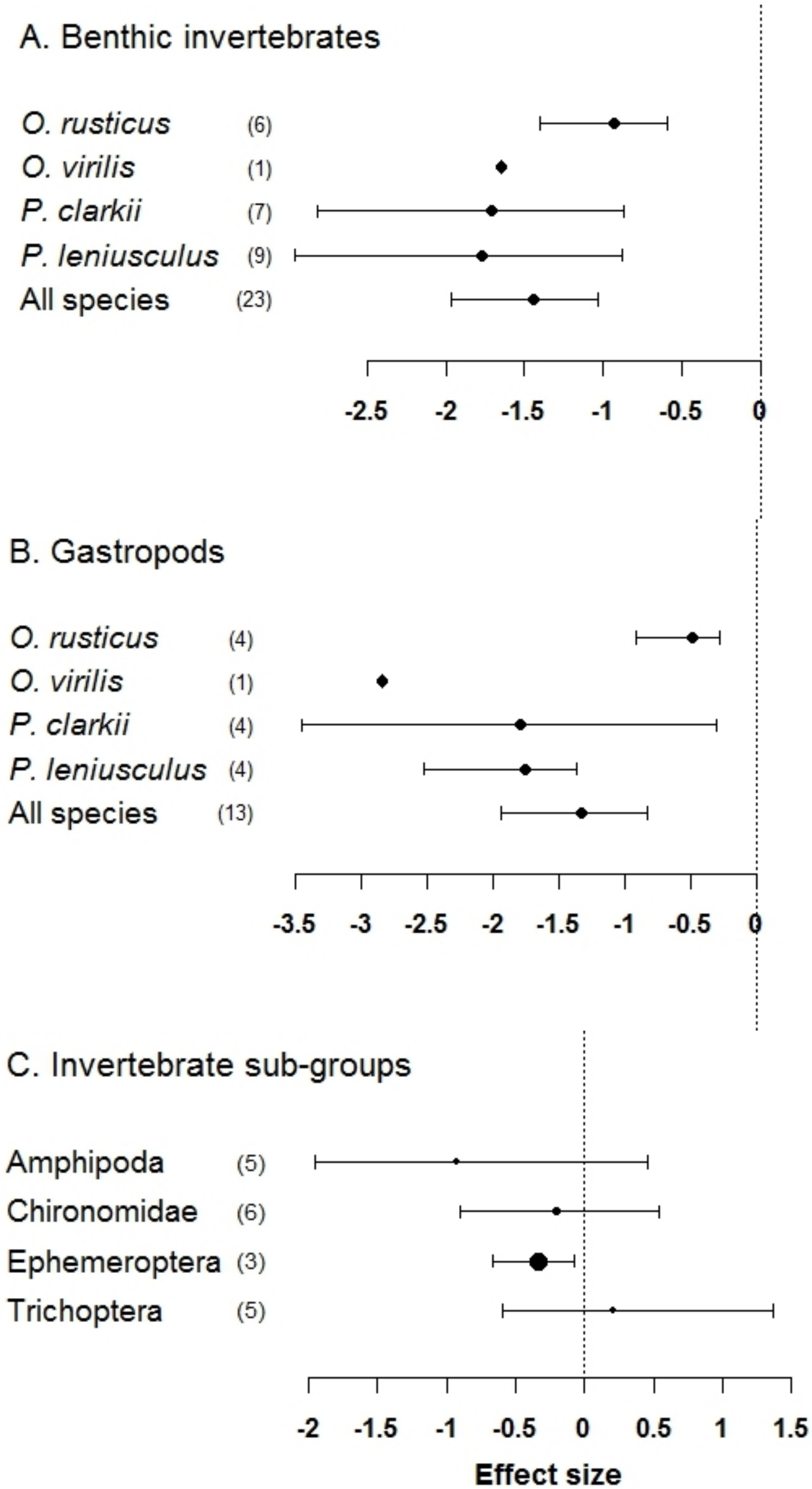
A. Benthic invertebrates
O. rusticus (6)
O. virilis (1)
P. clarkii (7)
P. leniusculus (9)
All species (23)
-2.5 -2 -1.5 -1 -0.5 0
B. Gastropods
O. rusticus (4)
O. virilis (1)
P. clarkii (4)
P. leniusculus (4)
All species (13)
-3.5 -3 -2.5 -2 -1.5 -1 -0.5 0
C. Invertebrate sub-groups
Amphipoda (5)
Chironomidae (6)
Ephemeroptera (3)
Trichoptera (5)
-2 -1.5 -1 -0.5 0 0.5 1 1.5
Effect size

Fig. 5

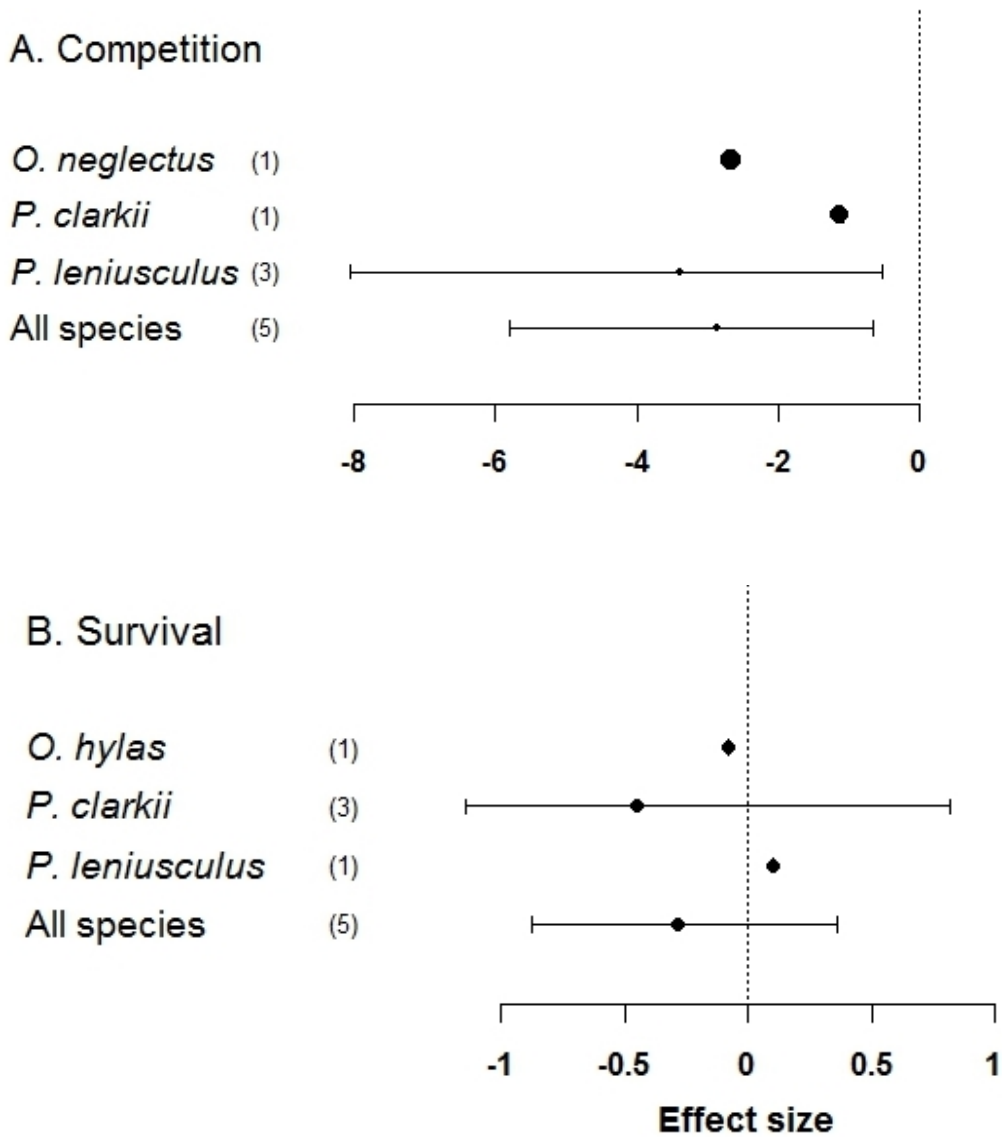
A. Competition
O. neglectus (1)
P. clarkii (1)
P. leniusculus (3)
All species (5)
-8
-6
-4
-2
0
B. Survival
O. hylas (1)
P. clarkii (3)
P. leniusculus (1)
All species (5)
-1
-0.5
0
0.5
1
Effect size

Fig. 6

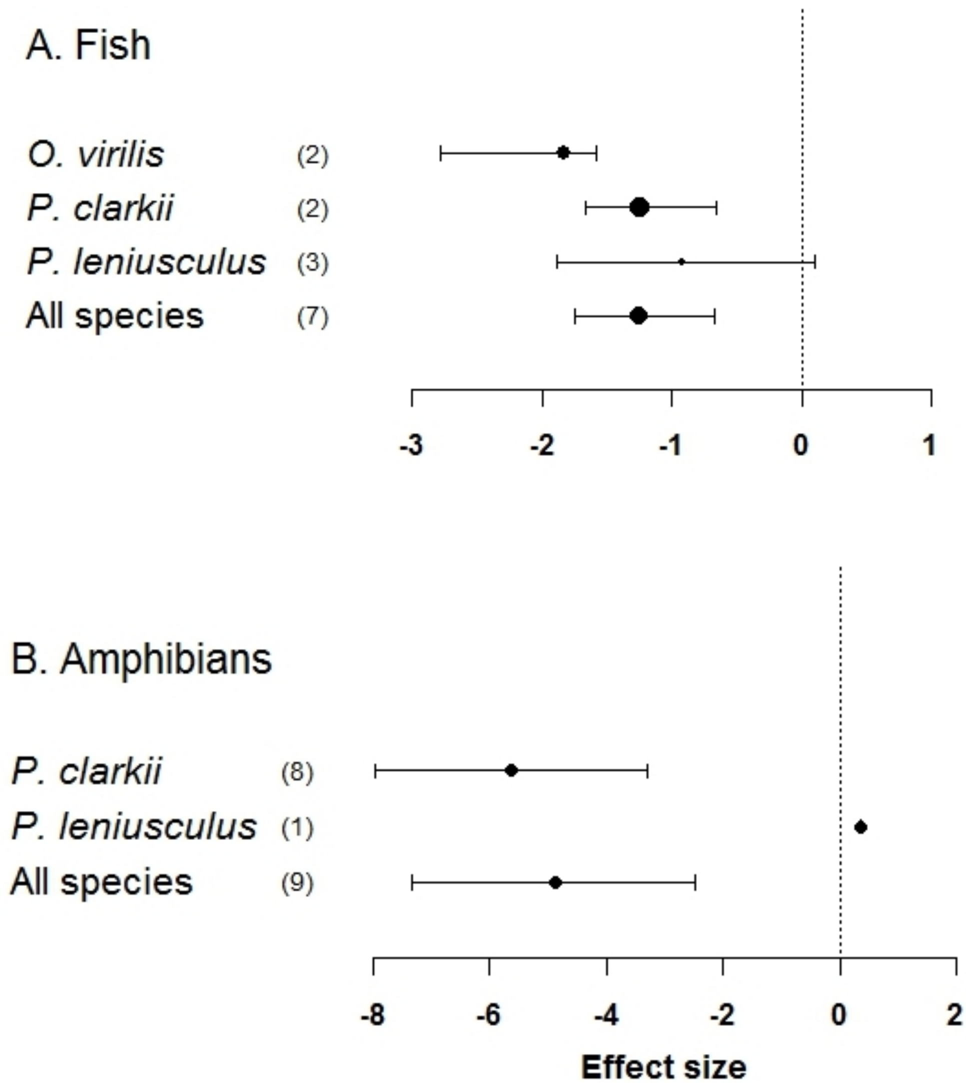
A. Fish
O. virilis (2)
P. clarkii (2)
P. leniusculus (3)
All species (7)
-3 -2 -1 0 1
B. Amphibians
P. clarkii (8)
P. leniusculus (1)
All species (9)
-8 -6 -4 -2 0 2
Effect size

Fig. 7

Appendix S1. Studies included in the meta-analyses. Invasive species include *Orconectes hylas*, *Orconectes rusticus*, *Orconectes virilis*, *Pacifastacus leniusculus*, and *Procambarus clarkii*.

Study	Nonnative species	Ecological response
Almeida, E., A. Nunes, P. Andrade, S. Alves, C. Guerreiro, and R. Rebelo. 2011. Antipredator responses of two anurans towards native and exotic predators. Amphibia-Reptilia 32:341–350.	*P. clarkii*	Amphibians
Anastácio, P.M., A.M. Correia, and J.P. Menino. 2005. Processes and patterns of plant destruction by crayfish: effects of crayfish size and developmental stages of rice. Archive für Hydrobiologie 162:37–51.	*P. clarkii*	Macrophytes
Anastácio, P.M., V.S. Parente, and A.M. Correia. 2005. Crayfish effects on seeds and seedlings: identification and quantification of damage. Freshwater Biology 50:697–704.	*P. clarkii*	Macrophytes
Arce, J.A., F. Alonso, E. Rico, and A. Camacho. 2006. A study of the possible effect of two crayfish species on epilithic algae in a mountain stream from Central Spain. Bulletin Francais de la Peche et de la Pisciculture 380–381:133–1144.	*P. leniusculus*	Algae
Banha, F., and P.M. Anastácio. 2011. Interactions between invasive crayfish and native river shrimp. Knowledge and Management of Aquatic Ecosystems 401:17.	*P. clarkii*	Invertebrates
Bobeldyk, A.M., and G.A. Lamberti. 2010. Stream food web responses to a large omnivorous invader, *Orconectes rusticus* (Decapoda, Cambaridae). Crustaceana 83:641–657.	*O. rusticus*	Algae, invertebrates
Brenneis, V.E.F., A. Sih, and C.E. de Rivera. 2011. Integration of an invasive consumer into an estuarine food web: direct and indirect effects of the New Zealand mud snail. Oecologia 167:169–179.	*P. leniusculus*	Invertebrates
Carpenter, J. 2005. Competition for food between an introduced crayfish and two fishes endemic to the Colorado River basin. Biology of Fishes 72:335–342.	*O. virilis*	Fish
Charlebois, P.M., and G.A. Lamberti.1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. Journal of the North American Benthological Society 15:551–563.	*O. rusticus*	Algae
Correia, A.M., and P.M. Anastácio. 2008. Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. Ecological Research 23:729–734.	*P. clarkii*	Invertebrates
Cruz, M.J., S. Pascoal, M. Tejedo, and R. Rebelo. 2006. Predation by an exotic crayfish, *Procambarus clarkii*, on natterjack toad, *Bufo calamita*, embryos: its role on the exclusion of this amphibian from its breeding ponds. Copeia 274–280.	*P. clarkii*	Amphibians
Cruz, M.J., and R. Rebelo. 2005. Vulnerability of southwest Iberian amphibians to an introduced crayfish, *Procambarus clarkii*. Amphibia-Reptilia 26:293–303.	*P. clarkii*	Amphibians
Ellrott, B.J., J.E. Marsden, J.D. Fitzsimons, J.L. Jonas, and R.M. Claramunt. 2007. Effects of temperature and density on consumption of trout eggs by *Orconectes propinquus* and *O. rusticus*. Journal of Great Lakes Research 33:7–14.	*O. rusticus*	Fish
Feminella, J.W., and V.H. Resh. 1989. Submersed macrophytes and grazing crayfish: an experimental study of herbivory in a California freshwater marsh. Holarctic Ecology 12:1–8.	*P. clarkii*	Macrophytes
Gamradt, S.C., and L.B. Kats. 1996. Effect of introduced crayfish and mosquitofish on California newts. Conservation Biology 10:1155–1162.	*P. clarkii*	Amphibians
Gamradt, S.C., L.B. Kats, and C.B. Anzalone. 1997. Aggression by non-native crayfish deters breeding in California newts. Conservation Biology 11:793–796.	*P. clarkii*	Amphibians

Gherardi, F., and P. Acquistapace. 2007. Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. Freshwater Biology 52:1249–1259.	*P. clarkii*	Macrophytes, invertebrates
Hanson, J.M, P.A. Chambers, and E.E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilis* and its impact on macroinvertebrates. Freshwater Biology 24:69–80.	*O. virilis*	Invertebrates
Hazlett, B.A., F.E. Anderson, L.A. Esman, C. Stafford, and E. Munro. 1992. Interspecific behavioral ecology of the crayfish *Orconectes rusticus*. Journal of Freshwater Ecology 7:69–76.	*O. rusticus*	Macrophytes
Johnson, P.T.J., J.D. Olden, C.T. Solomon, and M.J. Vander Zanden. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. Oecologia 159:161–170	*O. rusticus*	Algae
Klose, K. 2011. Snail responses to cues produced by an invasive decapod predator. Invertebrate Biology 130:226–235.	*P. clarkii*	Invertebrates
Larson, E.R., and D.D. Magoulick. 2009. Does juvenile competition explain displacement of a native crayfish by an introduced crayfish? Biological Invasions 11:725–735.	*O. neglectus*	Native crayfish - *Orconectes eupunctus*
Light, T. 2005. Behavioral effects of invaders: alien crayfish and native sculpin in a California stream. Biological Invasions 7:353–367.	*P. leniusculus*	Fish
Lodge, D.M., S.K. Rosenthal, K.M. Mavuti, W. Muohi, P. Ochieng, S.S. Stevens, B.J. Mungai, and G.M. Mkoji. 2005. Louisiana crayfish (*Procambarus clarkii*) (Crustacea: Cambaridae) in Kenyan ponds: non-target effects of a potential biological control agent for schistosomiasis. African Journal of Aquatic Science 30(2):119–124.	*P. clarkii*	Invertebrates
Lodge, D.M., and J.G. Lorman. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. Canadian Journal of Fisheries and Aquatic Sciences 44:591–597.	*O. rusticus*	Macrophytes
Lodge, D.M., M.W. Kershner, J.E. Aloi, and A.P. Covich. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology 75:1265–1281.	*O. rusticus*	Algae, macrophytes, invertebrates
Luttenton, M.R, M.J. Horgan, and D.M Lodge. 1998. Effects of three *Orconectes* crayfishes on epilithic microalgae: a laboratory experiment. Crustaceana 71:845–855.	*O. rusticus*	Algae
Maezono,Y., R. Kobayashi, M. Kusahara, and T. Miyashita. 2005. Direct and indirect effects of exotic bass and bluegill on exotic and native organisms in farm ponds. Ecological Applications 15:638–650.	*P. clarkii*	Macrophytes
Mazlum, Y., and A. Eversole. 2005. Growth and survival of *Procambarus acutus acutus* (Girard, 1852) and *P. clarkii* (Girard, 1852) in competitive settings. Aquaculture Research 36:537–545.	*P. clarkii*	Native crayfish - *Procambarus acutus acutus*
Matsuzaki, S.S., M. Sakamoto, K. Kawabe, and N. Takamura. 2012. A laboratory study of the effects of shelter availability and invasive crayfish on the growth of native stream fish. Freshwater Biology 57:874–882.	*P. clarkii*	Fish
Mueller, K.W., and L.R. Bodensteiner. 2009. Shelter Occupancy by Mixed-Species Pairs of Native Signal Crayfish and Non-Native Red Swamp Crayfish Held in Enclosures. Journal of Freshwater Ecology 24(1):67-76.	*P. clarkii*	Native crayfish - *Pacifastacus leniusculus*
Mueller, G.A., J. Carpenter, and D. Thornbrugh. 2006. Bullfrog tadpole (*Rana catesbeiana*) and red swamp crayfish *Procambarus clarkia*) predation on early life stages of endangered razorback sucker (*Xyrauchen texanus*). Southwestern Naturalist 51:258–261.	*P. clarkii*	Fish
Nakata, K., and S. Goshima. 2003. Competition for shelter of preferred sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference, and body size. Journal of Crustacean Biology 23:897–907.	*P. leniusculus*	Native crayfish - *Cambaroides japonicus*
Nakata, K., and S. Goshima. 2006. Asymmetry in mutual predation between the endangered Japanese native crayfish *Cambaroides japonicus* and the North	*P. leniusculus*	Native crayfish - *Cambaroides japonicus*

American invasive crayfish *Pacifastacus leniusculus*: a possible reason for species replacement. Journal of Crustacean Biology 26:134–140.		
Nyström, P., and J.R. Perez. 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. Hydrobiologia 368:201–208.	*P. leniusculus*	Invertebrates
Nyström, P., O. Svensson, B. Lardner, C. Brönmark, and W. Granéli. 2001. The influence of multiple introduced predators on a littoral pond community. Ecology 82:1023–1039.	*P. leniusculus*	Algae, macrophytes, invertebrates, amphibians
Nyström, P., and J. Strand. 1996. Grazing by a native and an exotic crayfish on aquatic macrophytes. Freshwater Biology 36:673–682.	*P. leniusculus*	Macrophytes
Olden, J.D., E.R. Larson, and M.C. Mims. 2009. Home-field advantage: native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). Aquatic Ecology 43:1073–1084.	*P. clarkii, O. rusticus*	Invertebrates
Pintor, L.M., A. Sih, and J.L Kerby. 2009. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology 90:581–587.	*P. leniusculus*	Invertebrates
Perry, W.L., D.M. Lodge, and G.A. Lamberti. 1997. Impact of crayfish predation on exotic zebra mussels and native invertebrates in a lake-outlet stream. Canadian Journal of Fisheries and Aquatic Sciences 54:120–125.	*O. rusticus*	Invertebrates
Perry, W.L., D.M. Lodge, and G.A. Lamberti. 2000. Crayfish (*Orconectes rusticus*) impacts on zebra mussel (*Dreissena polymorpha*) recruitment, other macroinvertebrates and algal biomass in a lake-outlet stream. American Midland Naturalist 144:308–316.	*O. rusticus*	Invertebrates
Peters, J.A., T. Kreps, and D.M. Lodge. 2008. Assessing the impacts of rusty crayfish (*Orconectes rusticus*) on submergent macrophytes in a north-temperate U.S. lake using electric fences. American Midland Naturalist 159:287–297.	*O. rusticus*	Macrophytes
Rogowski, D.L., and C.A. Stockwell. 2006. Assessment of potential impacts of exotic species on populations of a threatened species, White Sands pupfish, *Cyprinodon tularosa.* Biological invasions 8:79–87.	*O. virilis*	Fish
Rudnick, D., and V. Resh. 2005. Stable isotopes, mesocosms and gut content analysis demonstrate trophic differences in two invasive decapod Crustacea. Freshwater Biology 50:1323–1336.	*P. clarkii*	Algae, macrophytes, invertebrates
Setzer, M., J.R. Norrgard, and T. Jonsson. 2011. An invasive crayfish affects egg survival and the potential recovery of an endangered population of Arctic charr. Freshwater Biology 56:2543–2553.	*P. leniusculus*	Fish
Söderbäck, B. 1994. Interactions among juveniles of two freshwater crayfish species and a predatory fish. Oecologia 100:229–235.	*P. leniusculus*	Native crayfish - *Astacus astacus*
Stenroth, P., and P. Nyström. 2003. Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. Freshwater Biology 48:466–475.	*P. leniusculus*	Invertebrates, fish
Stewart, T.W., J.G. Miner, and R.L. Lowe. 1998. An experimental analysis of crayfish (*Orconectes rusticus*) effects on a *Dreissena*-dominated benthic macroinvertebrate community in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 55:1043–1050.	*O. rusticus*	Invertebrates
Usio, N., R. Kamiyama, A. Saji, and N. Takamura. 2009. Size-dependent impacts of invasive alien crayfish on a littoral marsh community. Biological Conservation 142:1480–1490.	*P. leniusculus*	Algae, macrophytes, invertebrates
Usio, N., K. Suzuki, M. Konishi, and S. Nakano. 2006. Alien vs. endemic crayfish: roles of species identity in ecosystem functioning. Archiv für Hydrobiologie 166:1–21.	*P. leniusculus*	Invertebrates
Vorburger, C., and G. Ribi. 2009. Aggression and competition for shelter between a native and an introduced crayfish in Europe. Freshwater Biology 42:111–119.	*P. leniusculus*	Native crayfish - *Austropotamobius torrentium*
Westhoff, J.T, R.J. DiStefano, and D.D. Magoulick. 2012. Do environmental	*O. hylas*	Native crayfish - *Orconectes*

changes or juvenile competition act as mechanisms of species displacement in crayfishes? Hydrobiologia 683:43–51.	*peruncus*

Appendix S2. Results of mixed-effects model analyses with Rosenthal's fail-safe number of invasive crayfish including *Orconectes rusticus*, *Orconectes virilis*, *Pacifastacus leniusculus*, and *Procambarus clarkii*.

Moderator variable	Sample size (no. of experiments)	Mean effect size	95% confidence interval	Q (heterogeneity)	p (χ^2)	Rosenthal's fail-safe number
Overall impacts						
All species	61	–1.63	–2.20 to –1.14	$Q_{\text{Total}} = 86.7$	0.01	1127
P. clarkii	27	–2.22	–3.41 to –1.28	$Q_{\text{b}} = 5.25$	0.15	
P. leniusculus	13	–1.72	–2.85 to –0.852	$Q_{\text{w}} = 81.4$	0.02	
O. rusticus	17	–0.960	–1.48 to –0.533			
O. virilis	4	–0.776	–2.23 to 2.13			
Algae						
All species	8	0.257	–0.456 to 1.14	$Q_{\text{Total}} = 7.00$	0.43	0
P. leniusculus	3	0.321	–0.771 to 2.53	$Q_{\text{b}} = 0.0117$	0.91	
O. rusticus	5	0.221	–0.523 to 1.03	$Q_{\text{w}} = 6.99$	0.32	
Macrophytes – effects of experimental venue						
All venues	20	–2.02	–2.84 to –1.29	$Q_{\text{Total}} = 25.1$	0.16	240
Laboratory	3	–2.58	–6.74 to –	$Q_{\text{b}} = 2.33$	0.31	

			0.819			
Cage	12	–1.59	–2.60 to –0.748	$Q_w = 22.7$	0.16	
Outdoor mesocosm	5	–2.96	–4.25 to –1.94			
Invertebrates – effects of experimental venue						
All venues	22	–1.49	–2.09 to –1.04	$Q_{Total} = 28.2$	0.13	289
Laboratory	7	–2.23	–3.92 to –1.39	$Q_b = 4.14$	0.13	
Cage	10	–1.33	–1.99 to –0.891	$Q_w = 24.1$	0.19	
Outdoor mesocosm	5	–0.771	–2.09 to 0.163			
Macrophytes – crayfish effects						
All species	21	–1.99	–2.74 to –1.32	$Q_{Total} = 27.6$	0.12	298
P. clarkii	10	–2.82	–3.70 to –1.20	$Q_b = 4.81$	0.09	
P. leniusculus	3	–2.06	–6.74 to –0.524	$Q_w = 22.8$	0.20	
O. rusticus	8	–1.16	–2.20 to –0.277			
Invertebrates – crayfish effects						
All species	21	–1.51	–2.14 to –1.02	$Q_{Total} = 25.9$	0.17	238

P. clarkii	6	–1.81	–3.11 to –0.759	$Q_b = 1.69$	0.43	
P. leniusculus	9	–1.73	–2.93 to –0.869	$Q_w = 24.2$	0.15	
O. rusticus	6	–0.98	–1.57 to –0.599			
Fish						
All species	7	–1.26	–1.78 to –0.702	$Q_{Total} = 5.73$	0.45	35
P. leniusculus	3	–0.92	–1.89 to –0.336	$Q_b = 1.60$	0.45	
P. clarkii	2	–1.23	1.67 to –0.666	$Q_w = 4.13$	0.39	
O. virilis	2	–1.93	–2.78 to –1.58			

Appendix S3. Results of random-effects model analyses testing effects of all crayfish on each taxonomic group.

Taxonomic group	Sample size (no. of experiments)	Mean effect size	95% confidence interval	Q_{Total}	p (χ^2)	Rosenthal's fail-safe number
All groups–native vs invasive crayfish comparison	11	0.144	–0.468 to 0.879	13.9	0.176	0
Algae	9	0.0114	–1.10 to 0.961	22.6	<0.01	0
Macrophytes	21	–2.01	–2.79 to –1.33	26.0	0.17	278
Invertebrates	22	–1.49	–2.08 to –1.03	27.8	0.15	284
Gastropods	13	–1.34	–1.95 to –0.829	13.7	0.32	110
Amphipoda	5	–0.927	–1.95 to 0.457	3.58	0.47	0
Chironomidae	6	–0.211	–0.900 to 0.542	5.17	0.40	0
Ephemeroptera	3	–0.331	–0.671 to –0.0730	0.67	0.72	0
Trichoptera	5	0.202	–0.598 to 1.36	5.22	0.27	0
Native crayfish	5	–0.288	–0.877 to 0.359	3.96	0.41	0
survival	5	–2.85	–5.80 to –2.85	2.78	0.60	0
Agonism	7	–1.26	–1.74 to –0.674	6.48	0.37	42
Fish	9	–4.87	–7.33 to –2.49	8.09	0.42	42
Amphibians						

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Ch. II. Non-native Chinese mystery snail (*Bellamya chinensis*) supports consumers in urban lake food webs

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Abstract. Non-native species are widely regarded as threats to ecosystem structure and function; however, these species may also provide benefits to ecosystems that have lost former functions to environmental degradation. This study is the first to evaluate whether non-native species provide prey resources or induce trophic cul-de-sacs (by diverting basal energy away from higher trophic levels) in developed ecosystems where native prey are in decline. We used stable isotopes of ^{13}C, ^{15}N, and ^{2}H to assess whether non-native Chinese Mystery snail (*Bellamya chinensis*) maintains the integration of benthic resources in food webs of lakes subjected to lakeshore development by providing an abundant prey resource. Consumers in undeveloped lakes were supported primarily by benthic resources, and lakeshore development dramatically reduced consumer reliance on these resources. This was at least partly due to a reduction in the availability of native snails, a high quality prey item, to the dominant littoral consumer, molluscivorous pumpkinseed sunfish (*Lepomis gibbosus*). In developed lakes with non-native *Bellamya*, generalist yellow perch (*Perca flavescens*) and piscivorous largemouth bass (*Micropterus salmoides*) consumed benthic resources in proportions similar to undeveloped lakes, and pumpkinseed sunfish consumed *Bellamya* in higher proportions than in undeveloped lakes. Thus, *Bellamya* provided a prey substitute in developed lakes where native snail populations were depressed; and *Bellamya*'s influence extended to higher trophic level consumers. Our study provides evidence that non-native species can ameliorate some effects of environmental degradation, and we suggest that future research consider how the effects of non-native species, either positive or negative, may vary across human-modified landscapes.

Key words: urban development, invasive, introduced species, prey subsidy, context-dependent impact

INTRODUCTION

Debate over the role of non-native species in contemporary landscapes has emerged in recent years (Schlaepfer et al. 2011, Vitule et al. 2012). On one side, non-native species are widely recognized to threaten native species, diversity, and ecosystem structure and function (Simberloff 2011, Ricciardi 2013), and their effects may be magnified by other anthropogenic disturbances, including habitat modification and climate change (Didham et al. 2007, Rahel and Olden 2008). Alternatively, non-native species may provide food, habitat, or engineering processes to ecosystems that have lost former functions to environmental degradation and other human activities, and thus could benefit native species in invaded ecosystems (e.g., Foster and Robinson 2007, Griffiths et al. 2010, Carroll and Peterson 2013). Examples include non-native mammals supporting native predatory birds in urban environments (Cava et al. 2012); non-native macrophytes benefiting fish by increasing prey production in coastal areas where native habitat has been destroyed (Martin and Valentine 2011); and non-native crayfish subsidizing wetland bird diets in rice fields (Tablado et al. 2010). Although non-native species can provide prey resources to consumers, it is unknown whether the quality of non-native prey can be equivalent to native prey that they replace and sufficient to support consumers. Further, research has yet to identify the environmental context in which non-native species may subsidize or replace existing prey resources.

Non-native species that are predator-resistant or have low nutritional value may be of little benefit to native consumers. For example, native rusty crayfish (*Orconectes rusticus*) and pumpkinseed sunfish (*Lepomis gibbosus*) exhibit low predation rates on non-native zebra mussels (*Dreissena polymorpha*) in laboratory settings (Nadafi and Rudstam 2014), and Vinson and Baker (2007) demonstrated that rainbow trout (*Oncorhynchus mykiss*) lose body mass when fed non-native New Zealand mudsnails (*Potamopyrgus antipodarum*). Low quality prey may induce what have been termed trophic cul-de-sacs (*sensu* Bishop et al. 2007) by out-competing other prey species for preferred resources and ultimately diverting basal energy away from consumers and back to lower trophic levels in the form of detritus (Bishop et al. 2007, Power et al. 2008). Non-native species may also have high feeding rates and be released from predation (Colautti et al. 2004, Dick et al. 2014), further increasing the likelihood of trophic cul-de-sacs. Alternatively, non-native species that are susceptible to predators may provide suitable prey resources in human-impacted environments where native prey have been extirpated or are in low abundance (Tablado et al. 2010, Cava et al. 2012).

Our study is the first to evaluate whether non-native species provide prey resources or induce trophic cul-de-sacs across a gradient in native prey availability due to human disturbance, and to explore the implications for higher trophic levels in freshwater ecosystems.
Freshwater ecosystems are particularly susceptible to human land use because they are highly connected to and reliant on adjacent terrestrial habitats that provide inputs of nutrients, habitat structure, and food resources (Allan 2004). Thus, degradation of the surrounding terrestrial environment disrupts supplies of organic material and habitat that support aquatic food web production (Brauns et al. 2011, Larson et al. 2011). Freshwater ecosystems are also subjected to ongoing species introductions that are positively associated with land use change (Strayer 2010), making freshwater food webs well suited for studying the potential for non-native species to ameliorate or magnify the effects of environmental degradation on ecosystems.

Lakes throughout North America have undergone extensive development characterized by removals of riparian and aquatic vegetation and coarse woody debris, which together reduce the retention of organic matter and densities of macrophytes that provide food and habitat for littoral-zone invertebrates and fish (e.g., Francis et al. 2007, Larson et al. 2011). Lakeshore development can dramatically alter compositions of benthic invertebrate communities, reduce the availability of benthic prey, and shift consumer diets from reliance on terrestrial and benthic to pelagic resources (Schindler and Scheuerell 2002, Francis et al. 2007). Concurrent with lakeshore development, Chinese mystery snail (*Bellamya chinensis*; hereafter *Bellamya*) has been introduced to many North American lakes and can achieve high densities in lake littoral zones (Solomon et al. 2010). *Bellamya* is a large snail (up to 70 mm shell height; Fig. 1c), and its thick shell and hard operculum may afford protection against some predators (Olden et al. 2009). In addition, the snail's ability to filter-feed and non-selectively scrape benthic periphyton (Jokinen 1982, Olden et al. 2013) provides the potential for large competitive effects on native grazing invertebrates. Together this evidence suggests that *Bellamya* has the potential to divert basal resources from, and intensify the effects of shoreline development on, higher trophic levels. Alternatively, *Bellamya* may help maintain the integration of benthic resources in lake food webs by providing an abundant prey resource in developed lakes where native benthic prey are in low densities.

Our objectives were to identify whether *Bellamya* induces trophic cul-de-sacs or maintains the integration of benthic pathways in food webs, and to assess whether the ecological role of this non-native species is consistent among lakes with varying degrees of shoreline development. We predict that *Bellamya* substitutes for declines in native invertebrates so that fish in developed lakes with *Bellamya* exhibit similar reliance on benthic resources compared to fish in undeveloped lakes; and *Bellamya* does not influence resource use by fish in undeveloped lakes due to the availability of native prey. Alternatively, if *Bellamya* induces a trophic cul-de-sac we expect to find an increase in reliance on pelagic resources and concurrent decrease in reliance on benthic resources among fish consumers in developed and undeveloped lakes.

METHODS

Study system

We studied 12 lakes in the Puget Sound lowlands of Washington State, United States, that span a gradient of shoreline urbanization ranging from undeveloped lakes with restricted public access to developed lakes with the entire shoreline containing residential houses and little riparian vegetation (Fig. 1a, b). The topography of the region was shaped 12,000 to 15,000 years ago by movements of the Puget Lobe of the Cordilleran ice sheet. Consequently, lake sediments are characterized by glacial till, an accumulation of clay and boulder, and areas of sandy gravel outwash. Lakes were selected using data from previous surveys (presented in Tamayo and Olden 2014), and by examining monthly monitoring data from King County's Lake Stewardship Program (www.kingcounty.gov), to ensure high similarity in surface area and mean depth; all lakes were small, summer stratified, and oligo- to mesotrophic (Table 1).

Shorelines of undeveloped lakes are characterized by a dense canopy of native evergreen and less abundant deciduous trees. Developed lakes are surrounded by open space, ornamental gardens and grass lawns, non-native shrubs, and native deciduous trees that typically outnumber evergreen tree species. Dominant aquatic vegetation in undeveloped and developed lakes includes submerged and floating-leaved pondweeds (*Potamogeton spp.*) and plant-like algae (*Chara spp.*).

Lakes contain similar assemblages of fish species that include native populations of rainbow trout, and naturalized populations of yellow perch (*Perca flavescens*), largemouth bass (*Micropterus salmoides*), and pumpkinseed sunfish (Fig. 1d). These species were first introduced to Washington State in the late 1800's and are the most abundant species across the lakes (Washington Department of Fish and Wildlife 2005). Further, they are representative of littoral (pumpkinseed sunfish) and littoral-pelagic (yellow perch and largemouth bass) consumers in the region. Assessing resource use by a specialized molluscivore, pumpkinseed sunfish, and a dietary generalist, yellow perch, allows for an examination of *Bellamya*'s influence on consumers with differing feeding specialties; in addition, we can assess whether *Bellamya*'s effects are transmitted through the food web via intermediate consumers to a higher trophic-level consumer, largemouth bass.

Site selection

Three lakes were selected from each of four categories contrasting developed vs. undeveloped and invaded vs. uninvaded by *Bellamya*. Lakes were considered undeveloped if 40% or less of the shoreline buffer (10 m from the water edge) was developed; developed lakes had greater than 60% shoreline development (Fig. 1a, b). Percent development was determined using aerial photographs combined with validation using direct field observations. Invasion categories were determined using presence/absence data for *Bellamya* from a 2009 survey (Olden, *unpublished data*). Our 2012 survey confirmed these category assignments with the exception of recently discovered populations of *Bellamya* in Martha and Sunday lakes (both developed), with mean densities of 0.08 and 0.17 individuals*m^{-2}, respectively. Each population was restricted to a single sampling location exhibiting very low abundances and thus is likely to impart minimal food-web scale impacts. Consequently, these lakes were assigned to the ‘developed/non-*Bellamya*’ category.

Sampling

At each lake, we collected allochthonous (terrestrial detritus) and autochthonous (aquatic macrophytes, periphytic algae) basal resources, benthic invertebrates, zooplankton, and fish, during July-August in 2012 and 2013. Lake physical and chemical characteristics were also assessed, including water temperature, dissolved oxygen, conductivity (YSI Model 85), and pH. We estimated lake clarity using Secchi depth (m), a standard limnological method to characterize water transparency. In addition, we collected duplicate water samples for analysis of total phosphorus concentrations (TP; $\mu g\ L^{-1}$) from the epilimnion using a Van Dorn bottle (Wetzel and Likens 1991). Water samples were transferred unfiltered to acid-washed polyethylene bottles, frozen, and analyzed at the University of Washington, School of Oceanography’s Marine Chemistry Laboratory. Analysis of TP followed methods of Valderrama (1981).

To estimate the importance of pelagic, benthic, and terrestrial pathways to consumers, we sampled all major primary and secondary producers for stable isotope analysis. Each lake was divided into four quadrants (according to the cardinal directions) to distribute the following sampling effort evenly. In each lake we deployed 4 hoop nets (7.9 m wing length and five hoops each 0.8 m in diameter) and 20 galvanized steel minnow traps (41.0 cm long with two 2.5 cm openings and 6.4 mm mesh size) for 24 hours to sample littoral fish. Two benthic, multi-mesh gill nets (58.5 m length x 1.8 m height, six panels each being 9.8 m long with mesh-sizes 25, 32, 38, 51, 64, 76 mm, stretched mesh) were set perpendicular to shore overnight to collect littoral and pelagic fish. Fish were identified to species, measured for total length (mm), and weighed (g).

Pelagic zooplankton were sampled using oblique net tows (35 cm diameter opening, 73 µm mesh cod end) prior to sunrise. We sampled invertebrates for isotopic analysis and community indices by sweeping a D-frame net (25 cm x 35 cm opening, 500 µm mesh) over the lake bottom within a 1 m^2 quadrat at depths between 0 and 1 m, and using a Ponar Grab (0.023 m^2 opening) at depths between 1 to 4 m. Within each quadrant we selected randomly 10 sites representing all habitat types (woody debris, macrophytes, gravel and cobble, sand) to sample invertebrates. In addition, we hand-collected *Bellamya* and native snails in the genus *Physella*, the most abundant native snail across the lakes, from sediment, logs, and vegetation. All benthic samples were sieved through a 500 µm-mesh bucket, and samples taken for community indices (n = 24 per lake) were preserved in 90% ethanol. Stable isotope samples (n = 16 per lake) were put on ice in the field and frozen at -20°C in the laboratory. Leaves from dominant evergreen and deciduous tree species surrounding each lake were collected to represent terrestrial end-members.

Environmental water contributes substantially to consumer δ^2H signature (Soto et al. 2013); therefore, we obtained water samples for δ^2H signatures from each lake with a Van Dorn bottle lowered to 0.5 m depth. Environmental water samples were filtered through GF/F 0.7 µm filters to remove organisms and particulate organic matter (POM) (Solomon et al. 2011). All isotope samples were returned to the laboratory on ice and frozen at -20°C until preparation for analysis.

Laboratory preparation

Benthic invertebrate community samples were processed according to EPA Bioassessment Benthic Macroinvertebrate Protocols. We developed a morphospecies curve using a high volume sample to estimate the minimum number of randomly selected individuals needed to detect all morphospecies in a sample. Based on this curve, we sub-sampled each sample until 300 (± 20%) individuals were counted. Samples containing fewer than 240 individuals were processed in entirety. In addition, we sorted *Bellamya* from the entire volume of each sample to estimate densities. Organisms were identified to genus (sub-family for Chironomidae) with an 80X dissecting microscope using published and online taxonomic guides (Thorp and Covich 1991, Merritt and Cummins 1996).

We prepared stable isotope samples by extracting plugs of dorsal muscle tissue from fish, avoiding contact with the digestive tract. Snail shells and opercula were removed from soft tissue prior to drying. Benthic invertebrate samples were sorted by order, and zooplankton samples were separated from POM. Small individuals were aggregated to reach target weights for isotopic analysis. Macrophytes and terrestrial leaves were sorted to genus or species. Scrapes of periphyton were sorted under a dissecting microscope to remove animal and wood material. All samples were dried at 55°C for 24 hours and ground to a fine powder with a mortar and pestle. Samples were weighed and encapsulated in tin for analysis of $\delta^{15}N$ and $\delta^{13}C$ and in silver capsules for analysis of $\delta^{2}H$.

Carbon and nitrogen samples were analyzed at UC Davis Stable Isotope Facility on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were compared to in-house laboratory standards calibrated against Vienna PeeDee Belemnite for carbon and air for nitrogen. The measurement error reported by UC Davis is the long-term standard deviation of 0.2 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$. Hydrogen samples were analyzed at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University) on a 1400 C thermal-chemical elemental analyzer coupled to a Thermo-Electron Delta Plus XL mass spectrometer. Samples were equilibrated for exchangeable hydrogen using a bench top equilibration technique, and raw data were corrected after analysis using the calibration standards and techniques of Wassenaar and Hobson (2003). Calibration standards were Caribou hoof (δ^2H = −197‰), Keratin (δ^2H = −121.1‰), and Kudo horn (δ^2H = −54.1‰). We report stable isotope values in standard delta notation,

$$\delta X = [(R_{sample} / R_{standard}) - 1] \text{ x } 10^3, \quad (1)$$

where X is the mass of the heavier isotope of the element, and R is the ratio of the heavy to light isotope in the sample and standard. The δ value is the amount of heavy and light isotopes in a sample.

Isotopic analyses

We used a Bayesian isotope mixing model (MixSIR; Moore and Semmens 2008) implemented in the R programming language (R Core Development Team 2014) to estimate contributions of benthic, pelagic, and terrestrial resources to consumer diets, and assess direct contributions of *Bellamya* to pumpkinseed sunfish diets. We modified MixSIR following the methods of Solomon et al. (2011) to account for contributions of environmental water to consumer δ^2H signatures. Environmental water has a compounding effect with trophic position, such that tissues of higher trophic level consumers reflect the environmental water that they ingest and the environmental water ingested by their prey; we accounted for the effect of environmental water on consumer δ^2H by calculating the total contribution of environmental water to consumer tissue as:

$$\omega_{compound} = 1 - (1 - \omega)^{\tau}, \quad (2)$$

where ω is the per trophic level contribution of environmental water and τ is the trophic position of the consumer. We estimated an ω value of 0.29 ± 0.11 (mean ± 1 SD) based on published experimental values (Solomon et al. 2009, Graham et al. 2014); trophic discrimination factors of 0.4 ± 1.3‰ per trophic level for $\delta^{13}C$ and 3.4 ± 1‰ for $\delta^{15}N$ (Post et al. 2002), and 0‰ for $\delta^{2}H$ (Solomon et al. 2009). Consumer trophic positions were estimated according to Post et al. (2002), and primary consumers were assigned a trophic position of 1.5. Consumer $\delta^{2}H$ signatures were then calculated as:

$$\delta^2 H_{consumer} = \omega_{compound} * \delta^2 H_{water} + (1 - \omega_{compound}) * (\phi_1 * \delta^2 H_1 + \phi_2 * \delta^2 H_2 + \phi_3 * \delta^2 H_3), \quad (3)$$

where $\delta^2 H_1$, $\delta^2 H_2$, and $\delta^2 H_3$ refer to the isotopic signatures of individual resources and ϕ is the proportion of the consumer's diet derived from each resource. Signatures of $\delta^2 H_{water}$ were similar across lakes, so we used a value of -60.10 ± 6.93 ‰ (mean ± 1 SD) in mixing models.

We examined the importance of resources from each habitat to juvenile and adult pumpkinseed sunfish, yellow perch, and largemouth bass diets, and included terrestrial detritus, zooplankton, and benthic invertebrates (amphipods and isopods) as representative end-members. Pumpkinseed sunfish included in mixing models as consumers ranged from 6.5 to 17.5 cm total length (TL), corresponding to sizes at which they feed on gastropods (Osenberg and Mittelbach 1989, Huckins 1997). Results from a 2012 study conducted in three of our study lakes (Padden, Walsh, Wilderness), for which we used stable isotopes to examine yellow perch trophic ontogeny, indicated that yellow perch undergo a non-significant ontogenetic shift at 13 cm TL from feeding on a mixed diet of zooplankton and benthic invertebrates to a diet composed of zooplankton, benthic invertebrates, and fish. We found that this non-significant shift varied by lake, but that yellow perch smaller than 13 cm TL consumed benthic prey as 40-60% of their diet, and yellow perch larger than 13 cm TL consumed benthic prey as 50-70% of their diet (S.

Linzmaier, L. Twardochleb, et al., *unpublished manuscript*). Thus, we included yellow perch between 9 and 27 cm TL in mixing models, which also correspond to documented sizes at which yellow perch are able to consume mixed pelagic-benthic prey (Graeb et al. 2005, Fullhart et al. 2011). Finally, we included largemouth bass 10 to 30 cm TL as consumers in mixing models. Young of year and small juvenile fish were excluded from mixing models because fish are limited to zooplanktivory early in ontogeny (Mittelbach and Persson 1998).

We also compared the contribution of *Bellamya* to pumpkinseed sunfish in undeveloped and developed lakes by parameterizing mixing models with terrestrial detritus, zooplankton, and the benthic invertebrate groups: collectors (amphipods and isopods), odonates, native snails *Physella*, and *Bellamya* as potential sources. We did not estimate the contribution of *Bellamya* to either largemouth bass or yellow perch diets because these species have a wide diet breadth, and mixing models that estimated the contributions of individual prey items to these two consumer species would not converge on a solution. We were unable to obtain isotope data for native snails from two lakes (Angle and Wilderness) in 2012 and instead parameterized mixing models using snail data sampled from the same lakes in 2009 (J. Olden, *unpublished data*). We ran two-sample t-tests to determine whether native snail isotopic signatures from other lakes included in mixing models differed significantly between 2009 and 2012, and found no significant differences between years (δ^{13}C, $t_{21} = -2.04$, $p > 0.05$; δ^{15}N, $t_{21} = 1.49$, $p > 0.05$). In addition, we used Welch two-sample t-tests to assess differences between *Bellamya* and *Physella* in their δ^{13}C, δ^{15}N, and δ^{2}H isotopic signatures. We found that isotopic signatures between these two species were highly significantly different (Table 2), and therefore determined that it was reasonable to include them as separate sources that could be distinguished from one another in isotope mixing models.

Statistical analyses

Lake size and transparency can influence the importance of benthic vs. pelagic and terrestrial energetic pathways to lake food webs (Vadeboncoeur et al. 2003, Larson et al. 2011); therefore, we tested for differences in lake surface area (km^2), epilimnetic TP ($\mu g\ L^{-1}$) and transparency as measured by Secchi depth (m) among lake categories using separate two-way analysis of variance (ANOVA). We tested for differences in densities of *Bellamya*, native snails, and the overall assemblage of non-molluscan invertebrates between lakes with developed and undeveloped shorelines using two-sample t-tests. Alpha levels were set at 0.05, all invertebrate data was log (x + 1) transformed prior to analyses, and data assumptions of homoscedasticity and normality were met for all analyses.

We assessed differences in the proportion of consumer diets derived from benthic resources across lakes, with lakes treated as replicates, using ANOVAs with binomial error structure with development and *Bellamya* presence/absence as categorical predictor variables. ANOVA models were weighted as a function of the variance in the posterior estimates of benthic resource use (as determined by Bayesian isotope mixing models) by fish for each lake. In addition, we ran weighted, one-way ANOVAs with binomial error structure to test the contribution of native snails and *Bellamya*, respectively, to pumpkinseed sunfish diets in undeveloped and developed lakes, with development as the categorical predictor variable. Three models were considered to explain the proportion of benthic resource use by consumers: individual main effects of lake development and *Bellamya*, and models including main and additive effects. Model selection was performed with maximum-likelihood values from ANOVA models using modified Akaike's Information Criterion (AICc) for small sample sizes. All statistical analyses were implemented in the R programming language (R Core Development Team 2014).

RESULTS

Invertebrate abundances and lake characteristics

Lake surface area, water clarity (measured as Secchi depth), and TP were comparable across lake categories (Table 1). We found no significant differences in lake surface area between developed and undeveloped lakes ($F_{1,8} = 0.592$, $p = 0.46$), between lakes with and without *Bellamya* ($F_{1,8} = 2.03$, $p = 0.19$), or any interaction therein ($F_{1,8} = 0.77$, $p = 0.41$). In addition, epilimnetic TP did not differ significantly between developed and undeveloped lakes ($F_{1,20} = 0.27$, $p = 0.61$), between lakes with and without *Bellamya* ($F_{1,20} = 0.83$, $p = 0.37$), nor was there a significant interaction ($F_{1,20} = 3.23$, $p = 0.09$). Similarly, we found no significant differences in water transparency between developed and undeveloped lakes ($F_{1,8} = 0.05$, $p = 0.83$), between lakes with and without *Bellamya* ($F_{1,8} = 0.64$, $p = 0.45$); nor did we find a significant interaction ($F_{1,8} = 1.77$, $p = 0.22$). Densities of *Bellamya* ($t_4 = -7.13$, $p < 0.01$) and native snails ($t_{10} = -2.50$, $p < 0.05$) were significantly higher in undeveloped than developed lakes (Fig. 2). By contrast, lake development did not have a significant effect on non-molluscan invertebrate densities (Fig. 2; $t_{10} = -0.93$, $p = 0.38$).

Benthic resource use by lake consumers

Isotope bi-plots revealed that benthic basal resources in developed lakes were enriched in ^{13}C compared to pelagic and terrestrial resources and that isotopic signatures of consumers differed between *Bellamya* and non-*Bellamya* lakes (Fig. 3). Figure 3 depicts a subset of resources and consumers for two developed lakes, Martha and Pine, to illustrate differences in carbon signatures between *Bellamya* and non-*Bellamy*a lakes. Pumpkinseed sunfish and largemouth bass were more enriched in ^{13}C in developed lakes with *Bellamya,* suggesting a higher reliance on benthic-derived resources compared to developed lakes without *Bellamya* (Fig. 3A, C; Appendix 1); however, yellow perch were not consistently more enriched in ^{13}C in developed/*Bellamya* lakes (Fig. 3B; Appendix 1). Benthic resources in undeveloped lakes were not as enriched in ^{13}C as in developed lakes, and were more isotopically similar to terrestrial and pelagic resources (Appendix 1). Although pumpkinseed sunfish and largemouth bass signatures were similar to $\delta^{13}C$ signatures of benthic resources (Appendix 1), $\delta^{13}C$ of yellow perch was intermediate between benthic and pelagic and terrestrial resources (Appendix 1) in undeveloped lakes. There were no clear systematic differences in consumer isotopic signatures between undeveloped lakes with and without *Bellamya*, suggesting that *Bellamya* does not have substantial influence on resource use by consumers in undeveloped lakes.

Results of three-isotope mixing models suggest that all consumers relied heavily on benthic resources in undeveloped lakes and that lake development substantially reduced the proportion of benthic-derived resources, and increased the proportion of pelagic resources in diets of yellow perch and largemouth bass, but not pumpkinseed sunfish (Fig. 4). Terrestrial resource use was low for all consumers (Appendix 2), such that resources for fish were obtained directly from benthic or pelagic food webs. In addition, there was a striking pattern of higher benthic resource use by all consumers in developed/*Bellamya* lakes compared to developed/non-*Bellamya* lakes (Fig. 4). AIC_c model selection on ANOVA models identified that for all consumers, a model including additive effects of lake development and *Bellamya* best explained the proportion of benthic-derived resources in consumer diets (Table 3).

Contributions of Bellamya and native snails to pumpkinseed sunfish diets

Pumpkinseed sunfish consumed higher proportions of *Bellamya* in developed lakes (Pine, Angle) where native snails have declined, and consumed native snails in higher proportions in undeveloped lakes (Cascade, Padden, Wilderness) where snails were present at high densities (Fig. 5; Appendix 3). However, *Bellamya* was a measureable component of the pumpkinseed sunfish diet in all lakes, comprising approximately 10% or more of the diet. ANOVAs indicated that pumpkinseed consumed significantly greater proportions of native snails in undeveloped than developed lakes, and significantly greater proportions of *Bellamya* in developed lakes (Table 4). We qualitatively assessed diets from frozen pumpkinseed sunfish and yellow perch from 6 lakes (n = 5 to 9 fish $lake^{-1}$) in the laboratory to confirm our mixing model results, and we found evidence that pumpkinseed sunfish and yellow perch consumed *Bellamya* and native snails in undeveloped and developed lakes. Native snails were identified by the presence of thin shell fragments, and *Bellamya* were identified by the presence of the operculum, which is lacking in other snails in these lakes. Our sample size was not sufficient to make a quantitative assessment of overall dietary components, but the most common dietary components for pumpkinseed sunfish and yellow perch were *Physella*, *Bellamya*, amphipods, isopods, cladocerans, and copepods.

DISCUSSION

This study indicates that a non-native species (*Bellamya chinensis*) does not induce a trophic cul-de-sac in lake food webs, but is accessible to fish consumers and substitutes for native snails, whose populations have declined in degraded habitats of developed lakes. We also provide evidence that the strength of the prey substitution represented by *Bellamya* depends on environmental context, such that *Bellamya* has greater effects on higher trophic levels in developed lakes where high-quality prey are less available to consumers. Overall, benthic resources were more important to food webs of undeveloped lakes than either terrestrial or pelagic resources, comprising 60-80% of fish diets. By contrast, lakes with developed shorelines had low abundances of native snails, and consumers displayed lower reliance on benthic resources and concurrently increased their use of pelagic resources. As predicted, *Bellamya* did not influence resource use in undeveloped lakes, where benthic reliance was high regardless of invasion history; however, *Bellamya* had detectable effects on the food webs of developed lakes, where fish consumed higher proportions of benthic resources in the presence of *Bellamya*, and their resource use was broadly similar to that of fish in undeveloped lakes. The dominant littoral consumer, pumpkinseed sunfish, consumed native snails and *Bellamya* as a large proportion of their diet in all lakes, but *Bellamya* was proportionally more important in developed lakes where native snails were rare.

Pumpkinseed sunfish are specialized molluscivores with pharyngeal jaws adapted for crushing snails, and their consumption increases directly with snail abundance (Wainwright et al. 1991). Our study suggests that pumpkinseed sunfish consume native snails and *Bellamya* in proportions from 30% to as high as 60% of the diet; levels that are consistent with the work of Huckins (1997) and Osenberg and Mittelbach (1989), in which snails contributed greater than 25 and 80%, respectively, of pumpkinseed diets in lakes with high snail densities. Results from our isotope mixing models suggest that *Bellamya* is a prey substitute in developed lakes, where it makes up a larger proportion of the pumpkinseed diet than native snails. *Bellamya* is larger-bodied (size range 4-65 mm shell height) than *Physella*, the most abundant native snail in these lakes (size range 1-6 mm; L. Twardochleb, *unpublished data*), and is highly visible on the surface of sediment and woody debris (L. Twardochleb, *personal observation*). Pumpkinseed sunfish have a higher encounter rate with large, visible snails and select positively for larger individuals that are more energetically dense (Osenberg and Mittelbach 1989), and therefore would be expected to have more encounters with, and consume higher proportions of *Bellamya* than native snails in developed lakes where overall snail densities are low. Although adult *Bellamya* likely reach a size refuge from predation due to pumpkinseed gape limitation, and their thick shell may confer resistance to predation, small thin-shelled juveniles (4-7 mm shell height) fall within the range of snail sizes that pumpkinseed sunfish are able to consume. For example, Mittelbach (1984) offered snails ranging from 2 to 11 mm shell length to pumpkinseed sunfish in feeding experiments, and fish consumed snails up to 1/10 of fish body length. Juvenile *Bellamya* are born during summer months when pumpkinseed feed actively on molluscs in lake littoral zones (Jokinen 1982, Stephen et al. 2013), and there is published literature to suggest that small pumpkinseeds feed on age-0 gastropods (e.g., Keast 1978). Taken together, this evidence indicates that juvenile *Bellamya* are accessible to pumpkinseed sunfish and provide a high quantity prey resource in developed lakes.

Our evidence that yellow perch consume more benthic resources in developed lakes with *Bellamya* is likely explained by the consumer's generalist feeding habits rather than a preference for snails. Yellow perch are widely considered 'secondary piscivores' that feed primarily on a variety of invertebrates in proportion to their abundance and secondarily on small fish (Liao et al. 2002, Graeb et al. 2005). Evidence from the literature indicates that perch consume snails when available; Liao et al. (2002) found that gastropods constitute one of the three most important prey sources to yellow perch in Spirit Lake, Iowa, USA, and Cobb and Watzin (1998) counted gastropods in proportions up to 25% of perch diets in Lake Champlain, Northeastern USA and Canada. In addition, we detected snails in the gut contents of more than half of the yellow perch dissected in the laboratory. Although we were unable to estimate proportions of native snails or *Bellamya* in yellow perch diets using mixing models, the preponderance of evidence suggests that perch eat snails in these lakes, and higher benthic resource use by perch in developed lakes with *Bellamya* may be due in part to the consumption of *Bellamya* as a substitute for native snails.

Bellamya also influenced benthic resource use by piscivorous largemouth bass in developed lakes, an effect that is probably mediated indirectly through prey fishes. Largemouth bass are piscivores that switch from foraging predominantly on invertebrates to fish after their first year of life (Mittelbach and Persson 1998), and display high rates of predation on pumpkinseed and bluegill sunfish (*Lepomis macrochirus*) in water-bodies where they are abundant (Olson 1996, Almeida et al. 2012). Pumpkinseed sunfish are the most abundant prey fish in our study lakes, and they display predominantly benthic isotopic signatures (Fig. 2, 3), with higher benthic resource use in developed lakes with *Bellamya* than in those without. The elevated benthic signature of pumpkinseed should be reflected in the isotopic signatures of their predator, largemouth bass, and consequently, our evidence that largemouth bass increase their use of benthic resources in developed/*Bellamya* lakes suggests that *Bellamya*'s effects may be transmitted up though the food web from prey fish to top consumers.

Consumers that exploit non-native prey can increase their fitness and provide biotic resistance against the population growth and spread of non-native species (Carlsson et al. 2009). King et al. (2006) showed that the threatened Lake Erie Water Snake (*Nerodia sipedon insularum*) increased their growth and body size after elevating their feeding on non-native round goby (*Neogobius melanostomus*). In addition to improving their fitness, consumers that are effective at feeding on non-native prey can regulate impacts of non-native species (Carlsson et al. 2009). For example, Carlsson et al. (2011) have found that native blue crabs (*Callinectes sapidus*) exert strong predation pressure on non-native zebra mussels (*Dreissena polymorpha*) in the Hudson River estuary, and as a result, the ecosystem shows signs of recovery from intensive zebra mussel grazing. By controlling populations of non-native prey, consumers may prevent the negative ecosystem-level impacts of non-native species in lakes.

Benthic energetic pathways are critical to the maintenance of higher trophic level consumers in lakes, and our study sheds light on the role a non-native species plays in helping maintain consumer access to benthic resources in degraded food webs. Our isotopic evidence revealed that food webs of undeveloped lakes were supported primarily by benthic pathways, which is consistent with a review by Vadeboncoeur et al. (2003) highlighting that zoobenthic prey constitute over half the diet of North American fishes, while piscivores derive 65% of their diets, both directly and indirectly, from benthic prey. In addition, Hecky and Hesslein (1995) used carbon isotopes to show that globally, fish assimilate 50% of their carbon from periphyton-based food chains. In small, shallow lakes, for which benthic production dominates, urban development reduces the efficiency of trophic transfers from benthic primary producers to higher trophic-level consumers and alters the balance of benthic and pelagic resources supporting lakes (Vadeboncoeur et al. 2003, Brauns et al. 2011). We found that development significantly reduced the contributions of benthic resources to consumers, which can have substantial food-web level consequences, including lower growth rates of fish and reduced benthic-pelagic habitat coupling that supports production of pelagic fish (Schindler et al. 2000, Schindler and Scheuerell 2002). However, our results also suggest that non-native prey can ameliorate some of these common, negative effects of development. Snails have high energy densities compared to other invertebrates, and fish that consume snails gain growth and competitive advantages over fish that do not (Mittelbach 1984). For example, pumpkinseed sunfish reach larger sizes and achieve higher population sizes in lakes where they can access snails (Huckins 1997). By providing a high-quality prey resource for fish in lakes with degraded benthic pathways, *Bellamya* may help maintain growth and population sizes of consumers.

Our study relied on stable isotope mixing models to estimate the importance of resource pools to food webs. Mixing models can provide a range of estimates for a finite number of individual prey contributions to consumer diets (Fry 2006), and as a result we were unable to quantify the proportional contribution of *Bellamya* to consumers such as yellow perch or largemouth bass that were expected *a priori* to consume a wide range of dietary items. However, the inclusion of hydrogen isotopes allowed us to effectively discriminate among benthic, pelagic, and terrestrial resource use by consumers (Doucett et al. 2007), and thus make inferences about how a non-native species influences broad patterns of ecosystem functioning. Future research would benefit from the integration of isotopic data with traditional diet data and emerging fatty acid analyses to allow for more detailed inferences about contributions of non-native species to consumer diets.

Non-native species are often assumed to have consistent, negative effects on ecosystems (e.g., Simberloff 2011, Ricciardi 2013), whereas their emerging positive roles in contemporary landscapes are under recognized (Schlaepfer et al. 2011). We did not find evidence that *Bellamya* induced trophic cul-de-sacs; in the contrary our results show that *Bellamya* had neutral or beneficial effects on lake food webs. Further, our study highlights that *Bellamya*'s importance to the food web was not consistent across a gradient of shoreline development. *Bellamya* did not play an important role in the dominant energetic pathway of undeveloped lakes, likely because native prey were abundant and available to consumers; however, *Bellamya* was an important resource in developed lakes with effects that were evident in higher trophic level consumers. Other studies have found evidence that non-native New Zealand mud snails are indigestible to rainbow trout (Vinson and Baker 2008) and induce trophic cul-de-sacs in streams (Moore et al. 2012); yet Hellmair et al. (2011) showed that endangered tidewater goby, *Eucycloglobius newberyi*, consume and digest New Zealand mud snails in estuaries, and the proportion of gobies consuming mud snails fluctuates throughout the year. These contrasting lines of evidence indicate that extrinsic factors, such as type of ecosystem, competitors, and predators in the environment, and time of year are important for determining the roles, both positive and negative, of non-native species in present-day food webs (Schlaepfer et al. 2011, Jeschke et al. 2014). Our evidence that a non-native species can ameliorate some effects of environmental degradation suggests that researchers and managers should consider the bidirectional effects of non-native species.

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Table 1. Physical characteristics, including lake surface area, mean depth, Secchi depth, a measure of water clarity, and mean epilimnetic total phosphorus concentrations for lakes in each of four sampling categories.

Lake	Surface area (km^2)	Mean depth (m)	Secchi depth (m)	Total phosphorus ($\mu g\ L^{-1}$)
Undeveloped- *Bellamya* absent				
Fern	0.10	4.6	4.1	10.7
Langlois	0.39	16.2	6.3	9.8
Walsh	0.43	5.2	3.2	8.3
Undeveloped- *Bellamya* present				
Cascade	0.68	8.2	5.8	15.4
Padden	0.64	8.3	3.2	7.5
Wilderness	0.28	6.4	3.2	12.5
Developed- *Bellamya* absent				
Martha	0.23	7.3	4.2	10.1
Shoecraft	0.53	5.5	3.6	8.3
Sunday	0.19	2.4	1.9	27.9
Developed-*Bellamya* present				
Angle	0.42	7.6	6.5	7.7
Pine	0.35	6.1	5.5	8.2
Star	0.34	7.6	3.1	9.8

Table 2. Mean and standard deviation of the mean (SD) isotopic signatures for non-native *Bellamya* and native *Physella* snails, and results of Welch two-sample t-tests for differences in their isotopic signatures.

	Bellamya		*Physella*				
Isotopic signature	Mean	SD	Mean	SD	t-statistic	df	*P*
$\delta^{13}C$ (‰)	-26.59	2.15	-22.50	2.45	-4.08	12.04	< 0.01
$\delta^{15}N$ (‰)	5.90	0.63	4.36	1.31	3.16	8.48	0.01
$\delta^{2}H$ (‰)	-152.50	10.90	-129.10	11.22	-4.96	13.17	< 0.01

Table 3. Summary of AICc model selection on three ANOVA models that explain differences in benthic resource use by consumers among lake categories. K is the number of model parameters; $\Delta AIC_c = AIC_c$ of the model - AIC_c minimum of all models under consideration; w_i is the probability that the model is the best of all models under consideration; and the evidence ratio is the weight of the best fit model divided by the weight of the model under consideration. For pumpkinseed sunfish, largemouth bass, and yellow perch, the best-supported models include additive effects of the categorical variables, *Bellamya* presence and lakeshore development.

Model	**K**	**AIC_c**	**ΔAIC_c**	**w_i**	**Evidence ratio**
Pumpkinseed sunfish					
Bellamya presence + development	3	141.0	0.0	1.0	1.0
Bellamya presence	2	151.5	10.5	< 0.01	187.7
Development	2	175.7	34.7	< 0.01	3.5e+7
Yellow perch					
Bellamya presence + development	3	230.4	0.0	1.0	1.0
Development	2	251.9	21.5	< 0.0001	4.7e+4
Bellamya presence	2	258.7	28.3	< 0.0001	1.4e+6
Largemouth Bass					
Bellamya presence + development	3	462.3	0.0	1.0	1.0
Bellamya presence	2	518.6	56.3	< 0.0001	1.7e+12
Development	2	542.9	80.6	< 0.0001	3.2e+17

Table 4. Summary of one-way ANOVAs with binomial error structure testing for effect of lake development on proportion of native snails and *Bellamya* in the diets of pumpkinseed sunfish. Lake development significantly decreased the proportion of native snails in fish diets, while development significantly increased the proportion of *Bellamya* in fish diets. *Z* scores and *P* values indicate whether a parameter coefficient is different from zero.

Model	**Coefficient**	**Estimate**	**SE**	***Z***	***P***
Native snails consumed	(Intercept)	-1.09	0.14	-7.69	<0.01
	Lake development	-1.94	0.20	-9.58	<0.01
Bellamya consumed	(Intercept)	-1.92	0.16	-11.78	<0.01
	Lake development	0.64	0.27	2.32	0.02

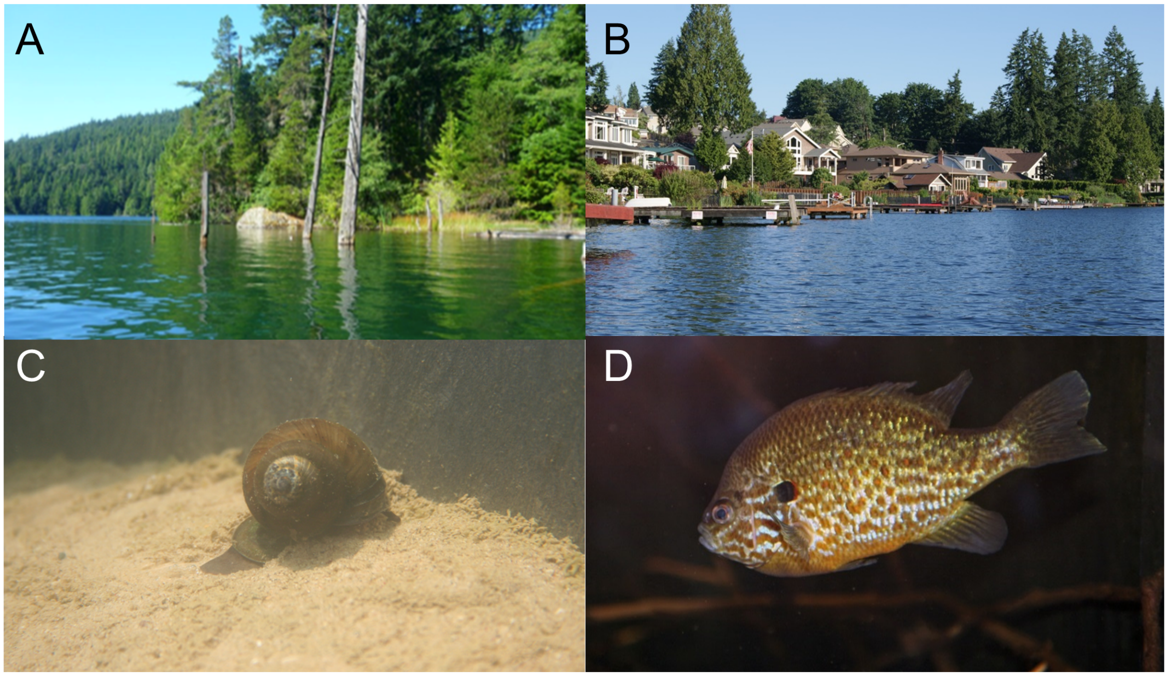


Figure 1. A) Undeveloped Cascade Lake, Orcas County, WA (L. Twardochleb); B) developed Pine Lake, King County, WA (S. Linzmaier); C) non-native Chinese mystery snail, *Bellamya chinensis* (J. Olden); D) molluscivorous Pumpkinseed sunfish, *Lepomis gibbosus* (Wikimedia Commons, M. Manske).

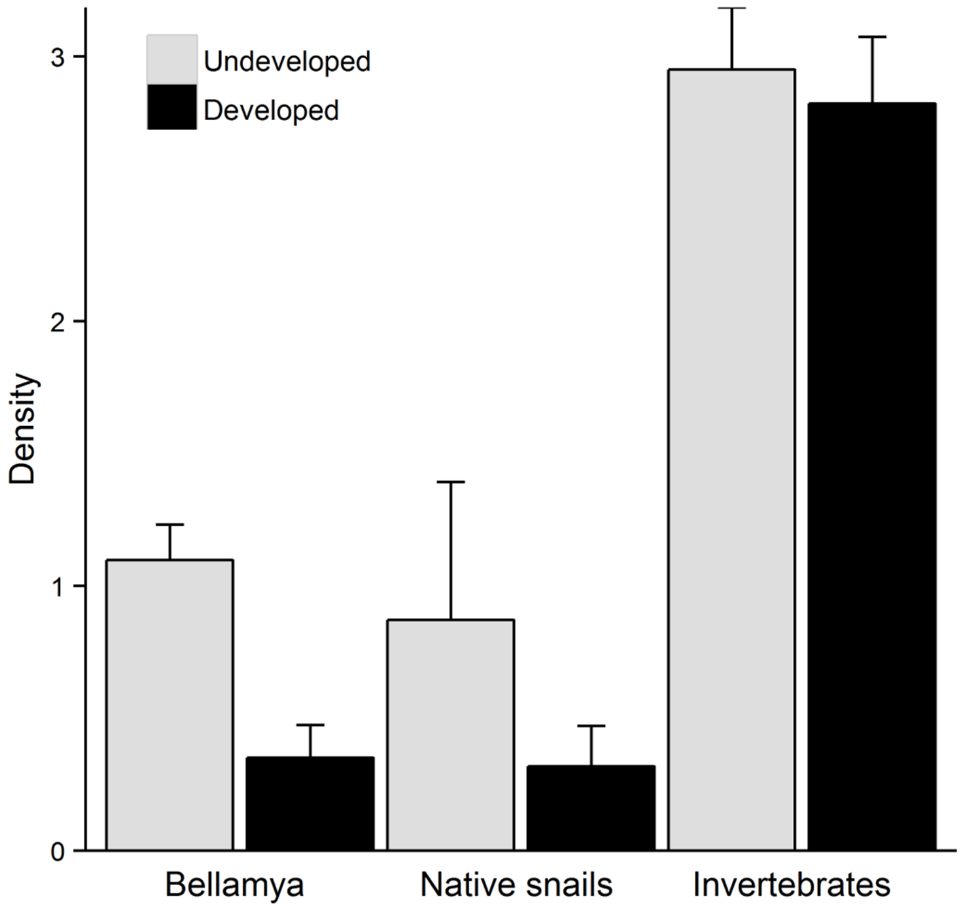


Figure 2. Densities (log + 1 m^{-2}) of *Bellamya*, native snails, and non-molluscan invertebrates in undeveloped and developed lakes (n = 6 lakes per category). Error bars represent 1 standard deviation of the mean.

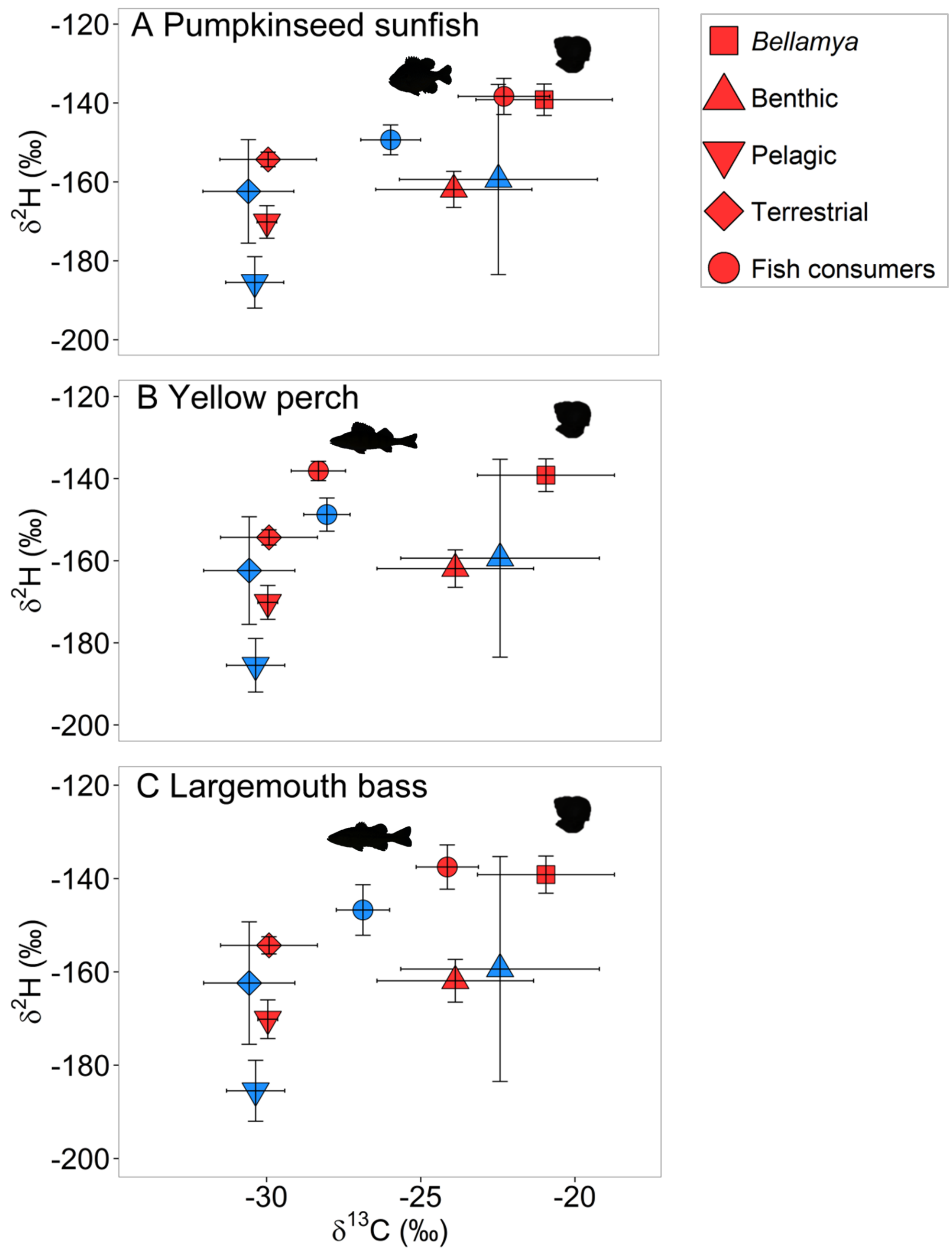


Figure 3. Source and consumer $\delta^{13}C$ and $\delta^{2}H$ signatures in developed lakes Martha (*Bellamya* absent; blue symbols) and Pine (*Bellamya* present; red symbols) for A) pumpkinseed sunfish; B) yellow perch; and C) largemouth bass. Note the enrichment in ^{13}C and ^{2}H for pumpkinseed sunfish and largemouth bass in the *Bellamya* compared to the non-*Bellamya* lake.

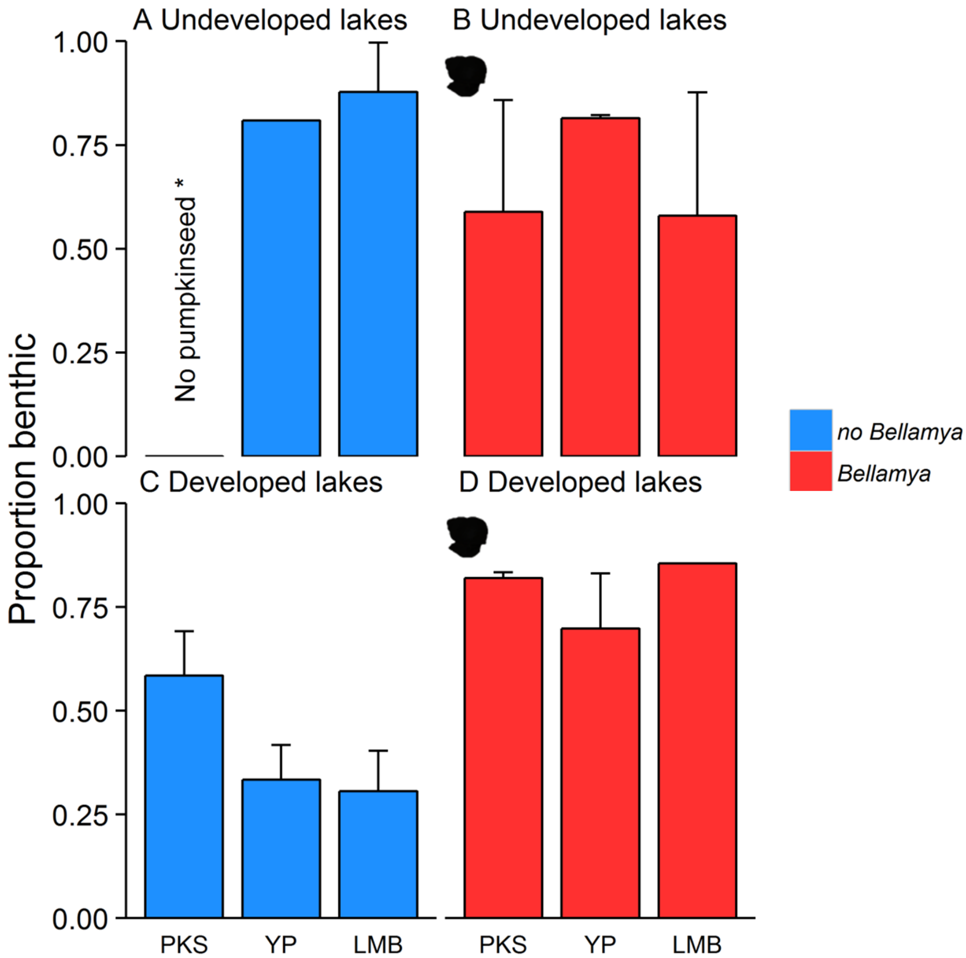


Figure 4. Benthic resource use was high among pumpkinseed sunfish (PKS), yellow perch (YP), and largemouth bass (LMB) in undeveloped lakes A) with and B) without populations of non-native *Bellamya*. Benthic resource use was much lower for all fish in C) developed lakes without *Bellamya* compared to undeveloped lakes. However, benthic resources comprised the majority of fish diets in D) developed lakes with *Bellamya*. Bars are mean ± 1 SD estimated proportion of fish diets composed of benthic prey for each lake category. *Note that there are no populations of pumpkinseed sunfish in undeveloped lakes without *Bellamya*.

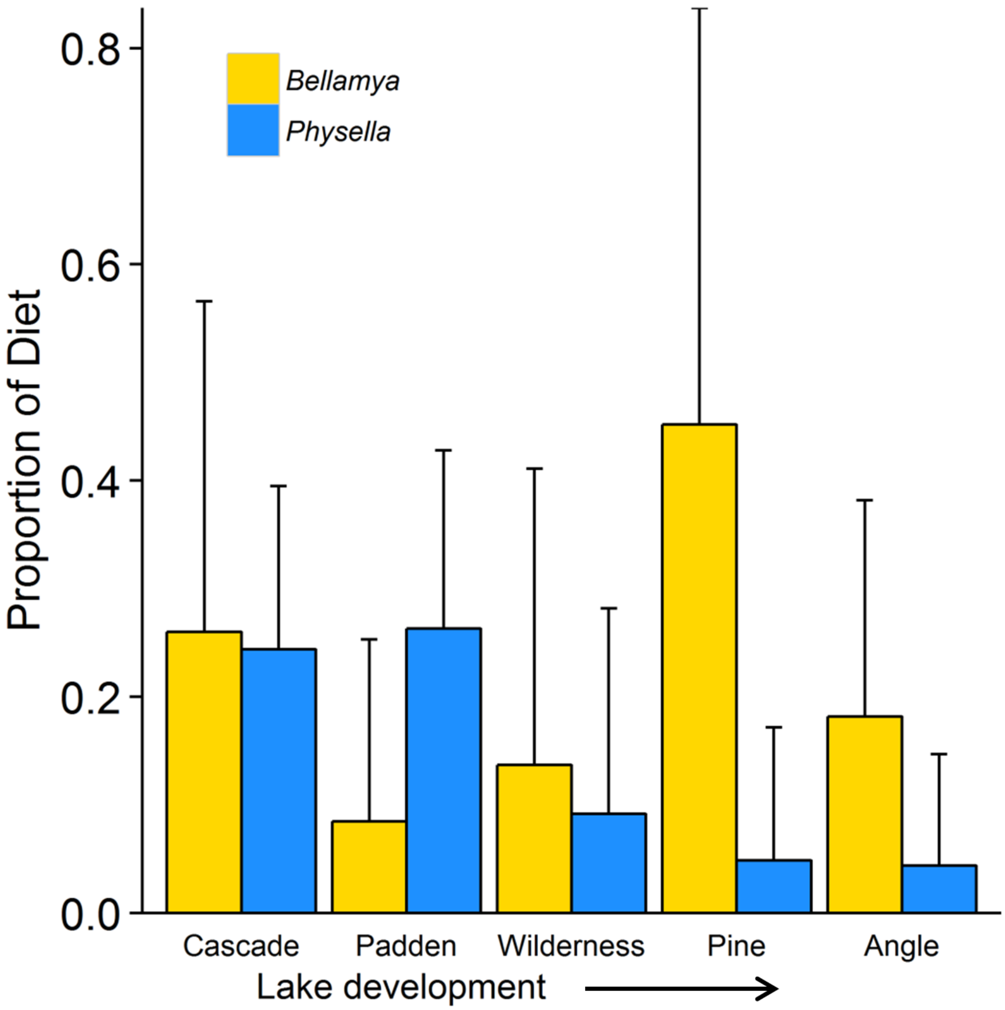


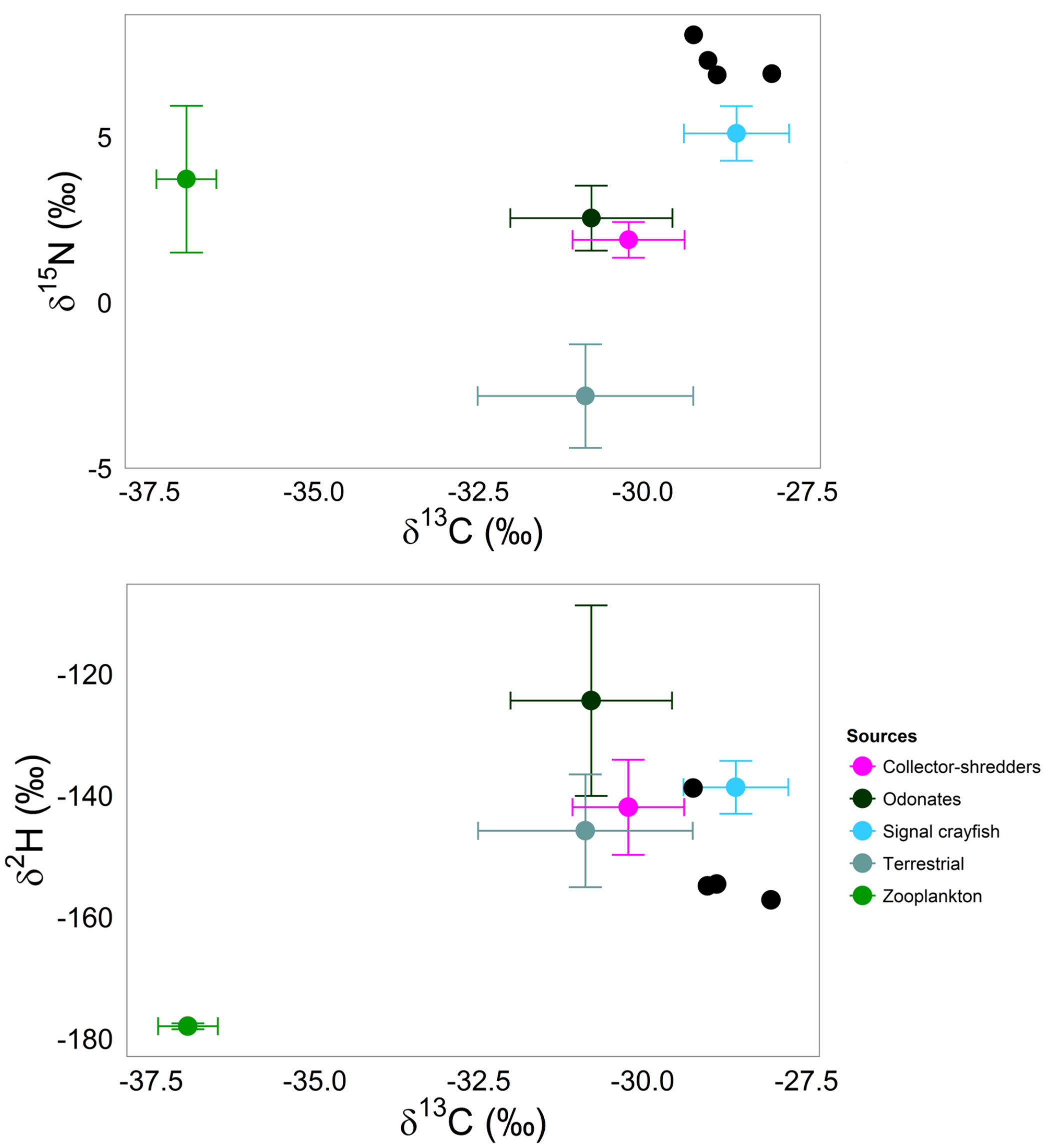
Figure 5. Pumpkinseed sunfish consumed *Bellamya* in higher proportions in developed (Pine, Angle) than undeveloped (Cascade, Padden, Wilderness) lakes and native snails, *Physella*, in higher proportions in undeveloped lakes. Bars show mean + 95% credible intervals for the proportion of the diet composed of *Bellamya* and native snails based on outputs of three-isotope mixing models.

Appendix 1

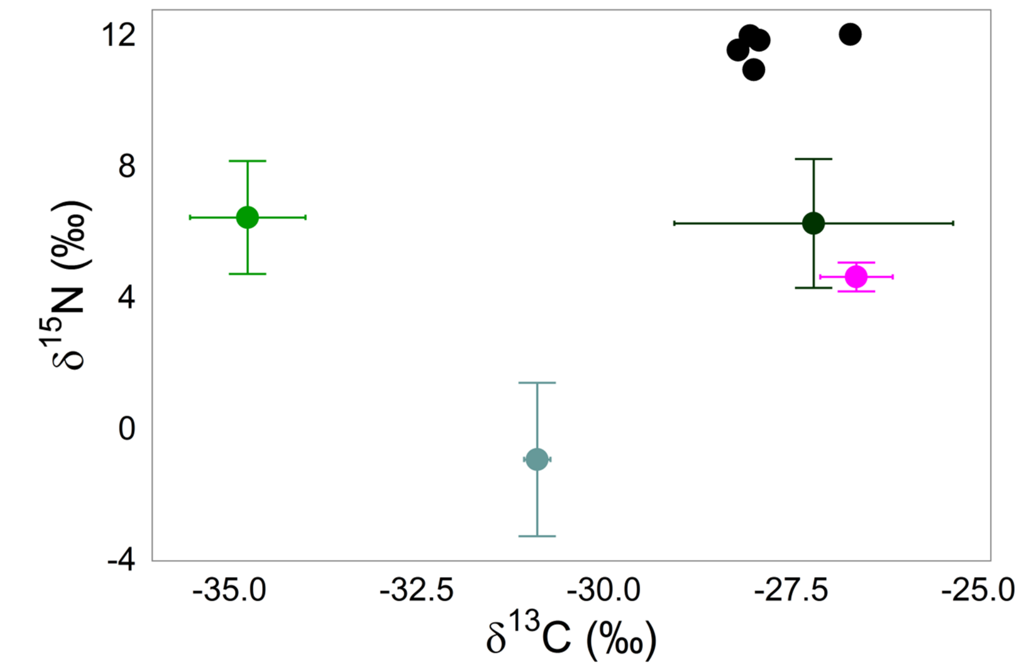
Biplots of ^{13}C, ^{15}N, and ^{2}H isotopic signatures for sources and consumers in each lake. Pumpkinseed sunfish are represented by squares, yellow perch by triangles, and largemouth bass by circles.

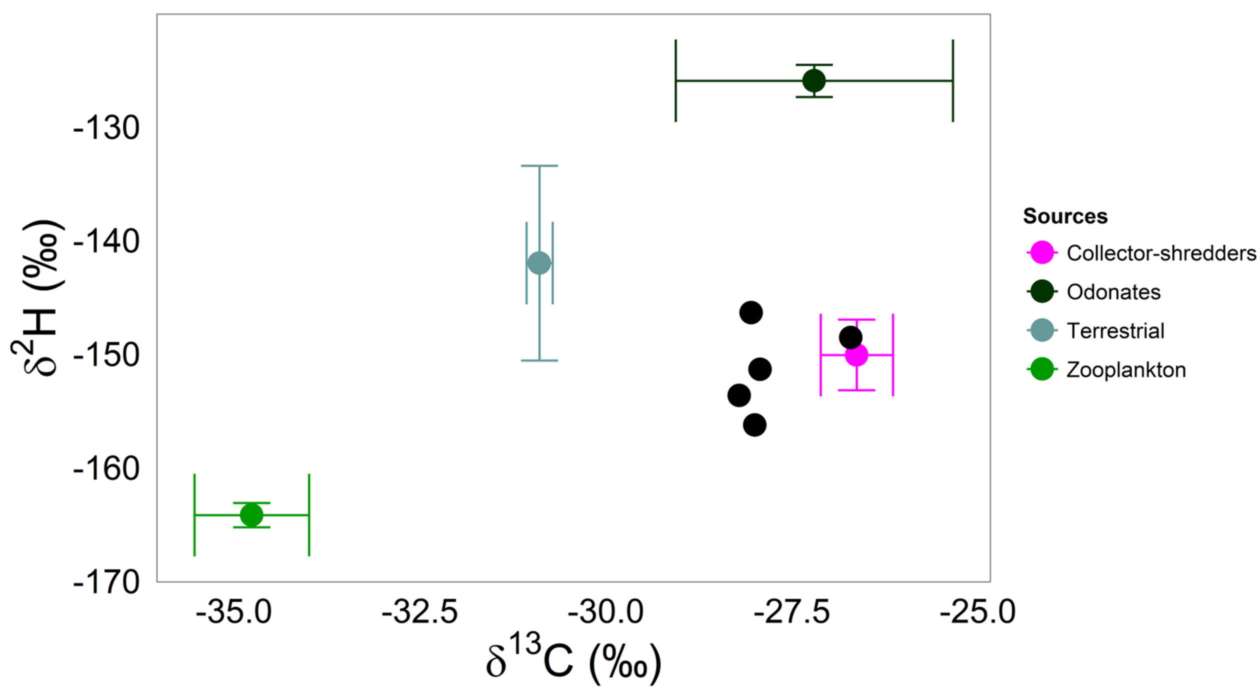
<u>Undeveloped/non-*Bellamya* lakes</u>

Fern Lake

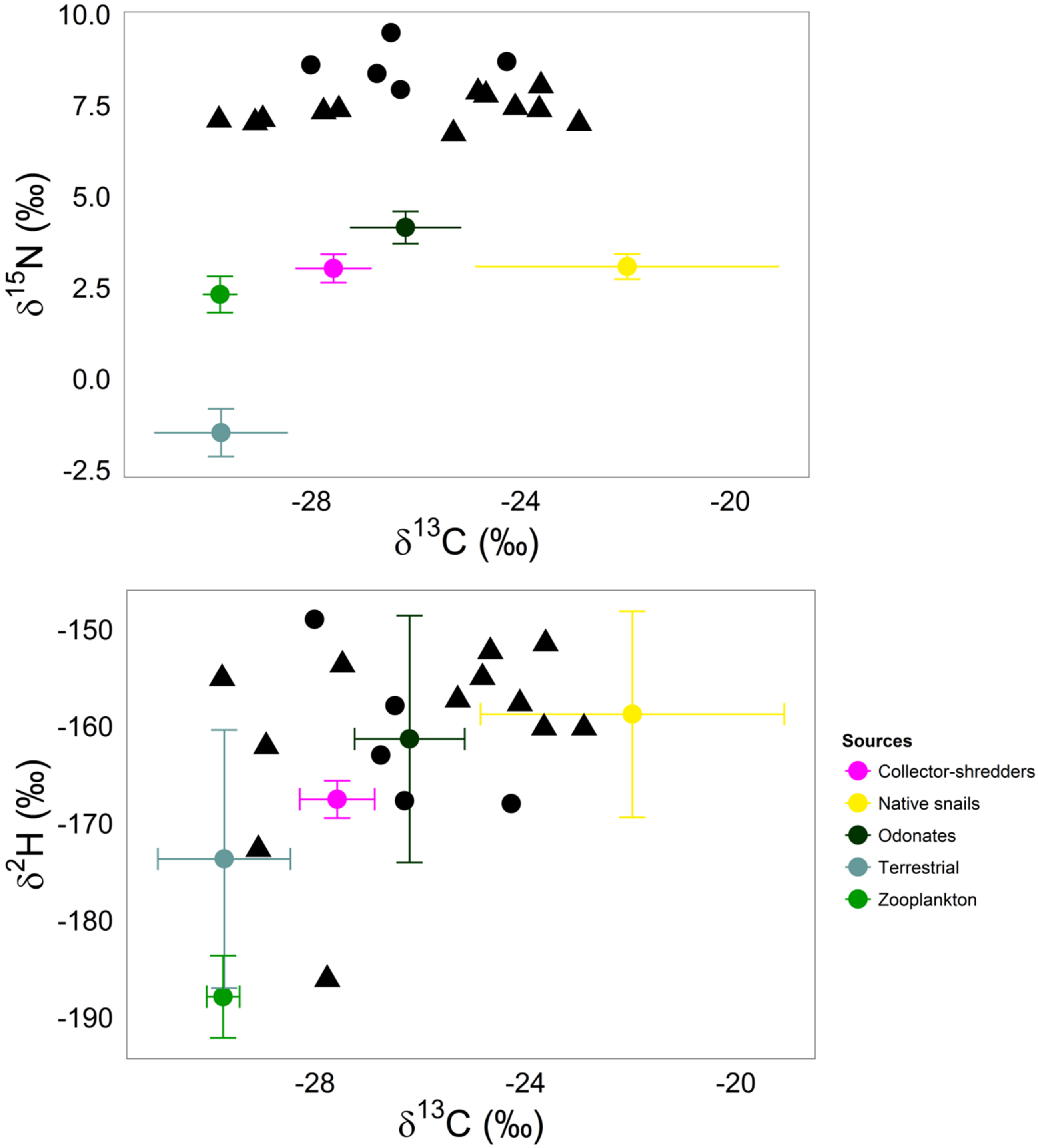


Langlois Lake



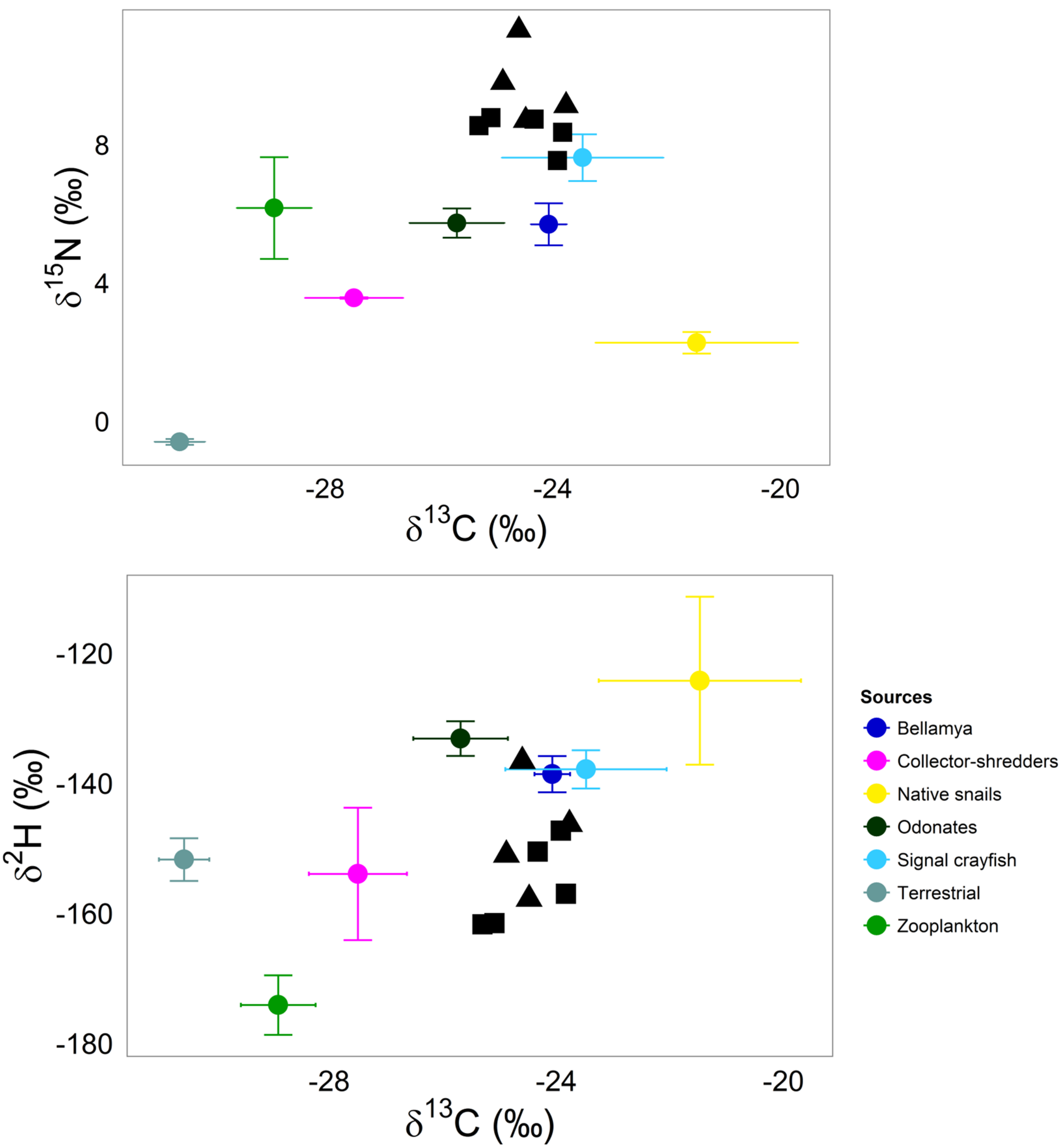


Walsh Lake

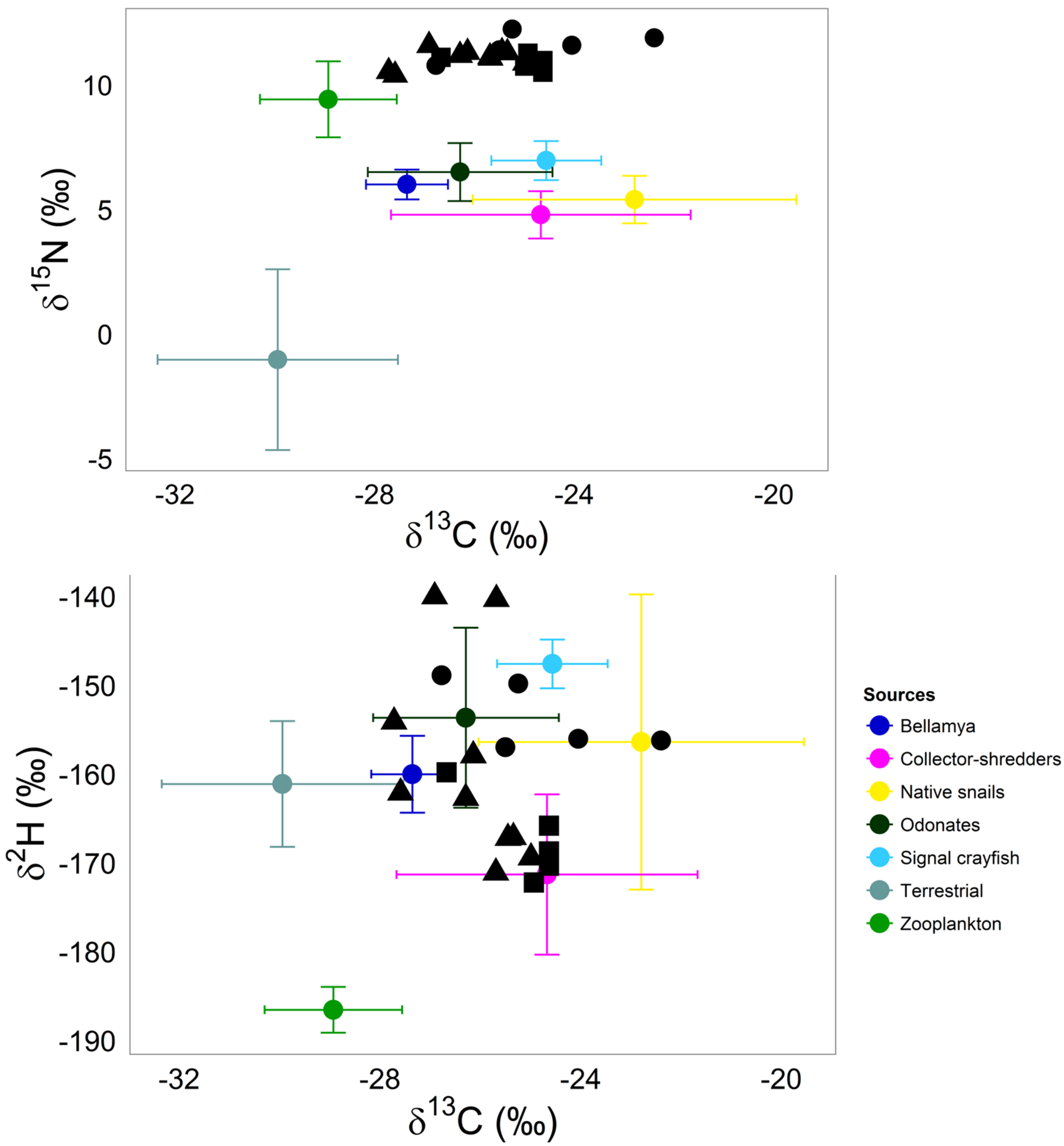

10.0
7.5
5.0
2.5
0.0
-2.5
$\delta^{15}N$ (‰)
-28
-24
-20
$\delta^{13}C$ (‰)
-150
-160
-170
-180
-190
$\delta^{2}H$ (‰)
-28
-24
-20
$\delta^{13}C$ (‰)
Sources
Collector-shredders
Native snails
Odonates
Terrestrial
Zooplankton

<u>Undeveloped/*Bellamya* lakes</u>

Cascade Lake

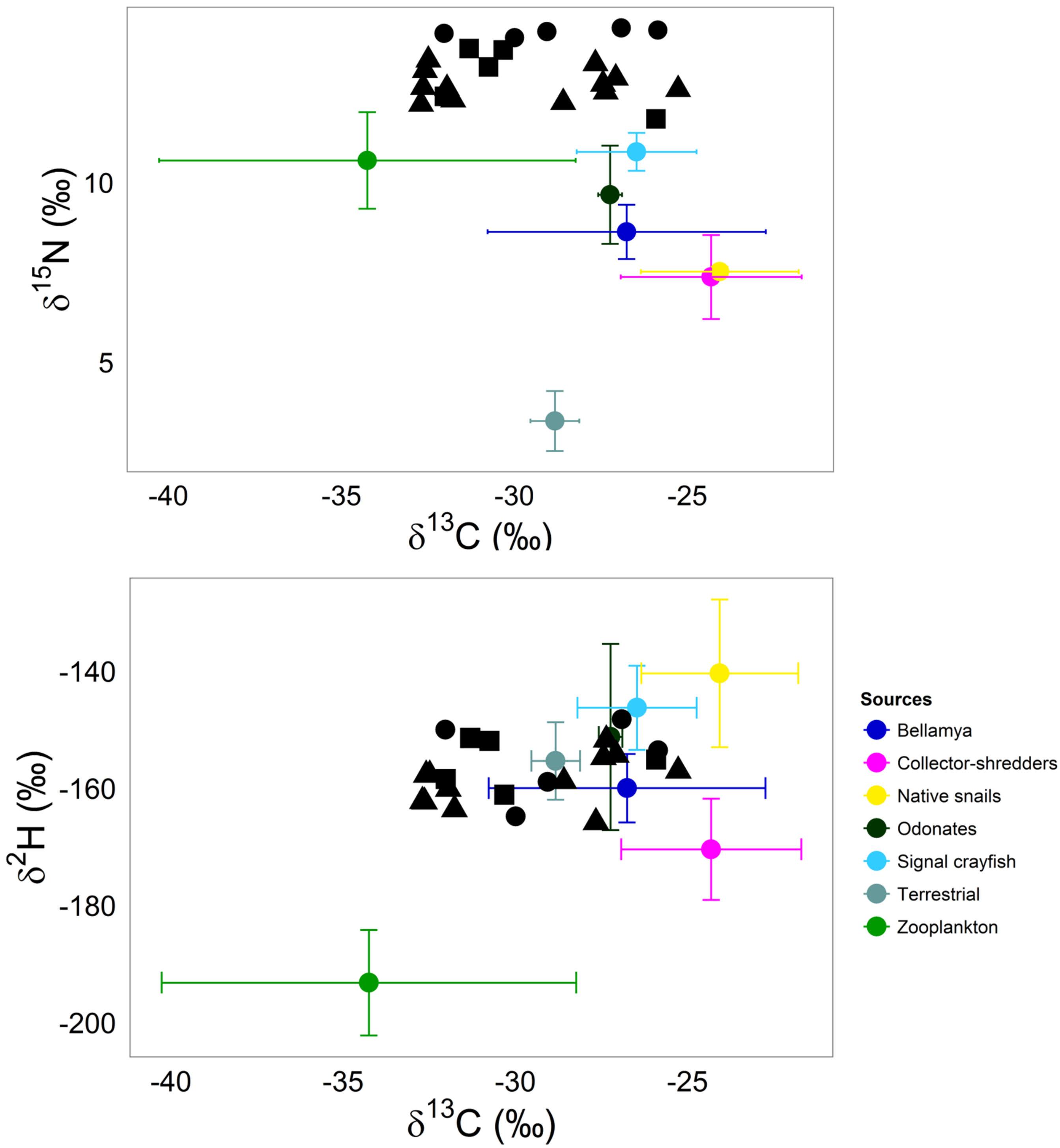


Lake Padden



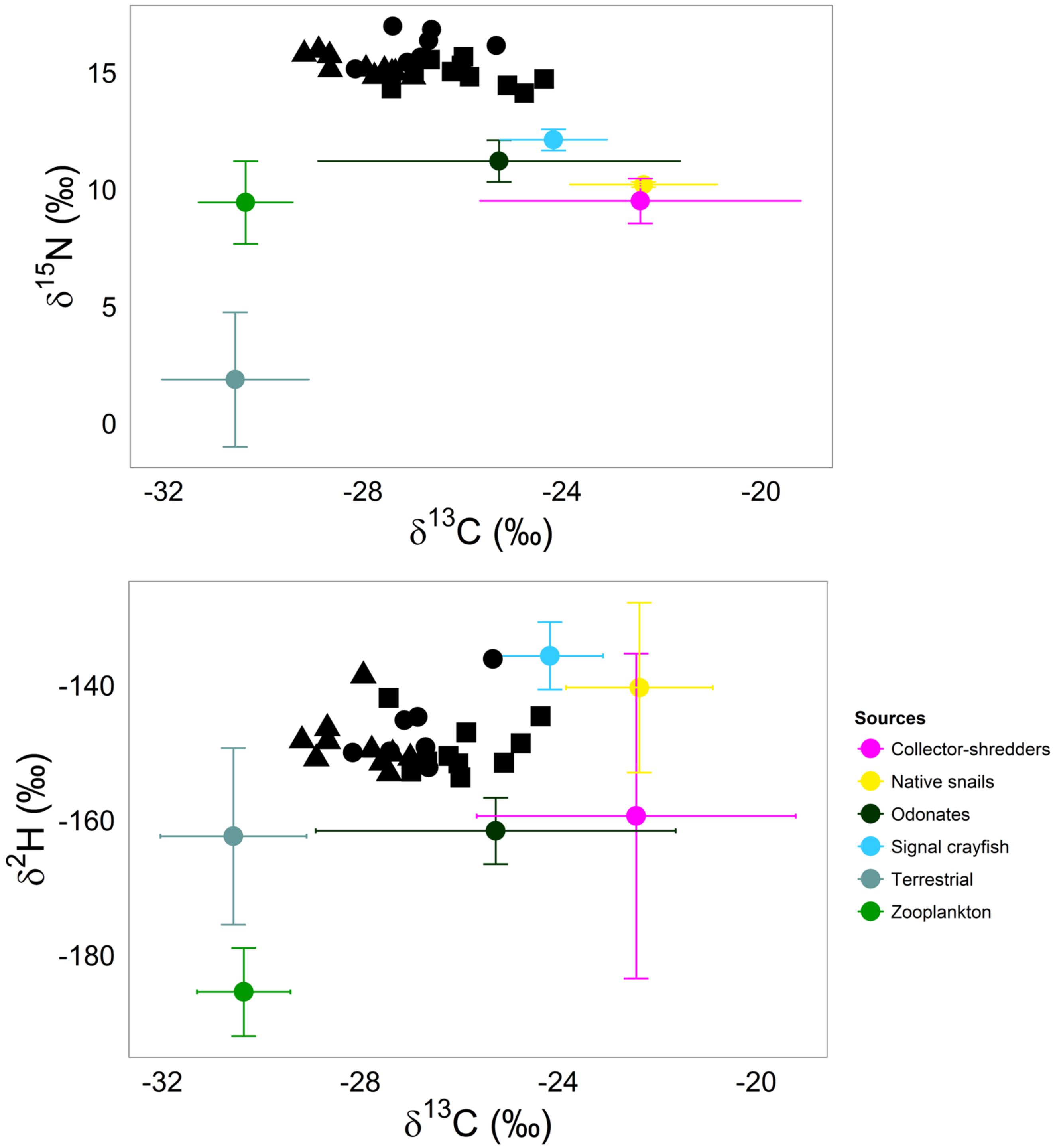
10
5
0
-5
δ15N (‰)
-32
-28
-24
-20
δ13C (‰)
-140
-150
-160
-170
-180
-190
δ2H (‰)
Sources
Bellamya
Collector-shredders
Native snails
Odonates
Signal crayfish
Terrestrial
Zooplankton

Lake Wilderness

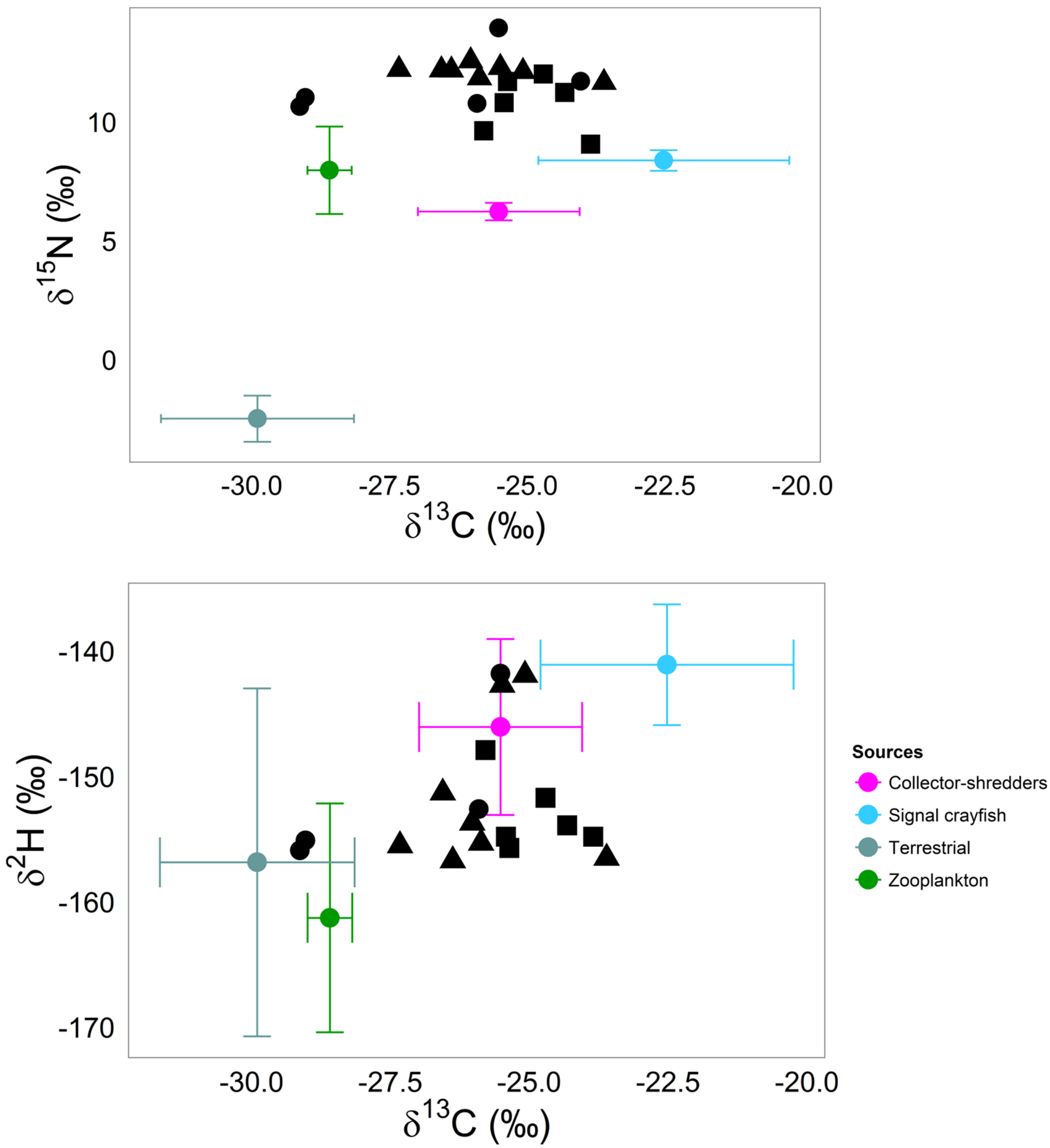


<u>Developed/non-*Bellamya* lakes</u>

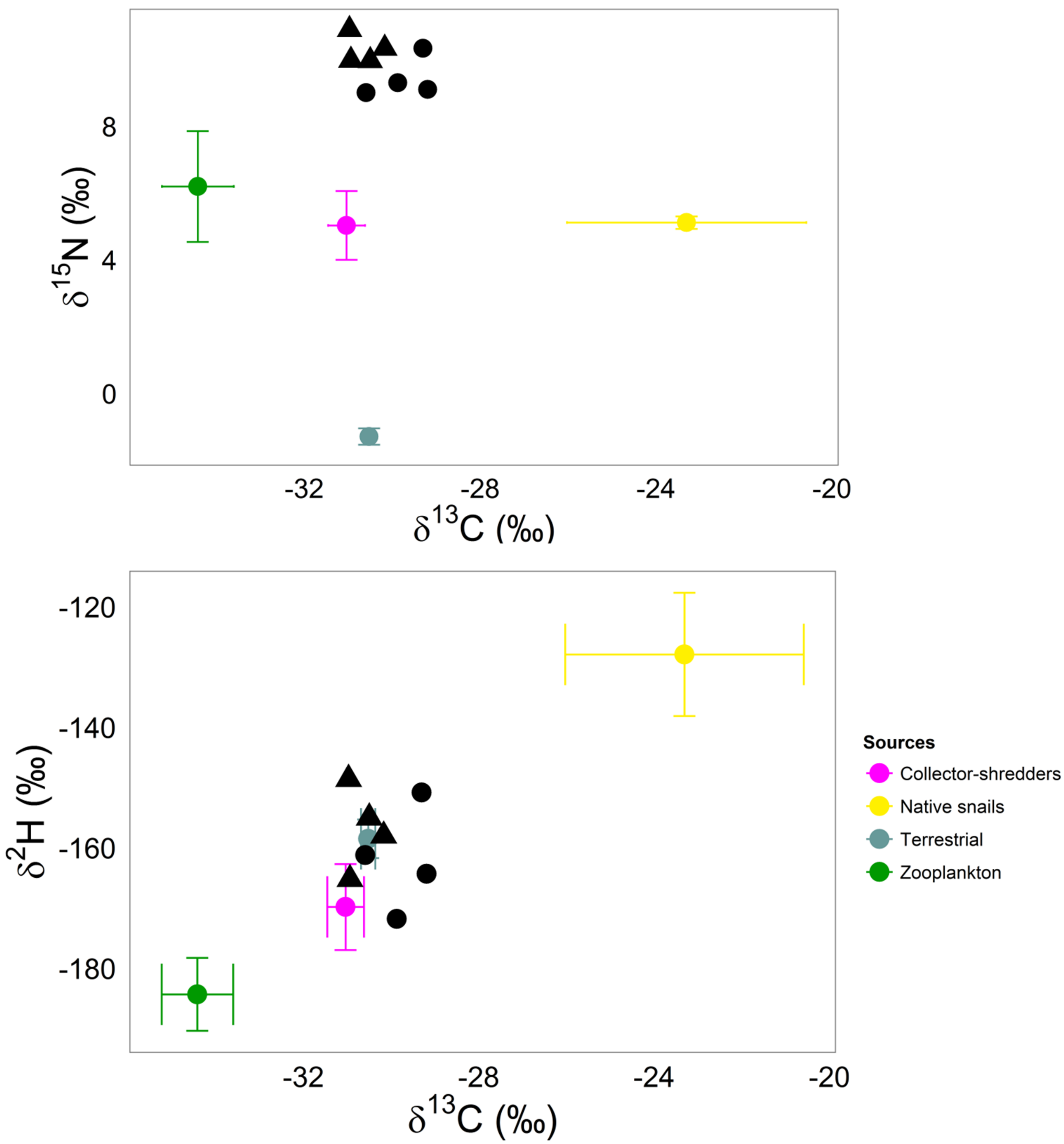
Martha Lake



Shoecraft Lake



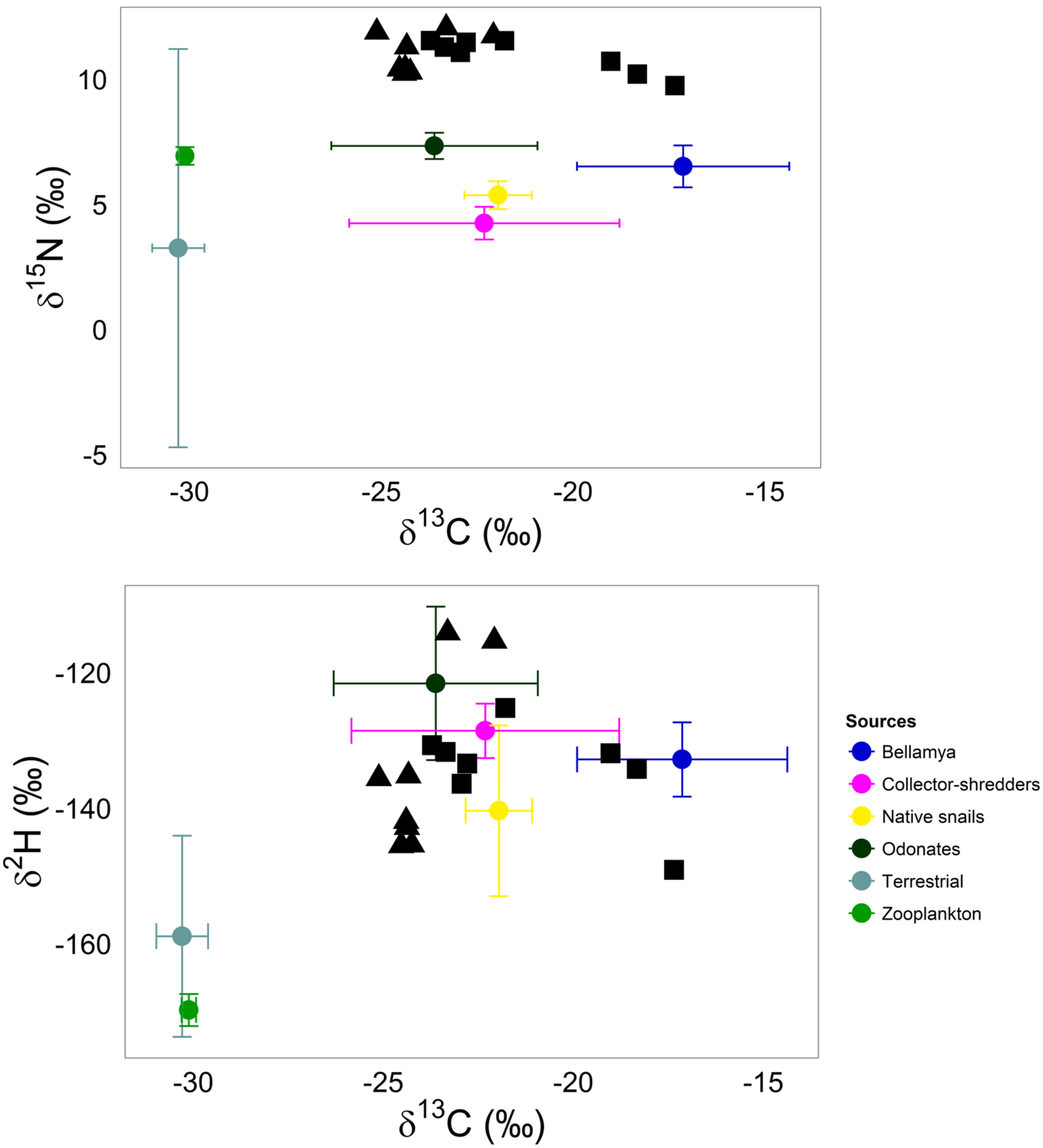
Sunday Lake



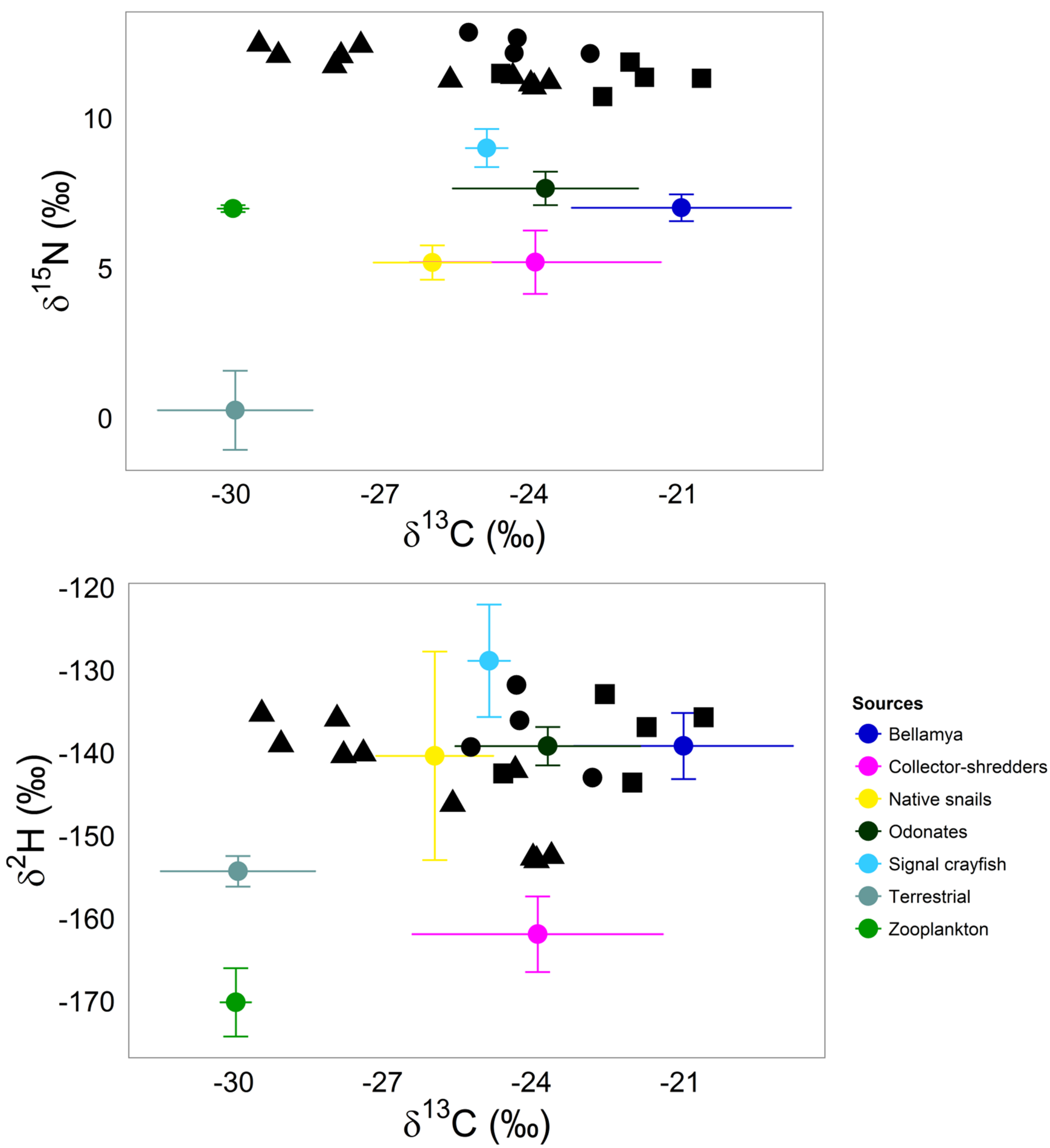
8
4
0
$\delta^{15}N$ (‰)
-32
-28
-24
-20
$\delta^{13}C$ (‰)
-120
-140
-160
-180
$\delta^{2}H$ (‰)
-32
-28
-24
-20
$\delta^{13}C$ (‰)
Sources
Collector-shredders
Native snails
Terrestrial
Zooplankton

Developed/*Bellamya* lakes

Angle Lake

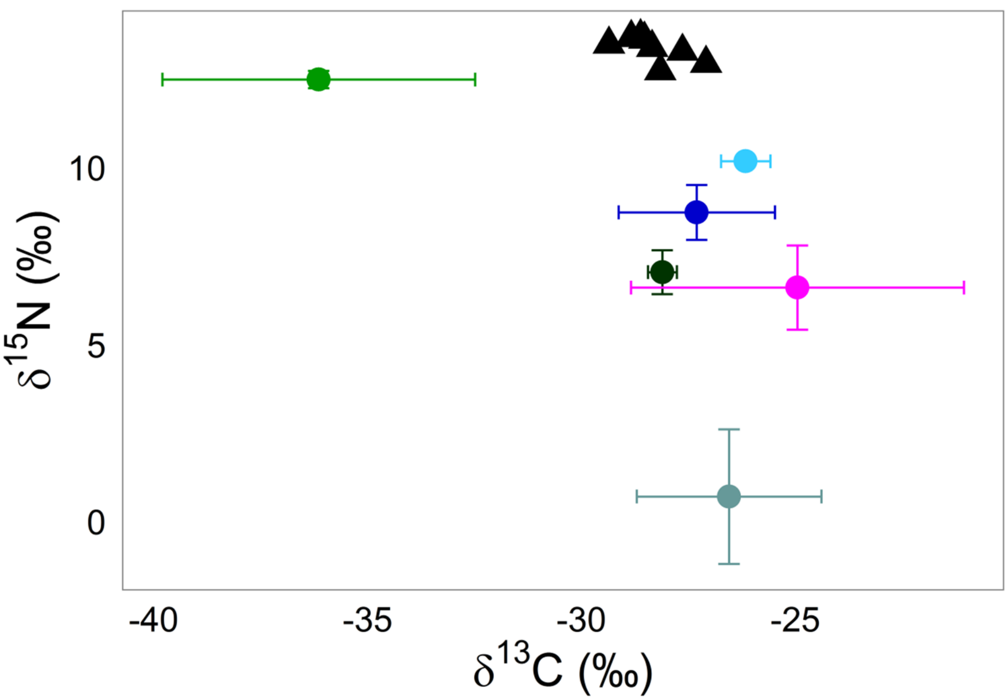


Pine Lake

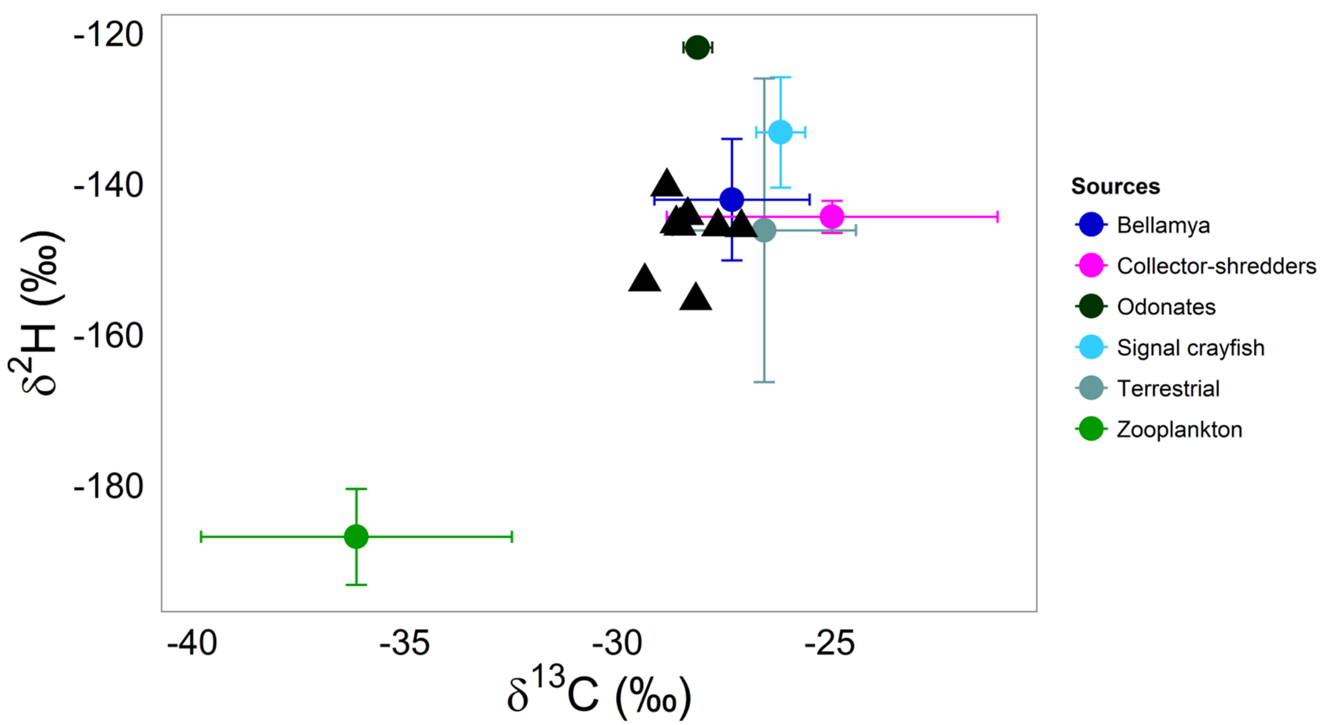


10
5
0
-30
-27
-24
-21
$\delta^{15}N$ (‰)
$\delta^{13}C$ (‰)
-120
-130
-140
-150
-160
-170
$\delta^{2}H$ (‰)
Sources
Bellamya
Collector-shredders
Native snails
Odonates
Signal crayfish
Terrestrial
Zooplankton

Star Lake



10
5
0
-40
-35
-30
-25
$\delta^{15}N$ (‰)
$\delta^{13}C$ (‰)



-120
-140
-160
-180
-40
-35
-30
-25
$\delta^{2}H$ (‰)
$\delta^{13}C$ (‰)
Sources
Bellamya
Collector-shredders
Odonates
Signal crayfish
Terrestrial
Zooplankton

Appendix 2

Tables of mean and 95% credible intervals from MixSIR model runs estimating contributions of resources from each habitat to fish consumers

Undeveloped/Non-*Bellamya* Lakes

Fern Lake

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.97	0.02	0.92	0.96	0.97	0.99	>0.99
Pelagic	0.02	0.01	0.00	0.01	0.01	0.02	0.05
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.02	0.05

Langlois Lake

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.74	0.08	0.61	0.69	0.74	0.79	0.91
Pelagic	0.25	0.08	0.08	0.20	0.25	0.30	0.39
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.01	0.03

Walsh Lake

Yellow perch

Source	Mean	Standard	2.5%	25%	50%	75%	97.5%

	Proportion	Deviation					
Benthic	0.81	0.08	0.65	0.75	0.81	0.87	0.95
Pelagic	0.17	0.08	0.16	0.11	0.17	0.23	0.33
Terrestrial	0.02	0.02	0.00	0.01	0.02	0.03	0.08

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.92	0.05	0.79	0.89	0.93	0.96	0.99
Pelagic	0.06	0.02	0.00	0.02	0.05	0.09	0.19
Terrestrial	0.02	0.05	0.00	0.01	0.02	0.03	0.07

<u>Undeveloped/*Bellamya* Lakes</u>

Cascade Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.88	0.07	0.72	0.83	0.88	0.93	0.98
Pelagic	0.09	0.08	0.00	0.04	0.08	0.13	0.25
Terrestrial	0.03	0.03	0.00	0.01	0.03	0.05	0.11

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%

Benthic	0.90	0.06	0.76	0.87	0.91	0.95	0.99
Pelagic	0.08	0.06	0.00	0.03	0.06	0.11	0.23
Terrestrial	0.02	0.02	0.00	0.01	0.02	0.03	0.07

Lake Padden

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.55	0.08	0.39	0.49	0.55	0.60	0.72
Pelagic	0.42	0.08	0.26	0.37	0.42	0.47	0.57
Terrestrial	0.03	0.02	0.00	0.01	0.02	0.04	0.09

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.68	0.06	0.57	0.64	0.67	0.72	0.82
Pelagic	0.31	0.06	0.18	0.27	0.31	0.35	0.42
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.02	0.04

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.52	0.09	0.35	0.46	0.52	0.57	0.70
Pelagic	0.46	0.09	0.28	0.41	0.47	0.52	0.62

Terrestrial	0.02	0.02	0.00	0.01	0.02	0.03	0.07

Wilderness Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.34	0.14	0.06	0.24	0.35	0.44	0.61
Pelagic	0.57	0.12	0.35	0.49	0.57	0.65	0.80
Terrestrial	0.09	0.06	0.01	0.04	0.08	0.13	0.23

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.37	0.10	0.17	0.31	0.38	0.44	0.54
Pelagic	0.53	0.07	0.40	0.48	0.53	0.58	0.68
Terrestrial	0.10	0.05	0.01	0.06	0.10	0.13	0.20

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.32	0.17	0.03	0.19	0.32	0.44	0.66
Pelagic	0.64	0.16	0.32	0.53	0.65	0.76	0.92
Terrestrial	0.04	0.03	0.00	0.01	0.03	0.05	0.13

Developed/Non-*Bellamya* Lakes

Martha Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.50	0.09	0.36	0.44	0.49	0.55	0.72
Pelagic	0.49	0.09	0.27	0.44	0.50	0.55	0.63
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.02	0.04

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.24	0.06	0.12	0.20	0.24	0.28	0.37
Pelagic	0.75	0.01	0.62	0.71	0.75	0.79	0.87
Terrestrial	0.01	0.06	0.00	0.00	0.01	0.02	0.04

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.38	0.16	0.20	0.29	0.34	0.40	0.92
Pelagic	0.62	0.16	0.06	0.59	0.65	0.70	0.79
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.01	0.03

Shoecraft Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.23	0.10	0.06	0.22	0.29	0.35	0.46
Pelagic	0.71	0.10	0.52	0.63	0.70	0.77	0.93
Terrestrial	0.02	0.01	0.00	0.01	0.01	0.02	0.05

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.39	0.09	0.18	0.34	0.39	0.45	0.54
Pelagic	0.61	0.09	0.46	0.55	0.60	0.66	0.81
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.01	0.03

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.24	0.15	0.45	0.64	0.75	0.86	0.97
Pelagic	0.74	0.15	0.01	0.11	0.23	0.35	0.54
Terrestrial	0.02	0.02	0.00	0.01	0.02	0.03	0.08

Sunday Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%

Benthic	0.54	0.12	0.34	0.46	0.53	0.62	0.82
Pelagic	0.42	0.12	0.15	0.35	0.43	0.50	0.61
Terrestrial	0.04	0.03	0.00	0.02	0.03	0.06	0.13

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.38	0.10	0.21	0.31	0.37	0.48	0.60
Pelagic	0.59	0.10	0.38	0.53	0.59	0.65	0.75
Terrestrial	0.03	0.03	0.00	0.01	0.03	0.05	0.11

<u>Developed/*Bellamya* Lakes</u>

Angle Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.81	0.08	0.67	0.76	0.81	0.86	0.95
Pelagic	0.14	0.08	0.01	0.07	0.13	0.20	0.30
Terrestrial	0.05	0.05	0.00	0.02	0.04	0.08	0.19

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.81	0.10	0.59	0.75	0.82	0.88	0.96

Pelagic	0.11	0.07	0.00	0.04	0.09	0.15	0.27
Terrestrial	0.09	0.10	0.00	0.02	0.05	0.12	0.36

Pine Lake

Pumpkinseed sunfish

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.83	0.09	0.65	0.77	0.84	0.90	0.97
Pelagic	0.14	0.09	0.01	0.07	0.14	0.21	0.33
Terrestrial	0.03	0.02	0.00	0.01	0.02	0.04	0.09

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.74	0.10	0.54	0.66	0.74	0.81	0.94
Pelagic	0.25	0.11	0.04	0.18	0.25	0.33	0.45
Terrestrial	0.01	0.01	0.00	0.00	0.01	0.02	0.04

Largemouth bass

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.86	0.09	0.65	0.80	0.87	0.92	0.98
Pelagic	0.13	0.09	0.01	0.05	0.11	0.18	0.33
Terrestrial	0.02	0.02	0.00	0.01	0.01	0.03	0.07

Star Lake

Yellow perch

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Benthic	0.55	0.08	0.38	0.50	0.55	0.60	0.69
Pelagic	0.42	0.07	0.29	0.37	0.42	0.46	0.55
Terrestrial	0.03	0.03	0.00	0.01	0.03	0.05	0.10

Appendix 3

Tables of mean and 95% credible intervals from MixSIR model runs estimating contributions of *Bellamya* and other resources to pumpkinseed sunfish diets in undeveloped and developed lakes.

Undeveloped/*Bellamya* Lakes

Cascade Lake

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Bellamya	0.26	0.15	0.02	0.14	0.25	0.37	0.57
Collectors	0.09	0.09	0.002	0.03	0.06	0.13	0.32
Odonates	0.24	0.16	0.01	0.10	0.22	0.35	0.57
Native snails	0.24	0.08	0.06	0.20	0.25	0.30	0.40
Terrestrial	0.02	0.02	0.001	0.01	0.02	0.03	0.07

Zooplankton	0.15	0.10	0.005	0.06	0.14	0.22	0.36

Padden Lake

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Bellamya	0.09	0.07	0.003	0.03	0.07	0.12	0.25
Collectors	0.10	0.08	0.004	0.04	0.08	0.14	0.30
Odonates	0.18	0.14	0.005	0.07	0.15	0.26	0.52
Native snails	0.26	0.09	0.05	0.21	0.27	0.33	0.43
Terrestrial	0.02	0.02	0.001	0.01	0.01	0.03	0.06
Zooplankton	0.36	0.18	0.18	0.31	0.36	0.33	0.43

Wilderness Lake

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Bellamya	0.14	0.11	0.004	0.05	0.11	0.20	0.41
Collectors	0.09	0.08	0.003	0.03	0.07	0.13	0.28
Odonates	0.18	0.12	0.01	0.08	0.17	0.27	0.44
Native snails	0.09	0.08	0.003	0.03	0.07	0.14	0.28
Terrestrial	0.05	0.04	0.002	0.08	0.04	0.07	0.14
Zooplankton	0.45	0.09	0.29	0.39	0.45	0.51	0.64

Developed/*Bellamya* Lakes

Angle

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Bellamya	0.40	0.19	0.20	0.32	0.41	0.53	0.62
Collectors	0.10	0.06	0.01	0.05	0.10	0.15	0.24
Odonates	0.25	0.12	0.15	0.21	0.25	0.34	0.50
Native snails	0.05	0.01	0.01	0.03	0.05	0.07	0.09
Terrestrial	0.02	0.01	0.00	0.004	0.01	0.02	0.05
Zooplankton	0.18	0.11	0.01	0.10	0.17	0.25	0.41

Pine

Source	Mean Proportion	Standard Deviation	2.5%	25%	50%	75%	97.5%
Bellamya	0.45	0.25	0.02	0.23	0.48	0.67	0.84
Collectors	0.04	0.03	0.001	0.01	0.03	0.05	0.12
Odonates	0.40	0.26	0.02	0.15	0.38	0.63	0.84
Native snails	0.05	0.05	0.001	0.01	0.03	0.07	0.17
Terrestrial	0.01	0.01	0.00	0.004	0.01	0.02	0.05
Zooplankton	0.06	0.05	0.002	0.02	0.04	0.08	0.19

Ch. III. Human development modifies the biological trait composition of lake littoral invertebrate communities

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Abstract. Residential shoreline and watershed development by humans are leading drivers of biodiversity loss in lake ecosystems that reduce abundances and diversity littoral invertebrates. Invertebrate biological and life history traits provide good indicators of environmental quality and ecosystem functioning, yet surprisingly few studies have utilized trait-based approaches to assess impacts of human development to lake littoral communities. We assessed environmental characteristics of human development of lakes that impact functional diversity and structure the biological trait composition of invertebrate communities. Multiple linear regressions revealed that functional diversity declined with increasing watershed development, concentrations of total phosphorus, and littoral macrophyte cover. Results from multivariate constrained ordination and fourth corner analysis indicated that high phosphorus concentrations and macrophyte cover filtered taxa with semivoltine life histories and filter feeders from lake communities, and that both regional, ecosystem-level and local, habitat characteristics of watershed development were important determinants of invertebrate community structure. Human development had particularly pronounced effects on invertebrate communities in the deep littoral zone, for which overall community abundances declined as a result of habitat removals and increased phosphorus concentrations. Our study indicates that lake shoreline development and phosphorus loading favor communities dominated by multivoltine taxa and herbivores, which may have important implications for energy flow between terrestrial, littoral, and pelagic food webs.

Keywords: functional diversity, life history traits, urban development, aquatic macroinvertebrates

Introduction

Residential shoreline and watershed development by humans are recognized as leading drivers of biodiversity loss in lake ecosystems (Carpenter et al., 1998; Hansen et al., 2005). Runoff, eutrophication, and replacement of complex shoreline habitat with beaches and retaining walls together erode the ecological integrity of invertebrate communities in lake littoral zones (Brauns et al., 2007; Donohue et al., 2009; McGoff et al., 2013a). Littoral invertebrates contribute to essential ecosystem processes by recycling nutrients and converting organic matter into energy for other organisms in littoral, pelagic, and riparian food webs (Covich et al., 1999; Schindler & Scheuerell, 2002; Vadeboncoeur et al., 2002). Therefore, by reducing or altering invertebrate abundances and diversity, human development of landscapes can negatively impact lake-ecosystem functioning (Brauns et al., 2011).

Invertebrate biological and life history traits provide good indicators of environmental quality and ecosystem functioning and are well suited for assessing the effects of urban development on ecological communities (Doledec et al., 2006; Poff et al., 2006). Invertebrate communities respond to the "habitat templet" such that species with traits that are unsuitable to survival in a given environment are filtered from the community, and thus, invertebrate assemblages comprise species with biological strategies that are adapted to the prevailing environmental conditions (Southwood, 1977; Townsend & Hildrew, 1994; Poff, 1997). Human development functions as a strong environmental filter by removing species with traits that are poorly adapted for survival in degraded environmental conditions, including traits that are sensitive to pollution and habitat modification (Statzner & Bêche, 2010). A rich body of literature has documented how the functional (trait) composition of communities responds to human development in river ecosystems (e.g., Townsend et al., 1997; Lange et al., 2014), yet surprisingly few studies have examined how invertebrate trait composition is structured by shoreline and watershed development in lake ecosystems.

Lakes throughout North America have undergone extensive shoreline development characterized by removals of riparian and aquatic vegetation and coarse woody debris resulting in lower availability of food and habitat for littoral invertebrates (Christensen et al., 1996; Francis et al., 2007; Larson et al., 2011). In addition, lakes receive runoff of nutrients and pollutants from human-dominated landscapes, which drives declines in abundances and diversity of sensitive taxa, such as those in the Odonata, Ephemeroptera, Plecoptera, and Trichoptera families (McGoff et al., 2013a; Miler et al., 2013). Although there has been considerable progress toward studying littoral invertebrate communities in European lakes to assess the effects of shoreline and watershed development on ecosystem integrity (e.g., Porst et al., 2012; Miler et al., 2013; McGoff et al., 2013b), these studies have been limited to taxonomic assessments of invertebrate community composition. A trait-based approach may be better suited to resolving the effects of multiple stressors on freshwaters because species' attributes react mechanistically to specific stressors such as eutrophication (Statzner & Bêche, 2010), and thus allow us to make *a priori* predictions of community responses (e.g., Mims & Olden, 2013).

Ecological theory predicts that species with traits that are unsuitable to survival in a given habitat are filtered from the environment at descending spatial scales. In this way, species should be filtered primarily by regional environmental characteristics, such as watershed development, and secondarily by local characteristics, including riparian cover and littoral habitat, leaving behind species with traits that are adapted to both watershed and local habitat conditions (Tonn, 1990; Poff, 1997). A limited number of studies suggest that changes in trait composition in response to regional and local environmental characteristics depend on the traits examined and the spatial extent. For example, Johnson and Goedkoop (2002) found that aquatic habitat explained a greater proportion of variation in invertebrate feeding diversity among Swedish lakes than did riparian, catchment, and ecoregion attributes. By contrast, Heino (2008) assessed functional group-environment relationships in a single drainage basin of Finland and determined that some functional groups responded strongly to watershed characteristics; in particular, gatherers were prevalent in lakes with relatively high concentrations of total phosphorus, whereas other functional groups were associated with abundant coarse organic matter and macrophyte cover in lake littoral habitats. These studies highlight the need to consider land-use variables at multiple spatial scales when determining how development structures littoral invertebrate communities (Olden et al., 2006).

Previous research suggests that the biological trait composition of invertebrate communities is structured predictably by environmental characteristics of human development. Littoral habitat disturbances, such as shoreline “tidying” (i.e., removal of woody debris and aquatic macrophytes), and recreational boating, are common on lakes with urban and residential shoreline development (Strayer & Findlay, 2010). Species with short generation times and high mobility are predicted to be resilient to these environmental disturbances, whereas species with long generation times and low dispersal ability should be filtered from communities by unstable conditions (Poff et al., 2006). Complex lake littoral zones that contain extensive macrophyte beds and coarse woody debris provide protection against predators and food resources to large-bodied invertebrates, and species in the shredder and predator feeding guilds (Weatherhead & James, 2001; Tolonen et al., 2003; Heino, 2008). Thus, predators and shredders would be expected to decline in developed lakes where macrophyte cover and inputs of terrestrial organic matter have been reduced along the shoreline (Brauns et al., 2007; Francis et al., 2007). In addition, organic matter that is normally retained close to shore by complex habitat structure is exported rapidly offshore and settles in deeper sediments of developed lakes, resulting in lower proportions of sediment organic matter in the near-shore (Francis et al., 2007). Differing availabilities of organic matter in shallow and deep littoral zones suggests that the degree to which human development shapes the functional composition of invertebrate communities depends upon littoral sampling depth.

We present one of the first studies to examine whether human development modifies the composition of invertebrate biological traits in lake littoral communities. We assessed the effects of regional and local development, and lake surface area, on invertebrate communities in shallow and deep littoral zones. We define characteristics of regional development as the proportion of the watershed converted to urban land use (watershed development) and epilimnetic total phosphorus concentrations ($\mu g\ L^{-1}$); and we define local development as the proportion of macrophyte and woody debris habitat around the lake littoral zone. Previous research indicates that these characteristics of development, and lake surface area, are most likely to shape the composition of biological traits in littoral invertebrate communities (Table 1). We predict that human development reduces invertebrate functional diversity and removes species with traits that are sensitive to habitat loss and pollution and favors invertebrate communities dominated by traits associated with population resilience. We further expect that regional environmental characteristics of development play a larger role than local characteristics in structuring invertebrate communities, but that development similarly affects communities in shallow and deep littoral zones (Table 1).

Methods

Study system

We sampled 12 lakes in the Puget Sound lowlands of Washington State, USA (Fig.1a), that span a gradient of shoreline and watershed development ranging from undeveloped lakes with restricted public access, intact riparian habitats, and forested watersheds (Fig. 1b), to developed lakes with the entire shoreline surrounded by residential buildings, sparse vegetation and woody debris, and a high proportion of the watershed developed (Fig. 1c). The topography of the region was shaped 12,000 to 15,000 years ago by movements of the Puget Lobe of the Cordilleran ice sheet. Consequently, lake sediments are characterized by glacial till, an accumulation of clay and boulder, and areas of sandy gravel outwash. Study lakes were carefully selected from hundreds of potential lakes in the Puget Sound lowlands to ensure high similarity in physiochemical conditions but differed in trophic status (Appendix 1).

The riparian zones of undeveloped lakes are characterized by a dense canopy of native evergreen and less abundant deciduous trees. Developed lakes are surrounded by open space, ornamental gardens and grass lawns, non-native shrubs, and native deciduous trees that typically outnumber evergreen tree species. Littoral zones of undeveloped lakes contain a high proportion of complex habitat, especially coarse woody debris, while developed lakes are characterized by open sandy beaches and cobble. Dominant aquatic vegetation in undeveloped and developed lakes includes submerged and floating-leaved pondweeds (*Potamogeton spp.*), non-native pond lilies (*Nuphar spp.*) and plant-like algae (*Chara spp.*).

Data collection

We sampled each lake over a period of three days during July-August in 2012 and 2013. We assessed watershed development using a lake database presented in Tamayo and Olden (2014). We collected duplicate water samples for analysis of total phosphorus concentrations (TP; $\mu g\ L^{-1}$) from the epilimnion using a Van Dorn bottle (Wetzel & Likens, 1991). Water samples were transferred unfiltered to acid-washed polyethylene bottles, frozen, and analyzed at the University of Washington, School of Oceanography's Marine Chemistry Laboratory. Analysis of TP followed methods of Valderrama (1981). Lake physical and chemical characteristics were also assessed, including water temperature, dissolved oxygen, conductivity (YSI Model 85), and pH, and parameters were found to be similar across lakes.

Each lake was divided into four quadrants according to the cardinal directions to distribute the following sampling effort evenly. We modified the US Environmental Protection Agency's (USEPA) lake habitat assessment protocols to characterize littoral habitat within each quadrant by scoring habitat cover from 1 to 4 for each of four types: woody debris, macrophytes, cobble, and sand (Baker et al., 1997). A score of 1 indicates low cover (<10%) and a score of 4 indicates high cover (>75%). This procedure was also followed to assess riparian vegetation cover associated with each quadrant. Within each quadrant we selected randomly 4 shallow (< 1 m depth) and 4 deep (1 to 4 m depth) sites along the littoral zone representing the four habitat types, for a total of 16 shallow and deep littoral samples in each lake. Shallow sites were sampled for invertebrates by sweeping a D-frame net (25 cm x 35 cm opening, 500 µm mesh; Wildco Wildlife Supply, Buffalo, NY) inside of a 1-m^2 quadrat placed on the lake substrate. Deep sites were sampled using a petite ponar grab (38.7 cm^2 opening; Wildco Wildlife Supply, Buffalo, NY). All samples were sieved through a 500 µm-mesh bucket and preserved in 90% ethanol for laboratory identification.

Laboratory preparation

Invertebrate samples were processed according to USEPA Bioassessment Benthic Macroinvertebrate Protocols (Barbour et al., 1999). We developed a morphospecies curve using a high volume sample to estimate the minimum number of randomly selected individuals needed to detect all morphospecies in a sample. Based on this curve, we sub-sampled each sample until 300 (± 20%) individuals were counted. Samples containing fewer than 240 individuals were processed in entirety. Organisms were identified to genus, subfamily for Chironomidae, and subclass for Annelida, with an 80X dissecting microscope using published and online taxonomic guides (Thorp & Covich, 1991, Merritt & Cummins, 1996). Traits were assigned to taxa on the basis of Poff et al. (2006) and the USEPA Freshwater Biological Traits Database (U.S. EPA, 2012).

Data analysis

We compared the importance of regional (watershed development, TP, lake surface area) and local (littoral macrophyte and woody debris cover) environmental characteristics in structuring the functional trait composition of invertebrate communities by analyzing data at two scales, samples aggregated by lake, and habitat samples from within the lake. In addition, we examined shallow and deep littoral samples separately to determine the effects of depth on trait-environment relationships. Invertebrate abundance data were log (x + 1) transformed prior to analyses to meet assumptions of normality and heteroscedasticity. In addition, environmental data matrices were column standardized, and TP values were log (x + 1) transformed. We created trait-by-site data matrices for shallow and deep littoral samples by conducting matrix multiplication of each log-transformed invertebrate abundance matrix (sample-by-taxa) and the transpose of the trait-by-taxa matrix.

We calculated two multidimensional indices of functional diversity to explore which environmental characteristics of development influence the trait composition and volume of functional space occupied by invertebrate communities. We calculated functional richness, the number of unique trait-category combinations in the community (Laliberté & Legendre, 2010). We also calculated Rao's quadratic entropy (Rao's Q), which measures functional diversity as pairwise differences in trait composition between taxa and accounts for relative abundances of taxa in a community (Botta-Dukát, 2005). Both diversity indices were calculated using the FD package in R, which provides a flexible approach to calculating multidimensional functional diversity indices. FD reduces the dimensionality of trait-combinations using principal coordinate analysis (PCoA) and selects a subset of axes to represent traits; the user can quantify the information lost when selecting a subset of axes by calculating an R^2-like ratio (Laliberté & Legendre, 2010).

We considered separate multiple regression models to explain which environmental characteristics of development influence functional richness and Rao's Q at each littoral zone depth. We examined correlation matrices for multicollinearity among predictor variables, and we found evidence for strong multicollinearity between the variables 'watershed development' and 'riparian cover', and between 'riparian cover' and 'woody debris'. Therefore, we removed the variable ‘riparian cover’ from further analyses. Regression models under consideration included the variables watershed development, logTP, lake surface area, woody debris cover, and macrophyte cover, and their interactive terms. We did not utilize formal model selection procedures using maximum likelihood estimates as our aim was to explain functional diversity-environment relationships rather than make predictions, and maximum likelihood procedures favor models with fewer parameters when sample size is small. Thus, our model selection procedure was as follows. We simplified models by eliminating non-significant terms, starting with interactive terms, and we selected models with the fewest parameters that maximized the proportion of explained variance (R^2-value). Poorly fitting models were eliminated based on visual examination of residual and normal Q-Q plots.

We used a constrained analysis on principal coordinates (CAP) on data aggregated by lake to assess how human development influences the overall biological trait composition of invertebrate communities. CAP is a method of constrained ordination that relates a matrix of response variables, species traits, with a matrix of explanatory variables, environmental characteristics. One of the primary advantages of CAP is that it permits the use of any distance or dissimilarity measure to address specific hypotheses (Anderson & Willis, 2003). We ran CAP using Bray-Curtis dissimilarity measures, which are appropriate for measuring dissimilarities in trait composition between sites (Bray & Curtis, 1957). Prior to running CAP, we assessed gradient length, the degree of turnover in traits across sites, using detrended correspondence analysis. Gradient length was less than 2, indicating a linear distribution of species traits and that CAP analysis was appropriate for constrained ordination. We ran partial CAP to examine the relative influence of regional and local environmental characteristics on the biological trait composition of invertebrate communities. We used Analysis of Variance (ANOVA) to test for overall significance of the ordination, in effect, whether the environmental characteristics together explain a significant proportion of variation in trait composition across lakes, and to test whether individual axes and environmental characteristics explain significant variation in the trait composition of invertebrate communities. CAP was run in Vegan for R version 3.1.2 (Oksanen et al., 2011; R Core Development Team, 2014).

We examined relationships between individual biological traits and environmental characteristics (Table 1) using fourth corner analysis (Legendre et al., 1997). Fourth corner analysis quantifies associations between traits and environmental characteristics by relating three data matrices: taxa-by-site, environmental characteristics-by-site, and trait-by-taxa and tests for statistical significance using a permutational approach. This approach tests the null hypothesis that taxa are distributed randomly with respect to environmental characteristics against the alternative hypothesis that the environment controls the distribution of individual taxa (Legendre et al., 1997). We assessed statistical significance for each trait by environment correlation by conducting 9,999 permutations, setting the significance level to alpha = 0.05 and employing a sequential Bonferroni technique for multiple pairwise comparisons to control the Type I error rate (Holm, 1979). The sequential Bonferroni technique has a lower Type II error rate than the classical Bonferroni technique, and thus has more power to correctly reject a false null hypothesis. Fourth corner analysis was run using the ade4 package in R (Chessel et al., 2004).

Results

Effects of human development on invertebrate functional diversity

We found moderate support based on multiple regression models for our hypothesis that invertebrate functional diversity declines with increasing human development of lakes (Table 2). Watershed development was an important predictor of diversity in three of four regression models, and both functional richness and Rao's Q were lower in lakes with highly developed watersheds. In addition, lakes with high TP had lower values of Rao's Q for both shallow and deep littoral invertebrate communities. Littoral habitat characteristics, woody debris and macrophyte cover, were also important predictors of functional diversity in three of four regression models, but their relationship with functional diversity depended on littoral zone depth, the diversity index (functional richness or Rao's Q), and interactive effects between the two habitat types.

Influence of human development on the biological trait composition of invertebrate communities

Constrained analysis on principal coordinates revealed that human development modifies the biological trait composition of invertebrate communities in both shallow and deep littoral zones. Regional and local environmental characteristics explained 67% of the variation in biological trait composition of shallow littoral invertebrate communities, and the first two CAP axes together accounted for 93% of the explained variation (Fig. 2). Although the overall ordination was not significant (ANOVA; $F_{5,6} = 2.43$; $p = 0.059$), CAP1 accounted for a significant proportion (73%) of explained variation in trait compositions (ANOVA; $F_{1,6} = 8.92$; $p = 0.024$). Watershed development, lake surface area, and woody debris cover were negatively correlated with CAP1, and TP and macrophyte cover were positively correlated with CAP1. Therefore, these environmental characteristics together explained significant variation in trait composition across lakes; however, individual environmental characteristics did not explain a significant proportion of variation in the traits of shallow littoral communities. Taxa with semivoltine life history (< 1 generation year^{-1}) and filter-feeding traits were negatively correlated with CAP1, indicating that these taxa were less abundant in lakes with high TP concentrations and macrophyte cover and were more abundant in lakes with high proportions of woody debris cover in the littoral zone. Taxa with multivoltine life histories (>1 generation yr^{-1}), small and medium sized bodies, and herbivores and predators were positively correlated with CAP1, and thus, were more abundant in lakes with relatively high TP and littoral macrophyte cover.

In the deep littoral zone, environmental characteristics explained 56% of the variation in biological trait composition of invertebrate communities, and the first two CAP axes accounted for 93% of the constrained variation (Fig. 3). The overall ordination including all environmental characteristics was not significant (ANOVA; $F_{5,6} = 1.53$; $p = 0.22$), but CAP1 explained a significant proportion (87%) of constrained variation in trait composition (ANOVA; $F_{1,6} = 6.63$; $p = 0.0083$). All environmental characteristics but macrophyte cover were strongly correlated with CAP1. Watershed development and TP were positively correlated with CAP1, and woody debris and lake surface area were negatively correlated with CAP1, indicating that these characteristics together significantly influenced variation in the biological trait composition of deep littoral invertebrate communities. However, no individual environmental characteristic explained significant variation in trait composition. All biological traits but large body size were negatively correlated with CAP1, which suggests that overall invertebrate abundances in the deep littoral zone were lower in lakes with high watershed development and TP concentrations.

Importance of regional and local environmental characteristics of development

We examined the relative influence of regional and local environmental characteristics on biological trait compositions using partial CAP on habitat-level data. In the shallow littoral zone, the full CAP model including regional environmental characteristics of watershed development, TP, and lake surface area, and local characteristics of littoral woody debris and macrophyte cover explained a significant proportion (26%) of variation in habitat-level, biological trait composition of invertebrate communities (ANOVA; $F_{5,42} = 2.89$; $p < 0.0010$). Further, watershed development (ANOVA; $F_{1,42} = 2.99$; $p = 0.025$), TP (ANOVA; $F_{1,42} = 4.01$; $p = 0.0069$), macrophyte cover (ANOVA; $F_{1,42} = 2.47$; $p = 0.0047$), and woody debris (ANOVA; $F_{1,42} = 3.24$; $p = 0.019$) each individually explained a significant proportion of variation in habitat-level trait composition. In addition, partial CAP revealed that regional and local environmental characteristics each independently explained a significant proportion of variation in trait composition. Regional characteristics independently explained 16% (ANOVA; $F_{3,42} = 2.91$; $p = 0.0013$) and local characteristics independently explained 10% of the variation in shallow littoral trait composition (ANOVA; $F_{2,42} = 2.64$; $p = 0.017$).

In contrast to the shallow littoral zone, the environmental characteristics we considered explained just 10% of the variation in habitat-level trait composition of deep littoral invertebrate communities (ANOVA; $F_{5,42} = 0.94$; $p = 0.53$). Further, individual environmental characteristics did not significantly influence trait composition. Regional environmental characteristics independently explained 5.5% (ANOVA; $F_{3,42} = 1.04$; $p = 0.39$) and local characteristics independently explained 3.4% (ANOVA; $F_{2,42} = 1.06$; $p = 0.36$) of variation in trait composition of deep littoral invertebrate communities.

Individual trait-environment relationships

We used fourth corner analysis to make 65 pairwise comparisons of trait-environment relationships for each littoral depth. Several invertebrate traits were strongly associated with individual environmental characteristics (Table 3). Notably, taxa with the life history trait, semivoltinism, were significantly negatively correlated with high TP in shallow littoral zones. Filter feeders were also correlated negatively with high TP, but the relationship was only marginally significant. No other trait-environment relationships were significant after sequential Bonferroni adjustment of p-values. However, this method of adjustment is highly conservative, and thus we also present relationships that were significant prior to adjustment (Holm 1979). Semivoltine taxa were correlated negatively with macrophyte cover and were correlated positively with woody debris cover, whereas multivoltine taxa were correlated positively with macrophyte cover and were correlated negatively with woody debris in shallow littoral zones. Herbivores and taxa with medium body sizes were also correlated positively with high macrophyte cover in the shallow littoral zone (Table 3).

We found qualitatively similar trait-environment relationships for invertebrate communities between shallow and deep littoral zones; however, the strength of these relationships was greater for communities in the deep littoral zone. For example, taxa with semivoltine life histories were significantly negatively correlated with both TP and macrophyte cover (Table 4). There was also a marginally significant positive relationship between semivoltinism and woody debris after Bonferroni correction for multiple pairwise comparisons. Filter feeders also were negatively correlated with environmental characteristics of development, namely high TP and macrophyte cover. By contrast, herbivores were correlated positively with macrophyte cover and were correlated negatively with woody debris cover. Trait-environment relationships for the deep littoral zone are shown if they were significant prior to Bonferroni correction of p-values for multiple pairwise comparisons (Table 4).

Discussion

Our study provides evidence that human development of lakes modifies the biological trait composition of littoral invertebrate communities. Multiple linear regression revealed that functional diversity generally declined with increasing human development, and both CAP and fourth corner analyses indicated that high TP concentrations, dense macrophyte cover, and reductions in woody debris in the littoral zone structured communities by filtering taxa that are sensitive to pollution and habitat removals. Notably, taxa with semivoltine life histories, a trait common among long-lived organisms, and filter feeders were sensitive to characteristics of human development. By contrast, taxa with certain resilience traits, especially multivoltine life histories, and herbivores were more common in lakes that had undergone human development.

Our results imply several mechanisms by which human development structures the biological trait composition of invertebrate communities at different spatial scales and depths in the littoral zone. Both regional and local environmental characteristics of development significantly influenced the biological trait composition of invertebrate communities, and the magnitude of regional and local effects on community structure was nearly equal. Among regional characteristics, watershed development was an important predictor of functional diversity, and TP was a significant filter of sensitive taxa. At the local scale, the availability of woody debris habitat and the extent of macrophyte cover in the littoral zone had the largest influence on community structure. We also found important differences in community responses to development between shallow and deep littoral zones. Although human development filtered taxa with similar biological traits at each depth, communities in the deep littoral zone were overall more strongly negatively influenced by characteristics of development.

High lake TP concentrations reduced abundances of taxa with semivoltine and filter feeding traits, but favored resilient taxa with small body sizes, multivoltine life histories, and herbivores. Previous research indicates that phosphorus loading increases lake primary production and thus can favor increased herbivore abundances in littoral zones (Heino, 2008). However, high primary production can also lead to sediment hypoxia due to high rates of microbial respiration that negatively impacts sensitive invertebrates (Søndergaard et al., 2003). Other evidence indicates that species richness and diversity display unimodal relationships with lake productivity, whereby invertebrate diversity peaks at moderate levels of productivity and then declines at higher productivity (Tolonen et al., 2005). TP concentrations in our lakes were fairly low (<28 mg L^{-1}), yet the loss of filter feeders and semivoltine taxa from higher TP lakes suggests that these biological traits confer disadvantages to survival at even moderate levels of nutrient loading. Although Hecky et al. (2004) found that non-native mussels in the family *Dreissenidae* perform well in lakes with high phosphorus loading, we found that filter feeders responded uniformly negatively to high TP concentrations. Filter feeders in our study lakes comprise primarily native pea clams in the genus *Pisidium* and were found only in oligotrophic lakes. *Pisidium*, and other native bivalve filter feeders have poor survival under conditions of eutrophication and low levels of dissolved oxygen, which explains their absence from study lakes with relatively high TP (Donohue et al., 2009; Cloherty & Rachlin, 2011).

Interestingly, our results suggest that TP and watershed development had opposing effects on the overall biological trait composition in shallow littoral communities. Although watershed development and TP both were negatively correlated with functional diversity, results from CAP and fourth corner analysis indicated that sensitive taxa responded negatively to TP but not watershed development. One explanation for the lack of relationship between trait composition and watershed development is that sensitive taxa responded more strongly to TP, which has a unimodal relationship with watershed development. Previous research on Puget Sound lakes demonstrated that lakes in highly urban watersheds have lower rates of phosphorus and nitrogen loading compared to lakes in semi-rural areas with high residential shoreline development. Such lakes are significantly more eutrophic stemming from residential septic leakage around the shoreline. This drives a unimodal distribution in lake nutrient concentrations and productivity, in which lakes with the best water quality have completely undeveloped shorelines and watersheds, followed by lakes in highly developed watersheds (Moore et al. 2003; Jankowski et al. 2012). The unimodal relationship between TP concentrations and watershed development complicates our study of the impacts of human development on invertebrate communities, but our results suggest that communities are impacted most strongly in high TP lakes with a high degree of shoreline development and low-moderate degree of watershed development.

We predicted that human development of lakes would reduce the availability of littoral macrophyte cover, and that this consequence of development would impact functional diversity. To the contrary, we found that lakes with high levels of watershed and shoreline development were characterized by relatively dense macrophyte cover in shallow littoral zones (Appendix 1), a pattern that was associated with lower functional diversity of invertebrate communities. We identified non-native, floating-leaved pond lilies (*Nuphar spp*.) in all lakes with high levels of shoreline development (Twardochleb, unpublished data). Floating-leaved macrophytes can lower the diversity of benthic invertebrate communities by several mechanisms, including forming stands of monocultures by shading out shorter, submerged macrophytes that contribute to complex littoral habitat; by reducing incident light available to sediments under macrophyte beds; and by respiring oxygen to the atmosphere and inducing sediment hypoxia (Caraco & Cole, 2002; Strayer et al., 2003). Our study was not designed to measure the specific ecosystem attributes of macrophyte beds, so we cannot with certainty attribute patterns of invertebrate diversity to the presence of *Nuphar spp*. However, the counterintuitive relationships observed here between macrophyte cover and functional diversity suggest that community and ecosystem attributes of macrophytes, such as bed composition and complexity, and water and sediment chemistry around beds, are also important determinants of the trait composition of invertebrate communities.

All feeding groups except for herbivores were associated with abundant woody debris habitat in lakes. We found that lakes with highly developed shorelines had substantially lower availability of woody debris habitat, and loss of this habitat had pronounced impacts on deep littoral invertebrate communities. Francis and Schindler (2006) also documented lower densities of coarse woody debris along developed littoral zones of Puget Sound lakes and concluded that this reduced the retention of sediment organic matter that is an important source of food for invertebrates (Francis et al., 2007). Woody debris also provides refuge against predation and accumulates biofilms that invertebrates consume, and therefore is one of the most important habitat characteristics structuring invertebrate communities across the human development gradient (Everett & Ruiz, 1993; Christensen et al., 1996). Our results are consistent with those of Francis et al. (2007), who found significant reductions in overall invertebrate abundances with removals of woody debris habitat.

We found evidence that human development filtered taxa from invertebrate communities primarily based on their life history and functional feeding traits. As predicted, we found evidence that semivoltine invertebrates were filtered from lakes with low woody debris cover and were replaced by higher abundances of rapidly reproducing, mutivoltine invertebrates. No studies have, to our knowledge, examined the impacts of human development on life history traits of lake invertebrates, but our results are consistent with previous taxonomic studies. Long-lived organisms, such as taxa in the orders Odonata and Trichoptera, decline in lakes that have been subjected to littoral habitat removals because slow reproductive rates inhibit their populations from rebounding quickly after a disturbance (e.g., Porst et al., 2012; Miler et al., 2013).

In contrast to previous research, we did not find strong evidence that invertebrate body sizes were influenced by lake environmental characteristics. Our results indicated that small invertebrates were more abundant in developed lakes, but large-bodied taxa did not respond consistently negatively to human development. McGoff et al. (2013a,b) found negative impacts of habitat removals on large invertebrates, but these effects were mediated by fish predation. CAP results indicated that littoral zones of developed lakes had lower woody debris cover but higher macrophyte densities, and therefore, developed lakes still provide refuge for large invertebrates that are sensitive to fish predation (Tolonen et al., 2003; Strayer & Malcolm, 2007).

Our study relied on a sample size of twelve lakes, and thus, the gradients of development that we examined do not capture the entire range of conditions across the Puget Sound region. In addition, we focused on measuring characteristics of development at the lake level, and therefore, we did not have the resolution to determine how microhabitat, such as sediment grain size or size and diversity of macrophyte beds, influenced biological trait compositions among habitats within lakes. Our habitat-level analyses comparing regional and local effects of human development indicate that other environmental factors not considered here are likely important determinants of trait composition between habitats within a lake. Taxonomic studies suggest that habitat complexity, the amount of organic material in the sediment, and the amount of fine sediment retained along the shoreline all can strongly influence freshwater invertebrate communities (Francis et al., 2007; Strayer & Findlay, 2010, and references therein). We suggest that future research explore additional questions, such as how the composition of biological traits differs across habitat patches ranging in size and quality, to improve our understanding of the interplay between ecosystem- and habitat-level effects of human development on communities. Despite these caveats, very few studies have used a traits-based approach to examine development impacts on lake invertebrate communities, and in doing so, our study lays important groundwork for future research by revealing influences of development by littoral depth and identifying important regional and local environmental drivers of biological trait composition.

Our research indicates that lake shoreline development and phosphorus loading due to watershed development favor communities of short-lived organisms and herbivores and act as environmental filters of other functional feeding groups. These changes may have implications for energy flow between terrestrial, littoral, and pelagic food webs. For example, herbivore dominance and declines in filter and shredder feeding groups in the deep littoral zone may interrupt terrestrial-aquatic energetic coupling, by reducing the ability of the benthic community to process coarse and fine terrestrial organic matter, invertebrates, and higher rates of energy transfer from littoral primary production to consumers via herbivorous invertebrates. In addition, declines in overall invertebrate abundances in the deep littoral zone may reduce prey availability to littoral zone fish and cause a switch to reliance on shallow benthic or planktonic prey (Twardochleb & Olden, unpublished manuscript). Thus, the biological trait composition of invertebrate communities provides important mechanistic insights into how human development impacts lake communities and ecosystem processes.

Table 1. Predicted changes in trait composition of invertebrate assemblages with lake development. Mechanisms and literature support are given for predicted responses. Evidence columns provide observed responses; a check indicates observed response matches predicted, and 'x' indicates the observed response was opposite predicted. No response is indicated for cases in which data did not provide strong evidence for a directional effect of environmental variables on trait composition.

Trait	Category	Predicted response	Mechanisms for trait response	Evidence: Shallow littoral <1m	Evidence: Deep littoral 1-4 m
Body size	Small	↑	Increased runoff of pollutants.[1, 2, 3] Removal of complex habitat that provides protection against predators.[4, 5]		
	Medium	↓		√	
	Large	↓			
Feeding guild	Filterer	↑	Increased fine particulate organic matter and nutrient inputs.[1, 2, 6]	X	X
	Gatherer	↑			
	Herbivore	↑	High primary production due to phosphorus loading.[1, 2, 4, 5]	√	√
	Predator	↓	Removal of complex habitat providing protection from predators, and increased pollution.[1, 2, 4, 5]		

	Shredder	↓	Reduced inputs of terrestrial organic matter that provide substrate for colonization and food.[4, 7, 8]		
Generation time	Multivoltine >1 generation yr^{-1}	↑	Removal of complex habitat providing protection from predators, and increased pollution.[1]	√	√
	Univoltine 1 generation yr^{-1}	↑		√	
	Semivoltine <1 generation yr^{-1}	↓		√	√
Swimming ability	No swimming ability	↓	Increased frequency of habitat disturbance favors species with ability to move to new habitat patch.[9]	X	√
	Swimming ability	↑		√	X
Functional richness		↓	Lower habitat complexity. Environmental filtering of species sensitive to pollution. Increased dominance of resistant and resilient taxa.[2, 9, 10]	√	√
Rao's Q		↓		√	√

1. Arce et al., 2014; 2.Donohue et al., 2009; 3.Cloherty & Rachlin, 2011; 4.Heino, 2008; 5.Tolonen et al., 2003; 6.Lange et al., 2014; 7. Brauns et al., 2007; 8.Francis et al., 2007; 9.Townsend et al., 1997. 10. Strayer & Findlay, 2010.

Table 2. Best-fitting multiple regression models of relationships between functional diversity and environmental characteristics of urban development. The coefficient 'watershed development' is the proportion of the watershed converted to urban land-use, 'wood' and 'macrophyte' are proportional woody debris cover, and proportional macrophyte cover, respectively, in the littoral zone; 'surface area' is lake surface area (km^2), and 'TP' is lake total phosphorus concentration ($\mu g\ L^{-1}$).

Diversity index	K	Coefficient	Estimate	Multiple R^2
Shallow littoral				
Functional richness	2	Intercept	0.24	0.25
		Watershed development	-1.68	
Rao's Q	7	Intercept	0.66	0.38
		Wood*macrophyte	6.75	
		Wood	-4.36	
		Macrophyte	-3.61	
		Surface area	-1.07	
		TP	-1.98	
		Watershed development	-3.05	
Deep littoral				
Functional richness	4	Intercept	2.30	0.56
		Wood	1.58	
		Macrophyte	1.32	
		Surface area	0.63	
Rao's Q	8	Intercept	-0.85	0.58
		Watershed development*TP	-1.73	
		Watershed development*wood	1.36	
		Wood*macrophyte	-1.43	
		Wood	1.41	
		Macrophyte	-1.15	
		TP	-1.72	
		Watershed development	-1.74	

Table 3. Results of fourth corner analysis relating traits to environmental characteristics in shallow littoral depths (< 1 m). The r-statistic indicates the strength and direction of correlation between each trait and environmental characteristic. Comparisons for which the sequential-Bonferroni, adjusted *p*-value is significant are indicated in bold and with an asterisk. All other comparisons shown here were significant prior to adjustment for multiple pairwise comparisons.

Shallow littoral

Trait	Habitat characteristic	r-statistic	*p*-value	*p*-value adjusted
Feeding guild				
Filterer	Phosphorus	-0.11	0.0010	0.064
Herbivore	Macrophyte	0.096	0.036	1.00
Generation time				
Multivoltine	Macrophyte	0.087	0.022	1.00
	Woody debris	-0.077	0.048	1.00
Semivoltine	Macrophyte	-0.099	0.0056	0.35
	Woody debris	0.093	0.0098	0.61
	Phosphorus	**-0.13**	**<0.0010**	**0.020***
Body size				
Medium	Macrophyte	0.090	0.032	1.00

Table 4. Results of fourth corner analysis relating traits to environmental characteristics for deep littoral samples (1 - 4 m). The r-statistic indicates the strength and direction of correlation between each trait and environmental characteristic. Comparisons for which the sequential-Bonferroni, adjusted *p*-value is significant are indicated in bold and with an asterisk. All other comparisons shown here were significant prior to adjustment for multiple pairwise comparisons.

Deep littoral

Trait	Habitat characteristic	r-statistic	*p*-value	*p*-value adjusted
Feeding guild				
Filterer	Macrophyte	-0.11	0.0012	0.074
	Phosphorus	-0.11	0.0010	0.063
Herbivore	Macrophyte	0.12	0.012	0.74
	Woody debris	-0.090	0.049	1.00
Generation time				
Semivoltine	**Macrophyte**	**-0.13**	**<0.0010**	**0.0065***
	Woody debris	0.11	0.0013	0.079
	Phosphorus	**-0.13**	**<0.0010**	**0.013***

Figure 1. Lakes sampled in the Puget Sound region of Washington State.

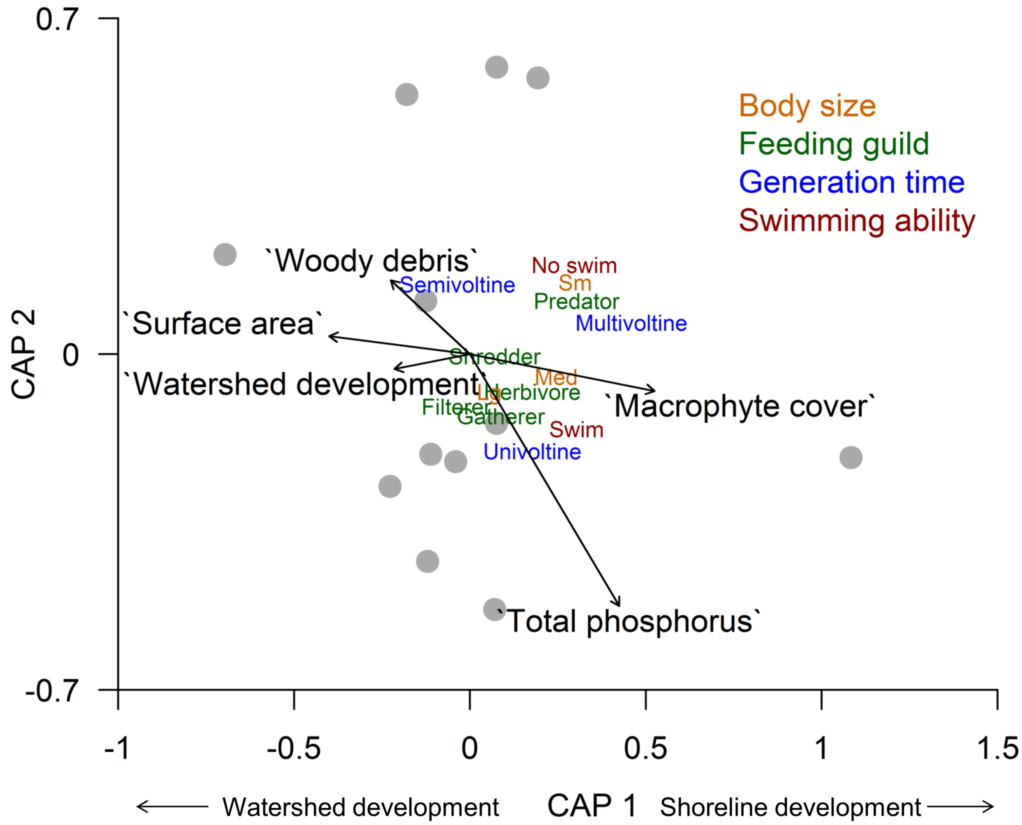

Figure 2. Results of constrained analysis on principal coordinates for shallow littoral macroinvertebrate communities. Lake total phosphorus concentrations and high macrophyte cover, which are consistent with high shoreline development, explained the greatest proportion of variation in invertebrate communities. Vector lengths and orientations indicate the amount of variation in trait composition explained by environmental characteristics and the correlations between environmental characteristics and the dominant axes of variation, CAP1 and CAP2. Trait locations on the ordination indicate their correlations with environmental characteristics. Lakes are represented by gray points and are oriented according to the trait composition of their invertebrate communities and their association with environmental characteristics.

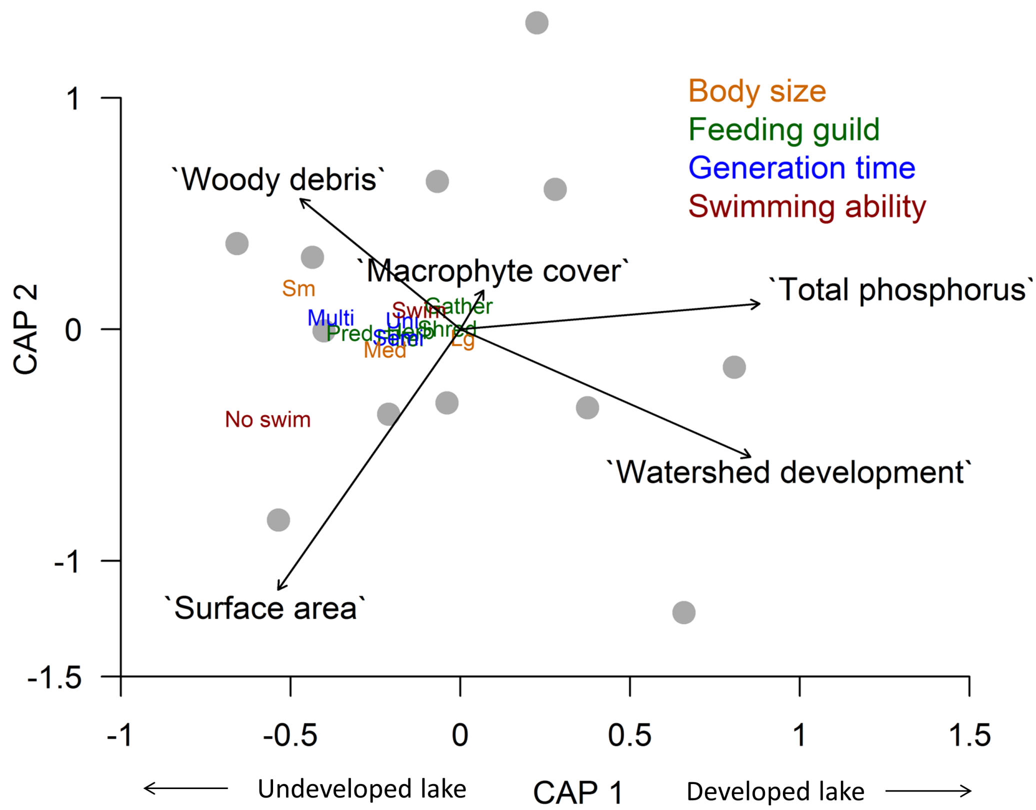

Figure 3. Results of constrained analysis on principal coordinates for deep littoral macroinvertebrate communities. Taxa from most trait categories were impacted negatively by high levels of lake total phosphorus and watershed development and were more abundant in lakes with abundant woody debris habitat, a characteristic of lakes with low shoreline development. Vector lengths and orientations indicate the amount of variation in trait composition explained by environmental characteristics and the correlations between environmental characteristics and the dominant axes of variation, CAP1 and CAP2. Trait locations on the ordination indicate their correlations with environmental characteristics. Lakes are represented by gray points and are oriented according to the trait composition of their invertebrate communities and their association with environmental characteristics.

Appendix 1. Lake-habitat characteristics hypothesized to influence the biological trait composition of invertebrate communities. For riparian cover, 1 indicates very low cover, whereas a score of 5 indicates high riparian cover. Macrophyte and woody debris cover are given only for shallow littoral samples. Lakes are listed in order from low to high watershed development.

Lake	Watershed development (%)	Phosphorus concentration ($\mu g\ L^{-1}$)	Riparian Cover	Macrophyte cover (%)	Woody debris cover (%)	Surface area (km^2)
Cascade	5	15.4	4.75	11.5	25.5	0.68
Langlois	13	9.8	5	18.8	41.3	0.39
Shoecraft	13	8.3	2.5	11.4	8.1	0.53
Fern	14	10.7	5	17	50.5	0.10
Walsh	15	8.3	5	38.5	12.5	0.43
Sunday	20	27.9	2.5	50.5	3.5	0.19
Padden	28	7.5	5	40.2	24.7	0.64
Wilderness	36	12.5	3.5	18.6	22.5	0.28
Pine	43	8.2	3.25	12.4	16.9	0.35
Star	64	9.8	1.5	27.5	21.3	0.34
Angle	64	7.7	1.75	20.5	4.8	0.42
Martha	69	10.1	3	48.4	7.8	0.23

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