

Effects of invasive macrophyte on trophic diversity and position of secondary consumers

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Abstract Invasive species are one of the widespread stressors of aquatic ecosystems. Several studies document food web effects of invasive fish, but little information is available on the effects of invasive macrophytes. We studied differences in food chain length as well as trophic position and trophic diversity of fish and odonates in lakes dominated by native plants or invasive Eurasian watermilfoil. Trophic position and food chain length were determined using baseline-adjusted $\delta^{15}\text{N}$ isotope signatures. Trophic diversity, or isotope niche width, was estimated from convex hull area analysis. Results show that trophic position of secondary consumers was not affected by the invasive macrophyte, whereas trophic diversity was greater in watermilfoil-dominated lakes. The direction of isotopic niche expansion was different in fish and odonates, suggesting potential decoupling in predator–prey interactions.

This study shows that dominant non-native macrophytes may cause significant changes in food web structure of invaded ecosystems. Trophic diversity may be a more sensitive indicator of environmental stress than trophic position and has the potential to be used for assessment of invasive species impacts and restoration success.

Keywords Odonata · Bluegill · Largemouth bass · Stable isotopes · Exotic · *Myriophyllum spicatum*

Introduction

Several types of anthropogenic disturbance can fundamentally change food web structure, nutrient dynamics, and energy flow in aquatic ecosystems. Among the most severe stressors are eutrophication, habitat destruction, and invasive species (Wilcove et al., 1998; Wetzel, 2001). Invasive species are often viewed from a population perspective, but their impacts can extend to affect trophic dynamics of invaded ecosystems (e.g., D’Antonio & Hobbie, 2005; Carvalheiro et al., 2010). In aquatic environments, much attention has been dedicated to food web effects of invasive fish (Vander Zanden et al., 1999a; Simon & Townsend, 2003; Britton et al., 2010), but considerably less is known about the effects of invasive macrophytes.

Invasive macrophytes often have deleterious effects on native macrophyte communities, and can

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affect their resident fauna by altering habitat, epiphyton production and nutrient cycling (Carpenter & Lodge, 1986; Jeppesen et al., 1997), all of which are likely to result in changes in trophic structure. If secondary consumers, which integrate changes over preceding trophic levels, are forced to rely on alternative or suboptimal food sources, we would see changes in their trophic position as well as ecosystem food chain length and trophic diversity, indicating potential decoupling of food web connections or shifts in resources at the base of the food web.

In this study, we were interested in understanding whether dominant non-native Eurasian watermilfoil (*Myriophyllum spicatum* L.) affects food web structure in invaded lakes. Of a number of approaches commonly used to address similar questions (e.g., carbon turnover, dietary analysis), we chose stable isotope analysis because it provides a rapid and relatively cost-effective assessment of trophic structure (Fry, 1991) and an integrated measure of changes in lower trophic levels (e.g., Layman et al., 2007b). Due to isotope fractionation, ^{15}N accumulates as trophic position increases, whereas carbon undergoes minimal fractionation and can be used to track basal resources (Fry, 1991). In addition, recently developed approaches allow quantitative descriptions of food web structure such as the total extent of trophic diversity and trophic redundancy, which are estimated, respectively, by the total area occupied by species in the isotope niche space and the nearest neighbor distance, measuring the overall density of species packing in isotopic space (Layman et al., 2007a).

Different response to environmental stress such as invasive species may be expected in consumers capable of exploiting various habitats as opposed to consumers with a more restricted effective dispersal distance. For example, fish can move between macrophyte patches, whereas some predatory macroinvertebrates (e.g., Odonata) exhibit limited dispersal during larval stage. Thus, we would expect those two types of secondary consumers to provide different levels of integration of nutrient flows in response to change in habitat due to invasive plants. Therefore, we tested whether the presence of a dominant invasive macrophyte affects (a) trophic diversity and (b) food chain length and trophic position of fish and predatory invertebrates.

Materials and methods

We examined trophic position of dominant fish and predatory invertebrates in four lakes in Minnesota, USA (60–100 ha in size). The littoral area of two of the lakes, Auburn and Pierson was dominated by invasive Eurasian watermilfoil. The other two lakes, Bush and Zumbra were treated with herbicides in 2004–2007 to control invasive watermilfoil and are presently dominated by native plants including coontail, *Ceratophyllum demersum*, and fragrant water lily, *Nymphaea odorata* (Skogerboe & Getsinger, 2006; Kovalenko et al., 2009). No invasive plants were present in our study sites in the treated lakes. Herbicide application at label rates should not directly affect fish or invertebrates (Washington State Department of Ecology, 2001a, b), and a previous study did not detect treatment-related changes in fauna, possibly due to selectivity of the treatment and timely restoration of native macrophytes (Kovalenko et al., 2010). Study lakes were similar in nutrient status (moderately eutrophic, Minnesota Department of Natural Resources, unpublished data) and fish community structure. A previous study showed that overall fish community in these lakes was dominated by invertivorous bluegill (*Lepomis macrochirus*, 74% of all fish collected in the littoral zone), whereas largemouth bass (*Micropterus salmoides*) was the most common piscivore (approximately 80% of all predatory fish abundance, Kovalenko et al., 2010).

Fish were collected from several randomly chosen macrophyte stands in each lake using electrofishing and selected to minimize size-related differences. Since bluegill can sometimes exhibit an ontogenetic niche shift (Mittelbach, 1981), we collected two size classes of bluegill (<7 cm and >7 cm total length), to account for potential shift in dietary habits. Largemouth bass had a mean length of 13.3 cm (SE 0.7 cm). Tissue samples for isotope analysis were collected from the dorsal muscle and immediately frozen. Samples were collected in early September 2008, at the peak of macrophyte development.

Predatory macroinvertebrates were represented predominantly by nymphs of dragonflies *Erythemis* sp. and *Leucorrhinia* sp. (Libellulidae) and damselflies *Enallagma* sp. (Coenagrionidae) and *Lestes* sp. (Lestidae). Previous dietary analysis showed that fish in these lakes fed mostly on Chironomidae (Kovalenko et al., 2009), which were therefore chosen as

the primary consumer for trophic position determination. Dietary analysis of fish stomachs collected in this study confirmed validity of baseline selection, as Chironomidae were the most abundant food item (63%). Invertebrates were collected using a dipnet, sorted in the field and kept in individual vials in lake water for 2–3 h to allow gut evacuation, after which they were rinsed, lightly blotted and frozen for future analysis. Nymphs of similar size and developmental stage were selected whenever possible. All samples were stored on dry ice until transported to the laboratory and thereafter kept in an ultra-cold freezer.

Samples for isotope analysis were dried in the oven at 60°C to constant mass (approximately 24 h) and ground with a mortar and pestle. Mass spectroscopy analysis was conducted by the Colorado Plateau Stable Isotope Laboratory. Analytical precision was determined by analyzing 30 samples (10%) in duplicate, and resulting SD was 0.07‰ for $\delta^{13}\text{C}$ and 0.06‰ for $\delta^{15}\text{N}$. Regression of trophic position against total length confirmed lack of size-related bias within the small range examined in this study ($P > 0.05$).

Trophic position (TP) was calculated as described in Vander Zanden et al. (1997)

$$\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}})/3.4 + 2,$$

where 3.4 is the most commonly used fractionation factor and 2 is the default trophic position of the primary consumer. Trophic position was analyzed using the analysis of variance. There were no significant differences between young and adult bluegill, so they were pooled for all analyses. Similarly, Anisoptera and Zygoptera were pooled due to lack of significant differences. Assumptions were tested using Shapiro–Wilk test for normality and a test for homogeneity of variance. Trophic diversity was measured as the convex hull area in $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ bi-plot space (Layman et al., 2007a) in PAST (Hammer et al., 2001) and compared between treatments using the permutation *t*-test.

Results

Fish trophic position was not significantly different among lakes, although one of the watermilfoil-dominated lakes tended to have a greater trophic

level. Bluegill TP was unaffected by the invasive macrophyte ($P > 0.05$, Fig. 1A). Largemouth bass trophic position was less than one trophic level greater than that of bluegill (average difference in TP = 0.4). As with bluegill, there were no lake-related differences in largemouth bass TP ($P > 0.05$, Fig. 1B) or total food chain length, since largemouth bass was the top predator in this system. The greatest food chain length of 4.7 trophic levels was observed in one of the invaded lakes (Pierson). Dragonflies and damselflies had an average trophic position of 2.5, with very low variability and no significant differences among lakes (Fig. 1C).

Convex hull area analysis of $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ signatures showed that fish niche width was approximately two times greater in watermilfoil-dominated lakes than in the reference lakes (average convex hull area 10.4 in watermilfoil-dominated lakes vs. 4.9 in reference lakes, permutation $P < 0.001$, Fig. 2A). For predatory invertebrates, trophic diversity was three times greater in watermilfoil-dominated lakes (average convex hull area was 15.3 in watermilfoil-dominated lakes vs. 4.8 in reference lakes, $P < 0.001$, Fig. 2B). Fish isotope signatures in invaded lakes were more ^{13}C depleted than those in the reference lakes, whereas Odonata signatures were shifted in the opposite direction.

Discussion

Our data show that invasive macrophytes may be associated with significant changes in trophic diversity without concurrent changes in trophic position of secondary consumers. Changes in trophic position would be expected if there were shifts in diet, in particular those involving changes in the proportional contribution of planktonic vs. benthic pathways (Hoeinghaus et al., 2008). In our study lakes, bluegill of all sizes were primarily benthivorous (Kovalenko et al., 2009), although in some systems they exhibit an ontogenetic shift from benthic invertebrates to zooplankton (Mittelbach, 1981). It is interesting that isotope-derived trophic position was higher than what could be inferred from diet data. Stomachs contained predominantly chironomids, with few Odonata and Trichoptera, yet there was considerable (more than one full trophic level) trophic enrichment between Odonata and fish, indicating an alternative food

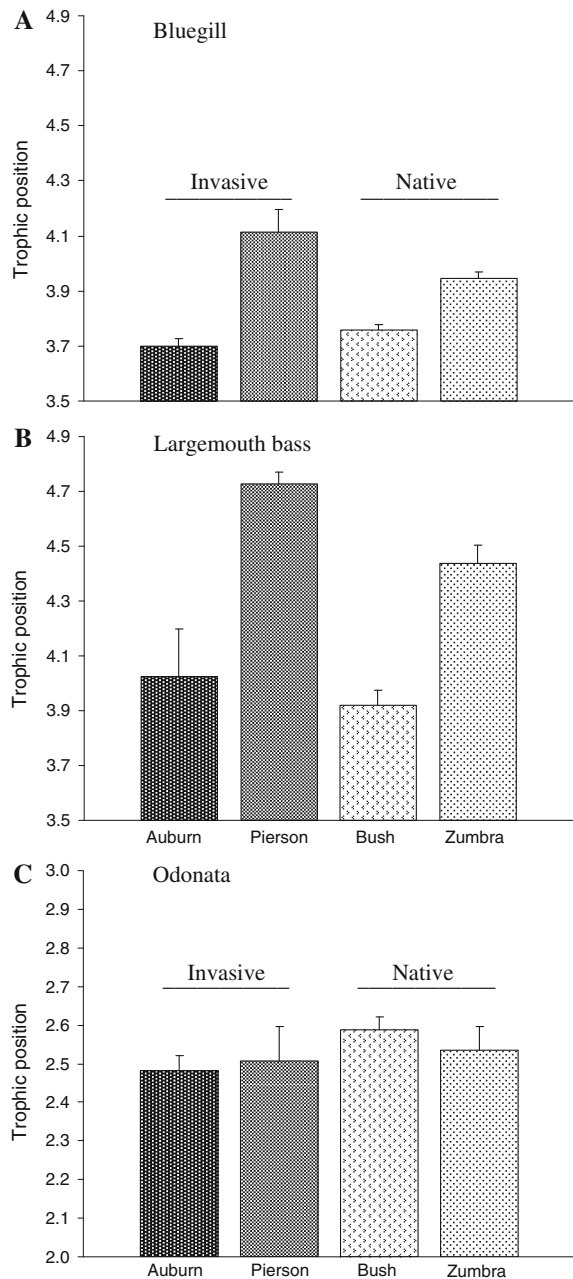


Fig. 1 Trophic position of secondary consumers in watermilfoil-dominated (*dark shaded*) and native plant-dominated lakes: **A** bluegill ($N = 14\text{--}18$ fish), **B** largemouth bass ($N = 4\text{--}7$), and **C** Odonata ($N = 17\text{--}18$ individuals or pooled samples). Values are means \pm SE

source, possibly cannibalism. Very few bluegill stomachs examined in this study contained fish remains. Nevertheless, bluegill were previously observed consuming conspecific eggs (Neff, 2003)

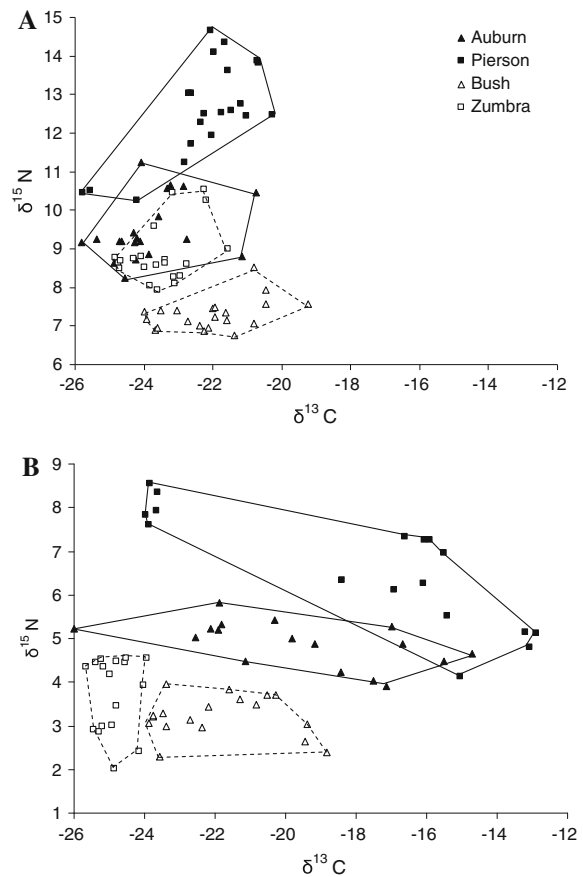


Fig. 2 Trophic diversity (niche width) of secondary consumers, estimated by convex hull area, in watermilfoil-dominated (*dark shaded*) and native plant-dominated lakes: **A** fish (bluegill + largemouth bass, $N = 21$ for each lake) and **B** Odonata (Zygoptera + Anisoptera, $N = 17$ for each lake)

and larvae (Kim & DeVries, 2001), and the latter study showed that dietary analysis may not be an adequate assessment of the true ingestion rates. Therefore, it is possible that isotope signatures reflect a substantial contribution of this seasonal subsidy to the total energy balance.

Largemouth bass consume invertebrates as well as fish (e.g., Dibble & Harrel, 1997), and it is therefore not surprising that this species had a higher trophic position than bluegill. Trophic position of largemouth bass was within the range reported in the literature (3.9–4.4, Vander Zanden et al., 1997), except for one of the invaded lakes, which had higher TP. Previous enclosure study demonstrated that largemouth bass tended to be more piscivorous in watermilfoil than in native pondweed (Dibble & Harrel, 1997); however, small-scale studies may not be easily extrapolated to the lake scale.

Since there was no difference in the trophic position of largemouth bass, the most abundant top predator in the system, we can conclude that food chain length was not different between lakes with and without the watermilfoil. Previous studies demonstrated that food chain length in aquatic ecosystems can be influenced by lake area and species richness (Vander Zanden et al., 1999b) as well as ecosystem type (Hoeinghaus et al., 2008). Major proximate mechanisms responsible for changes in food chain length are change in apical predators, insertion of intermediate predators and change in the degree of omnivory, and often two or more mechanisms are necessary to elicit a change in food chain length (Post & Takimoto, 2007). Since our lakes were similar in size and community structure, even if changes in omnivory had occurred, they may not have been sufficient to translate into differences in food chain length.

Similar to the situation with fish, no difference in trophic position was detected for invertebrate predators in the invaded lakes. Odonata are highly generalist predators, feeding on chironomids, macrophyte-associated cladocerans and many other aquatic invertebrate taxa (Pritchard, 1964; Thompson, 1978). There is evidence that most prey are consumed in proportion to their availability in the environment (Thompson, 1978; Blois, 1985), whereas other studies show that certain prey are preferred by some taxa (e.g., Folsom & Collins, 1984, for *Anax*). We observed no differences in odonate $\delta^{15}\text{N}$, which could be due to insufficient differences in prey isotopic signatures, making it impossible to resolve small dietary changes. Indeed, as most of Odonata's prey are primary consumers of detritus, periphyton or phytoplankton (e.g., Thompson, 1978 and references therein), similar in TP and presumably $\delta^{15}\text{N}$, the hypothesized differences in Odonata TP would most likely result from different degrees of cannibalism. Cannibalism in Odonata may be intraspecific as well as intraguild, e.g., when larger dragonflies with tearing mouthparts, such as Aeshnidae, feed on damselfly larvae (Pritchard, 1964). Yet, even though cannibalism was observed in controlled conditions (Robinson & Wellborn, 1987), particularly coupled to food limitation (Johansson, 1993), it was negligible in many field studies, possibly due to differences in habitat use and activity levels (Thompson, 1978 and references therein). It is therefore not surprising that we observed low variability in odonate trophic position in our study.

Unlike the trophic position, isotopic niche width was significantly different in watermilfoil-dominated lakes. A previous study documented decreased niche width of a generalist fish predator as a result of habitat fragmentation (Layman et al., 2007b). On the contrary, in our study, which explored habitat degradation due to invasive species, isotopic niche was greater in lakes dominated by non-native plants. It is interesting that the expansion of trophic diversity did not occur in the same direction: as $\delta^{13}\text{C}$ of invertebrate predators became less depleted, fish in fact shifted the other way. This could potentially indicate utilization of new, less ^{13}C -depleted sources by the invertebrates, uncoupled from the fish compartment of the food web. This result is supported by a concurrent study examining carbon flows from basal resources to abundant macroinvertebrates in invasive-plant dominated stands (Kovalenko and Dibble, unpubl.). Other variables that could affect consumer $\delta^{13}\text{C}$ signatures are water $\delta^{13}\text{C}$ and consumer isotopic fractionation; however, both of these could have resulted in a lake-specific change in consumer $\delta^{13}\text{C}$ signatures but not in isotopic niche width.

An increase in isotopic niche without a concurrent increase in TP indicates an increase in non-omnivorous generality, or feeding on several carbon sources within a trophic level (sensu Romanuk et al., 2006). Higher non-omnivorous generality has been previously associated with increased population stability (Romanuk et al., 2006). It is possible that populations may experience selective pressure for increasing generality to counteract stress of habitat change. This interesting possibility needs to be examined across a gradient of invasive plant abundance or other disturbance.

Isotopic niche, or trophic diversity, is different from the traditional dietary niche: the latter shows number of prey types, whereas the former relates to number of distinct basal sources utilized in the food web. We have previously observed a negative relationship between habitat complexity and the number of prey types consumed by the fish, yet this pattern was not observed with increasing density of highly complex watermilfoil (Kovalenko et al., 2009). Coupled with the present data, this suggests that some invasive plants are not used by the fish in the same way as native plants, confirming results of previous studies (Dibble & Harrel, 1997; Kelly & Hawes, 2005). One could argue that, because native

plant dominated lakes were repeatedly treated for watermilfoil prior to this study, observed differences may be due to the treatment rather than watermilfoil dominance per se. However, we consider this unlikely, because a previous study showed that even immediately following the treatment there was no change in the number of prey types consumed by the fish (Kovalenko et al., 2009).

We conclude that presence of invasive watermilfoil was associated with changes in trophic diversity but not trophic position of secondary consumers. To our knowledge, this is the first study to attempt analysis of odonate trophic position and isotopic niche in response to environmental stress. In both odonates and fish, trophic diversity appears to be a more sensitive indicator of environmental disturbance than trophic position. Overall, this study contributes to understanding of the food web impacts of invaders, which is essential for assessing functional recovery of aquatic ecosystems. This approach could be adapted for assessment of fish and invertebrate community restoration after removal of non-native plants, although we recommend it to be tested in more lakes, including those without the history of invasion.

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