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MULTIPLE STRESSORS AND MULTIPLE INVADERS IN LINKED STREAM-
RIPARIAN ECOSYSTEMS: COMBINED RESEARCH AND PEDAGOGY
CONTRIBUTIONS

by

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Dedication

In memory of my grandmother, Shirley Ann Heinrich

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Multiple stressors and multiple invaders in linked stream-riparian ecosystems: Combined research and pedagogy contributions

Dissertation Abstract—Idaho State University (2015)

Streams and riparian areas are closely linked and essentially operate as one ecosystem with reciprocal fluxes of energy, materials and organisms. The linked stream-riparian ecosystem is inherently complex, dynamic and affected by factors that interact across multiple spatial and temporal scales. Multiple stressors can alter these stream-riparian ecosystems with effects that propagate in both directions between land and water. Additionally, multiple invasive species are involved in a variety of complex interactions that can lead to profound consequences for native communities and ecosystem processes. I studied the effects of multiple stressors and multiple invasive species on southeastern Idaho stream-riparian ecosystems. My work had three components. First, I investigated the effects of both riparian vegetation loss and invasion by New Zealand mudsnail (*Potamopyrgus antipodarum*) on in-stream macroinvertebrates, emergence of adult aquatic insects, and the abundance of terrestrial insectivores along the Portnuef River. I found that riparian habitat loss did reduce web-weaving spider abundance. Unexpectedly, sites with high mudsnails were more productive in that they had greater flux of insect emergence. Abundances of spiders and birds were positively related to emergence flux. Second, in Deep Creek, a representative cold desert stream and site of long term studies, I investigated the interaction between two non-native species widespread in the western USA: common carp (*Cyprinus carpio*) and Russian olive (*Elaeagnus*

angustifolia), an invasive riparian tree associated with di-nitrogen fixation. I found that the carp population increased ~4X subsequent Russian olive invasion. Russian olive made up nearly 40% of carp diets and sustained ~1/3 of carp production. In turn, subsidized carp were associated with 2-3-fold reductions in chlorophyll-a, benthic organic matter, and aquatic macrophytes, and carp that consumed Russian olive recycled up to 2X more nitrogen than those that did not. This scenario is characteristic of an “invasional meltdown” that extends across the land-water boundary. Third, I developed a guide for an inquiry-based outreach activity and collaboration between stream ecologists and students whose aim is to stimulate student understanding of ecosystem connectivity. Traditionally, exploration of ecosystems has been restricted to connections within conventionally defined ecosystem boundaries (i.e., within a stream, within a forest). Further, investigations that have treated linked ecosystems highlight unidirectional inputs. I employed a constructivist approach to explore connections across ecological boundaries and how these linkages couple habitats and organisms in their vulnerability to agents of environmental change.

For: *Aquatic Sciences*

AN INVESTIGATION OF HABITAT DEGRADATION, NEW ZEALAND
MUDSNAIL INVASION, AND PATTERNS OF INSECT EMERGENCE AND
RIPARIAN INSECTIVORES IN A SEMI-ARID WATERSHED

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Abstract

Multiple stressors can alter stream-riparian ecosystems, with effects that propagate in both directions between land and water. Riparian spiders and insectivorous birds may respond to such changes in connections between streams and riparian zones, because they not only rely upon these habitats but also consume emerging adult aquatic insects. Individual or multiple ecological stressors that decrease emergence and/or habitat may have indirect effects that reduce the abundance of riparian spiders and insectivorous birds. We examined the influence of habitat degradation and New Zealand mudsnail (*Potamopyrgus antipodarum*, NZMS) invasion on web-building riparian spiders at 4 site types (low relative abundance of NZMS with intact vegetation, low NZMS/vegetation removed, high NZMS/intact vegetation, and high NZMS/vegetation removed), along the Portneuf River in southeastern Idaho during summer 2012. We also examined in-stream invertebrate communities and insectivorous bird responses to NZMS invasion. Spider abundance was ~7X greater at sites with intact vegetation, and unexpectedly, ~3X greater at those with high NZMS. We detected no interaction between habitat degradation and NZMS invasion. Sites with high NZMS were more productive in that they had greater flux of insect emergence. In turn, abundance of spiders and birds were positively related to emergence flux of Ephemeroptera, Plecoptera, and Trichoptera taxa. However, emergence flux could have been greater prior to invasion, making it difficult to assess the effect of NZMS. Nevertheless, our results demonstrate that riparian

habitat loss can reduce spider abundance, and that riparian spider and bird abundance respond positively to emergence production.

Introduction

Streams and riparian areas are closely linked and essentially operate as one ecosystem with reciprocal fluxes of energy, materials, and organisms (Baxter et al. 2005). For example, many terrestrial insectivores such as birds, lizards, and spiders rely on the emergence of adult aquatic insects as a food source (Nakano and Murakami 2001; Sabo and Power 2002; Kato et al. 2003). Specifically, riparian spider populations can closely track availability of emerging stream insects (Marzcak and Richardson 2007) and insectivorous birds respond with increased abundance to elevated emergence production (Gray 1993; Heinrich et al. 2014). Such linkages create the potential for ecological stressors to have effects that propagate across the land-water interface.

Most stream-riparian ecosystems are subjected to more than one stressor simultaneously, yet these combined effects are less commonly studied than are those of single stressors (Townsend et al. 2008, Ormerod et al. 2010). Streams are closely connected to, and strongly influenced by their watersheds (Hynes 1975, Allan 2004). Therefore, streams are easily altered by the many stressors that affect the adjacent riparian habitat. The main stressors for streams worldwide include water extraction by diversion or groundwater pumping, channelization, dams, riparian deforestation, water pollution, biological invasion, and changes in water temperature and precipitation patterns via climate change (Poff et al. 2007, Vorosmarty et al. 2010). Of these, habitat degradation and invading species are among the leading causes of global environmental change

and biodiversity loss in ecosystems, including streams, worldwide (Vitousek et al. 1997).

Habitat degradation and invasive species are often investigated as independent stressors (Fazey et al. 2005), but may act synergistically (Didham et al. 2007). One of the most common types of habitat degradation in riparian ecosystems is the loss of vegetation. Removal of riparian vegetation decreases shade which alters stream temperature and primary production, alters channel morphology by reducing bank stability and delivery of wood, and decreases input of terrestrially-derived organic material important to the stream food web (Gregory et al. 1991; Wallace et al. 1997, Poole and Berman 2001). Often coincident with such degradation, biological invasions are common in freshwater systems and include a wide variety of organisms that can develop enormous populations (Strayer et al. 2010). For example, in the Intermountain West of the United States, the invasive New Zealand mudsnail (NZMS) has been found at densities exceeding 500000 individual m^{-2} (Richards et al. 2001; Hall et al. 2003). These snails can dominate in-stream macroinvertebrate secondary production, shift assemblages of primary producers, and alter the flow of energy and nutrients in stream ecosystems (Strayer et al. 1999; Hall et al. 2003; 2006; Cross et al. 2010; 2013). Because both vegetation removal and invasive species can alter primary production and stream food webs, the two together may have cumulative effects, including those that propagate to the riparian ecosystem via changes in insect emergence, with consequences for terrestrial insectivores. However, this possibility has not been investigated.

The goal of this study was to investigate the effects of single and multiple stressors on linked stream-riparian ecosystems, by examining insect emergence abundance and flux, and terrestrial insectivore (spiders and birds) abundance responses to habitat degradation and NZMS invasion. We hypothesized that the two stressors' combined effects would reduce insect emergence and riparian insectivore abundance. Our specific objectives were to quantify and compare aquatic insect abundance, biomass, and emergence flux, and spider and insectivorous bird abundance at sites with and without riparian habitat degradation and the invasive NZMS.

Methods

The Portneuf River, located in southeastern Idaho, is a fifth order river that flows approximately 150 km from headwaters to its confluence with the Snake River at American Falls Reservoir and drains 3,500 km², with elevations ranging from 1,330 to 2,825 m. Mean discharge ranges from 1.6 to 24.8 m³ sec⁻¹. The river, like many in the Intermountain West, has experienced both riparian habitat degradation and the recent invasion of NZMS. The river has been characterized as impaired by the Idaho Department of Environmental Quality, is heavily affected by agricultural, urban, and industrial land uses, and is subjected to direct and indirect nutrient loading, riparian and river channel degradation, and hydrologic regime alteration (IDEQ 1999). The upper drainage includes areas where riparian fencing has allowed naturally occurring vegetation to flourish, creating patchiness in streamside vegetation conditions. The Portneuf River was extensively studied both before (Minshall and Andrews 1973) and after the invasion of NZMS (Hopkins 2007; Hopkins et al. 2011). Mudsnaills were first documented in the Portneuf River in 2000, and have since been identified as a high priority for control because of their potential impact on the diversity and abundance of native species, the ecological stability of infested waters, and the commercial, agricultural, and recreational activities dependent on these waters (Idaho Invasive Species Council 2007). In 2004-2006, Hopkins (2007) found that NZMS dominated the community structure at most sites along the Portneuf River, reaching densities greater than 400000 individuals m⁻². Hopkins (2007) hypothesized that competition among NZMS and native macroinvertebrates

could have reduced the abundances of riverine insects, particularly Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa. Mudsails are able to survive out of water for several weeks and can move rapidly (as much as 60 m over a 3-month period), allowing their transportation to new environments (Zaranko et al. 1997). These traits allow it to dominate invertebrate communities and out-compete native invertebrates (Kerans et al. 2005). Our study was conducted at 12 sites, during the summer of 2012, along the upper Portneuf River between Chesterfield Reservoir and the city of Pocatello, ID (Figure 1).

We used 4 site types: low relative abundance of NZMS with intact vegetation, low NZMS with vegetation removed (due to grazing or other agricultural land use), high NZMS with intact vegetation, and high NZMS with vegetation removed ($n = 3$ for each site type). Sites labeled 1, 5, 7, 11, and 12 were the same as those used in previous studies with recognized differences in NZMS densities (Minshall and Andrews 1973, Hopkins et al. 2011) and encompassed 150 m of stream length. Originally, the study was designed to examine NZMS effects on emerging insects and riparian insectivores (spiders and birds) and only included sites 1, 5, 7, 11, and 12, but preliminary results caused us to re-evaluate our design/comparison. Thus, we added 7 additional sites to also explore the effect of habitat degradation (Figure 2) and test for a possible interaction with effects of NZMS. These additional sites were chosen to encompass similar gradients in stream size and channel morphology but differed in NZMS densities and presence of riparian vegetation.

Because of their potential importance in mediating the river insect assemblage and productivity, we estimated average stream temperature, channel width, depth, habitat type, substrate composition, and in-stream macrophyte cover at all sites. Water temperature data were collected with HOBO data loggers (Onset Corporation, Bourne, MA, USA) at each site through the duration of the study. Transects were established every 10 m along the entire length of each site. Channel width was estimated by measuring the wetted widths and channel depth was an average of 5 depths measured across each transect. Habitat type and macrophyte cover were estimated for each 10-m section of the site and summed to calculate the proportion of each major habitat type (depositional, erosional) and percentage macrophyte cover. To estimate substrata composition in the stream, we measured substrate particle sizes where in-stream macroinvertebrate samples were collected (see below). Substrata categories were based on a modified Wentworth scale (Cummins 1962).

We sampled in-stream macroinvertebrates at all 12 sites at the end of the summer in 2012. Ten samples were collected to quantify and compare the invertebrate abundance and biomass, and to determine NZMS densities. Samples were collected at random locations within each study reach using a Surber sampler (sampling area = 0.096 m², 250-µm mesh). Substrata within the sampler frame were disturbed and larger particles scrubbed in the flowing water through the entrance of the collecting net. All of the sample contents were rinsed onto a 250-µm sieve and placed into a plastic bag and preserved in ~70% ethanol. In the lab, macroinvertebrates were sorted from the sample, identified to family-

level, counted, and measured to the nearest mm (total body length). Biomass estimates for all taxa were calculated using taxon-specific length-mass relationships obtained from Benke et al. (1999), Hall et al. (2006), and Miyasaka et al. (2008).

We sampled spider (Arachnida) abundance by conducting two observer counts (Nichols et al. 2000) at night starting at approximately 2200, under complete darkness when spiders are most active (Kato et al. 2003). Counts were made on the same or successive nights covering 60 m of stream edge. The observers worked upstream, side-by-side, counting all spiders above the active channel and along the riparian zone within 1 m of the stream edge and up to a maximum height of 2.5 m above water. Individual spiders were identified to taxonomic family based on web and body morphology (Ubick et al. 2005). Spiders were divided into two groups (riparian-specialist tetragnathids and other spider families that are not riparian specialists and capture both terrestrial and aquatic prey). We focused on spiders belonging to the family Tetragnathidae, because they are known to track availability in aquatic insect emergence (Gillespie 1987; Power et al. 2004).

In order to quantify habitat conditions for orb weaving spiders (Araneoidea) at sites with intact and removed vegetation, we used methods described and developed by Benjamin et al. (2011). We counted branch density within 2.5 m above the stream surface into categories (0, 1-5, 6-25, 26-50, and >50 branches) in each 2 m segment along the spider survey section. We included live and dead branches that were <5 cm diameter at their base and >50

cm in length. We estimated total branch density in the spider survey section by summing the midpoints of the categories for each segment; we used 75 as a conservative estimate for the midpoint of the largest category (Benjamin et al. 2011).

We sampled aquatic insect emergence at sites 1, 5, 7, 11, and 12 four times throughout the summer of 2012 using sticky traps, an established method that has been used to measure emergence in a variety of ecosystems (Power et al. 2004). Along the river at each site, 10 fence posts were deployed at approximately 15-m intervals located near the wetted edge on alternating sides of the bank. Using wire, 100 cm² acetate cylinders were attached to the posts and painted with a sticky substance made of castor oil, waxes, and resins (Tanglefoot, Contech Inc., Victoria, B.C.). Traps were deployed for two week periods and all adult aquatic insects were identified to order in the lab and emergence flux was calculated using taxon-specific length-mass regressions (Sabo et al. 2002). We also investigated patterns in composition of emergence, as a function of body size because this is known to affect vulnerability and predator preference. For example, EPT taxa which are relatively large-bodied can lead to positive responses from riparian insectivores (Heinrich et al. 2014). Insectivorous birds were also counted visually and aurally at these sites to estimate abundance (birds ha⁻¹) using 50-m fixed-radius point counts between 0600 and 0900 hours for 5 minutes during early summer 2012.

Statistical Analyses

We used two-way analysis of variance (ANOVA) to assess the effects of habitat degradation and NZMS invasion on the average stream temperature, channel width, depth, habitat type, substrate composition, in-stream macrophyte cover, and two groups of spiders (tetragnathids and other spider families). All data were log transformed to reduce heteroscedasticity, and then analyzed using PROC GLM in SAS (version 5; SAS Institute, Cary, NC, USA). Regressions were used to examine relationships between insect emergence flux and benthic invertebrate biomass with NZMS density, and the relationships between emergence and riparian insectivores (spiders and birds). Site 1 was omitted from spider versus emergence analysis because it had no intact vegetation. An a priori α of 0.05 was used for all statistical tests.

Results

Stream habitat characteristics were similar across all sites types with the exception of substrate composition and % macrophyte cover (Table 1). Substrate differed between sites with high and low NZMS densities ($F_{[1,8]} = 6.59$, $p = 0.033$) with higher densities of NZMS associated with more coarse substrate.

Macrophytes were associated with high NZMS densities ($F_{[1,8]} = 28.25$, $p < 0.001$), but were highest at sites that had high NZMS densities and where vegetation had been removed (NZMS x Vegetation, $F_{[1,8]} = 5.80$, $p = 0.043$).

Mean NZMS abundance at sites ranged from ~100 to >1000000 individuals m^{-2} (Table 2). High NZMS sites averaged 340000 ± 160000 (individuals $m^{-2} \pm SE$), and low sites 1200 ± 400 . Mean macroinvertebrate abundance (individuals $m^{-2} \pm SE$), not including NZMS, ranged from 4900 ± 990 to 148000 ± 29000 . Biomass (mg dry mass [DM] $m^{-2} \pm 1 SE$) of non NZMS macroinvertebrates was between 900 ± 20 and 77000 ± 50000 and, contrary to our expectation, was positively correlated with NZMS density ($R^2 = 0.861$, $p < 0.001$) (Figure 3).

Two families dominated the web-weaving spider assemblages at the sites, Tetragnathidae (58% of all individuals, $n = 437$) and Araneidae (37%), but three other families were also observed; Lycosidae, Linyphiidae, and Pholcidae. Average tetragnathid densities were significantly higher at sites with high NZMS densities ($F_{[1,8]} = 6.16$, $p = 0.038$) and at sites with intact vegetation ($F_{[1,8]} = 11.95$, $p = 0.009$), and we detected no NZMS x Vegetation interaction (Figure 4). For the other spider families the NZMS effect was not significant, but densities

were higher at sites with intact vegetation ($F_{[1,8]} = 5.98$, $p = 0.040$), and, again, there was no NZMS x Vegetation interaction. The average branch density (branches $m^{-2} \pm 1$ SE) at sites where vegetation was intact was 8.19 ± 1.67 , versus 0.09 ± 0.05 at sites where vegetation had been removed (Table 2).

Mean insect emergence abundance (individuals $trap^{-1} \pm 1$ SE) ranged from 56.4 ± 11.6 to 181.7 ± 44.0 . Abundance was higher at sites with low NZMS, but was dominated by smaller bodied taxa (e.g., Chironomidae) relative to those at sites with high NZMS. The average flux of emerging biomass (mg DM $trap^{-1} \pm 1$ SE) ranged from 79.5 ± 20.5 to 438.2 ± 214.4 , and exhibited a strong positive relationship ($R^2 = 0.828$, $p = 0.032$) with NZMS density (Figure 5). Mean emergence (individuals $trap^{-1} \pm 1$ SE) of just EPT taxa ranged from 16.4 ± 4.1 to 60.6 ± 19.3 and EPT flux (mg DM $trap^{-1} \pm 1$ SE) from 19.3 ± 9.3 to 322.2 ± 206.7 (Table 2).

Spider and bird abundance were not correlated with total insect emergence. However, both tetragnathid spiders ($R^2 = 0.906$, $p = 0.048$) and insectivorous birds ($R^2 = 0.949$, $p < 0.001$) were positively associated with emergence flux of EPT taxa (Figure 6). Bird abundance (individuals ha^{-1}) ranged from 12.8 to 42.3 (Table 2). Common species (>10% of all birds counted) included the Barn Swallow (*Hirundo rustica*), American Robin (*Turdus migratorius*), Tree Swallow (*Tachycineta bicolor*), Cedar Waxwing (*Bombycilla cedrorum*), and Red-winged Blackbird (*Agelaius phoeniceus*). These species are primarily insectivores, with the exception of the frugivorous Cedar Waxwing and the granivorous Red-winged Blackbird which supplement their diets with

emergent insects during summer (Rodewald 2015). Bird abundance did not differ between sites with high versus low NZMS.

Discussion

We investigated the potential effect of both riparian vegetation loss and invasion by NZMS on in-stream macroinvertebrates, emergence of adult aquatic insects, and the abundance of terrestrial insectivores. Unexpectedly, we observed a positive relationship between NZMS invasion and emerging stream insects, as well as riparian spiders and birds, likely because of their positive response to EPT emergence flux. Our original hypothesis was that high NZMS abundance would reduce the number of macroinvertebrates and subsequent insect emergence. We did not observe this pattern. However, we observed clear evidence of the importance of vegetation for riparian spiders; there were 5-9X more spiders at sites with intact vegetation than at those where riparian vegetation had been removed (typically by grazing or other agricultural land use). Previous studies have shown that web-weaving spiders that occupy the riparian zone rely on terrestrial habitat, especially woody branches, to support their webs (Power et al. 2004, Laeser et al. 2005).

Emerging aquatic insects subsidize riparian food webs (Nakano and Murakami 2001, Sabo and Power 2002, Balingier and Lake 2006) and our findings further corroborate studies that have documented positive responses of insectivores to emergence. Spiders have been shown to significantly decrease in abundance when aquatic insects are reduced, with horizontal orb weavers (Tetragnathidae) exhibiting the strongest response (Marczak and Richardson 2007). Meanwhile, positive responses of insectivorous birds to aquatic insects have been shown repeatedly (Jackson and Fisher 1986, Gray 1993, Whitaker et

al. 2000, Nakano and Murakami 2001, Iwata et al. 2003, Burdon and Harding 2008). Specifically, greater emergence of larger-bodied insect taxa (e.g., EPT taxa) has resulted in a positive numerical response by riparian birds (Heinrich et al. 2014).

Interactions between channel form and riparian vegetation have consequences for emergence and insectivores. The physical template of freshwater ecosystems has a pervasive influence on biological communities and processes (Vannote et al. 1980, Wallace et al. 1995). Changes in depth, velocity, and substratum have direct and indirect influences on aquatic insects (Minshall 1984, Wallace et al. 1995). Stream geomorphology affects aquatic insect flux and insectivorous bird abundance (Iwata et al. 2003). For example, stream meanders increase sinuosity and increasing sinuosity slows velocity of a stream and allows for nutrient retention. This, in turn, results in an increase in aquatic insect emergence, which leads to a higher abundance of insectivorous birds (Iwata et al. 2003). Likewise, increased habitat heterogeneity within streams enhances aquatic insect diversity and production, positively affecting insect emergence production and responses by riparian insectivores (Heinrich et al. 2014). Additionally, relationships between insect emergence and birds have been compared among different terrestrial habitat types. For example, insect emergence and bird density were both higher within gallery forest habitats compared to prairie/shrub habitats, suggesting that bird abundance was related to vegetation, but densities of insectivorous birds in both habitat types were highly correlated with stream insect emergence (Gray 1993).

There were not any noticeable differences in channel form between our sites along the Portneuf River, but sites with high NZMS densities were more productive in general. This might be due to the influence of groundwater springs and substrate composition associated with the high NZMS sites. Previous studies on the Portneuf River found NZMS to be the most common taxon at spring-influenced sites (Hopkins et al. 2011). As a result, these sites also experience little variation in temperature. The NZMS and in-stream macroinvertebrates likely benefit from the stable and productive environment provided by the influence of groundwater springs. Further, Hopkins (2007) found NZMS density correlated with flow velocity, rock substrate, and macrophyte abundance all of which co-varied with spring influence. Other sites that have experienced the invasion of and high densities of NZMS commonly have high rates of primary production, stable hydrology, and warm temperatures (Hall et al. 2006). The highest density previously documented, to our knowledge, was 800000 NZMS m⁻² (Dorgelo 1987) in Lake Zurich, Switzerland. At site 9 on the Portneuf River, the average was 1000000 NZMS m⁻² and we counted densities as high as 2700000 NZMS m⁻². Hall et al. (2006) found that invasive NZMS exhibit some of the highest values ever reported for a stream invertebrate, and our observation are certainly consistent with this claim.

The effects of riparian habitat degradation and NZMS invasion may extend beyond the scales that we considered. For example, we did not measure fish or other terrestrial invertebrates. Riparian vegetation loss can reduce terrestrial invertebrates (Kawaguchi et al. 2003). Birds exploit aquatic prey more when their

terrestrial prey resources are scarce (Nakano and Murakami 2001). They may aggregate along streams as a result of increased food availability (from emergence) but often still exhibit preference for terrestrial insects (Murakami and Nakano 2002). Further, decreased terrestrial prey input can lead to a reduction in stream consumers, like fish whose diets are regularly composed of ~50% of this resource (Cloe and Garman 1996, Kawaguchi and Nakano 2001). Therefore, fish predation could be masking the effects of NZMS on aquatic insects. Sites with intact vegetation could be supplying more terrestrial invertebrate prey to the stream and buffer aquatic insects from predation because fish may prefer them. Furthermore, we do not know to what extent fish influence NZMS.

Linked stream-riparian ecosystems, like most ecological systems, are inherently complex, dynamic, and affected by factors that occur and interact across multiple spatial and temporal scales. Further, individual stressors can have strong negative effects, beyond which additional stressors may not have added influence (Fausch et al. 2010). There is a need to study responses to multiple stressors, including those that may propagate across the land-water interface, and its implications should be integrated into policies governing the introduction of non-native species and the management of streams and forests.

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Table 1. Stream habitat characteristics ($\pm$ SE) at the 4 site types: low relative abundance of NZMS with intact vegetation, low NZMS with vegetation removed, high NZMS with intact vegetation, and high NZMS with vegetation removed ($n = 3$ for each site type).

Site type	Stream temp (°C)	Channel width (m)	Stream depth (m)	Habitat type (% depositional)	Substrate* (% coarse)	Macrophyte** (% cover)
Low NZMS Veg Removed	18.7 (0.4)	11.8 (4.6)	0.6 (0.4)	24.4 (21.2)	20.2 (10.1)	1.7 (1.1)
Low NZMS Veg Intact	18.5 (0.6)	14.7 (3.9)	0.6 (0.2)	6.7 (6.7)	20.4 (4.5)	5.2 (3.6)
High NZMS Veg Removed	18.6 (1.4)	18.7 (3.0)	0.8 (0.2)	0.0 (0.0)	64.4 (11.6)	96.0 (1.9)
High NZMS Veg Intact	18.2 (1.3)	16.7 (4.2)	0.5 (0.1)	12.2 (0.6)	66.2 (4.0)	38.2 (22.8)

* Indicates difference ($p < 0.05$) between low and high NZMS sites.

+ Indicates NZMS x Veg interaction ($p < 0.05$) on variable.

Table 2. Mean density ($\pm$ SE) for New Zealand mudsnails (NZMS), non NZMS invertebrates, branches, spiders (Tetragnathids and other families), and insectivorous birds; and mean biomass ($\pm$ SE) of non NZMS invertebrates, emerging insects (Emg flux) and emerging Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT flux).

Site (UTM Coordinates)	NZMS m ⁻²	Non NZMS m ⁻²	Non NZMS Biomass	Branch Density m ⁻²	Tetra	Other spiders	Emg flux	EPT flux	Birds ha ⁻¹
1 (423787 4746501)	870.8 (515.9)	51126.2 (12872.0)	33003.6 (15521.3)	0.0 (0.0)	0.7 (0.4)	2.3 (0.9)	162.5 (50.2)	80.2 (20.6)	24.4
2 (423700 4746588)	1723.1 (840.8)	71076.9 (12996.3)	68442.7 (51535.9)	0.0 (0.0)	0.3 (0.2)	0.7 (0.3)	--	--	--
3 (419816 4737602)	111.5 (86.9)	52107.7 (8952.2)	12541.8 (3870.6)	4.1 (0.4)	2.8 (1.6)	2.8 (1.4)	--	--	--
4 (417965 4732810)	76000.0 (24562.7)	148276.9 (29270.6)	66165.2 (29274.6)	0.0 (0.0)	4.4 (3.1)	4.0 (2.4)	--	--	--
5 (417983 4732592)	12578.5 (2680.0)	79716.9 (12941.6)	16045.0 (3458.2)	7.3 (0.7)	36.0 (14.2)	14.2 (4.3)	438.2 (214.4)	322.2 (206.7)	42.3
6 (418040 4720403)	215.4 (52.2)	4963.5 (990.9)	927.1 (23.3)	10.1 (0.9)	1.0 (0.5)	1.5 (0.7)	--	--	--
7 (418135 4718979)	2601.5 (953.9)	47049.2 (16320.6)	76950.0 (50206.3)	3.3 (0.7)	7.2 (3.2)	9.8 (4.4)	195.3 (62.5)	53.5 (24.7)	19.2
8 (417863 4719042)	1684.6 (881.2)	13076.9 (6696.0)	8752.0 (6967.3)	0.2 (0.1)	0.0 (0.0)	0.5 (0.2)	--	--	--
9 (415926 4719515)	1024246.2 (385840.0)	28800.0 (6646.2)	12421.1 (5126.1)	0.0 (0.0)	0.2 (0.2)	0.3 (0.2)	--	--	--
10 (415309 4719451)	487446.2 (176402.3)	24738.5 (6572.4)	20599.0 (13980.6)	0.3 (0.2)	2.0 (0.8)	0.7 (0.3)	--	--	--
11 (415416 4719302)	456664.6 (82888.1)	26043.1 (4328.4)	11563.7 (2669.1)	10.2 (0.6)	4.8 (1.6)	1.5 (0.6)	87.3 (12.3)	19.3 (9.3)	17.9
12 (402042 4723780)	3485.0 (831.9)	5652.3 (985.5)	1077.6 (256.2)	14.1 (0.4)	14.2 (2.2)	16.0 (2.0)	79.5 (20.5)	25.4 (7.4)	12.8

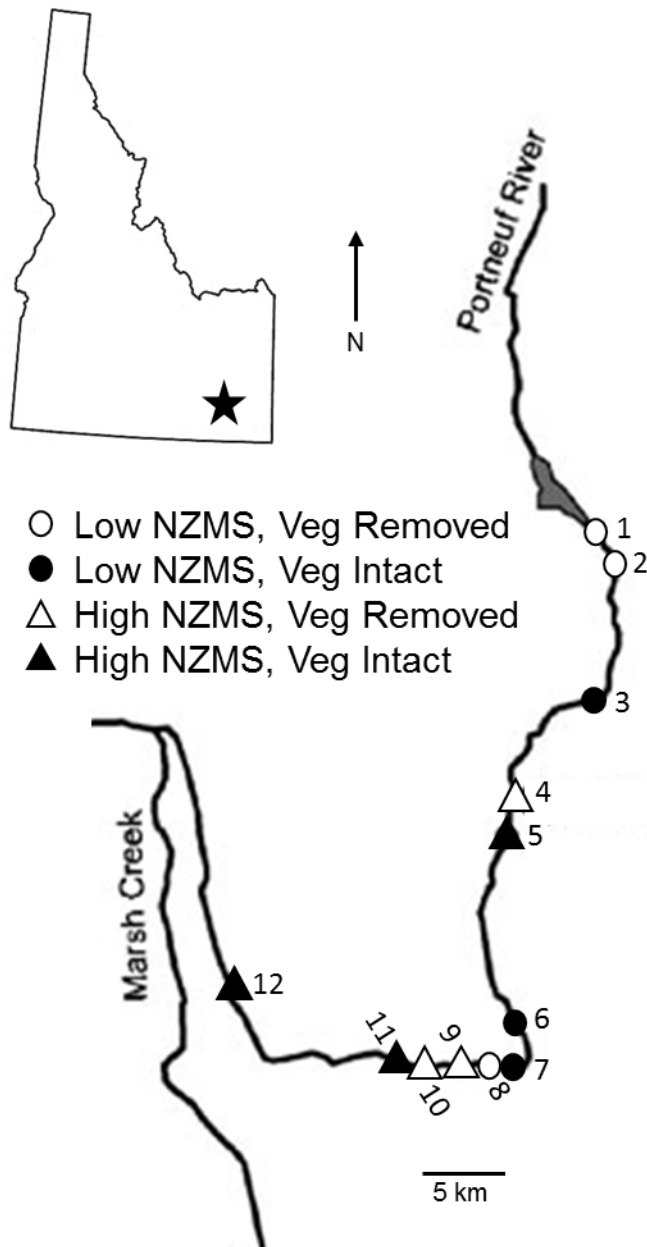


Figure 1. Map of study area and location of study sites on the upper Portneuf River in southeast Idaho.



Figure 2. Representatives of spider survey and emergence trap sites along the Portneuf River showing a site with intact vegetation (top, site 11) and a site where vegetation has been removed (bottom, site 1).

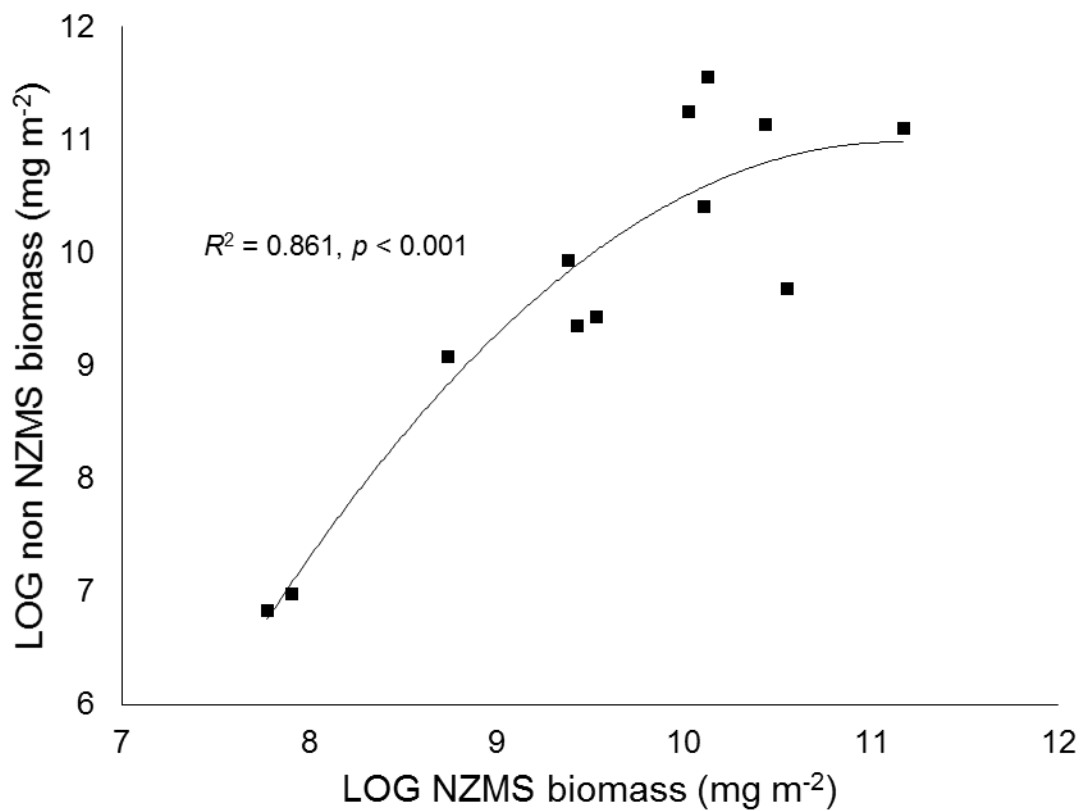


Figure 3. Relationship between mean non New Zealand mudsnail biomass and mean New Zealand mudsnail density at all sites on the Portneuf River in 2012.

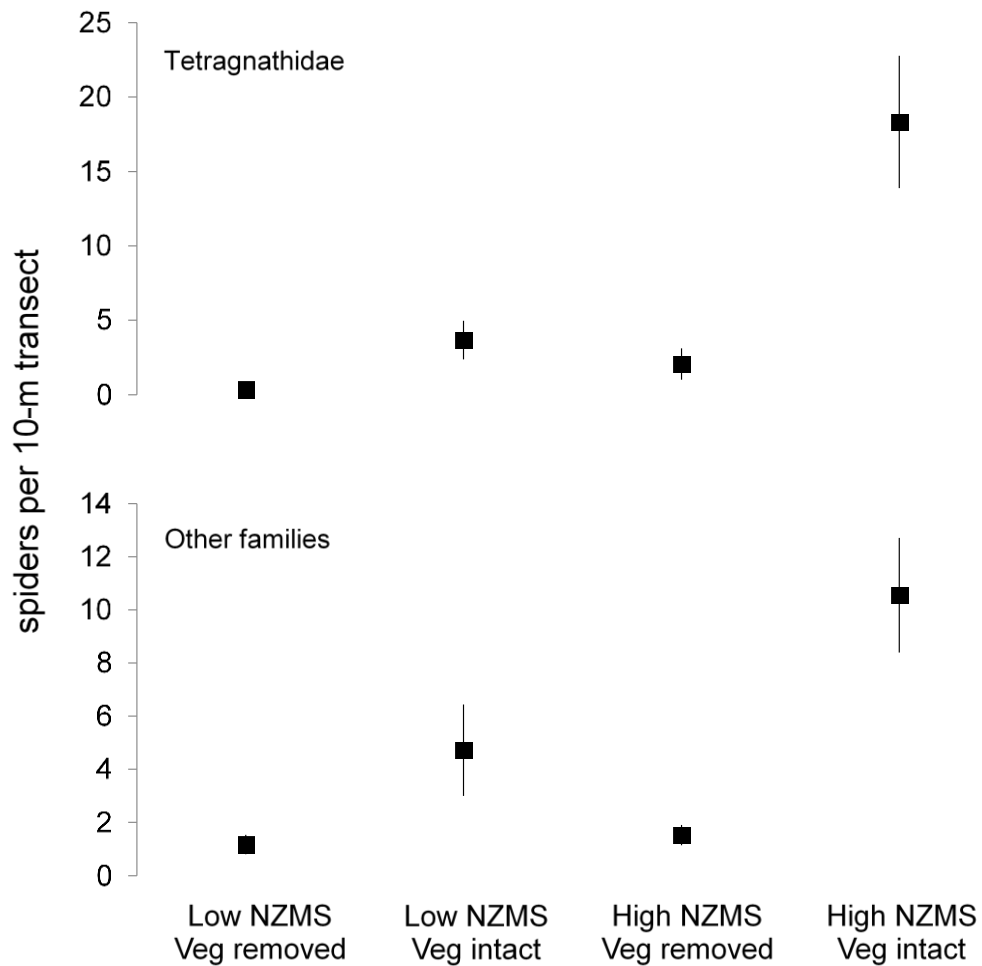


Figure 4. Numbers of tetragnathid spiders and those belonging to other web-weaving families found at sites of four types (low relative abundance of New Zealand mudsnails [NZMS] with vegetation removed, low NZMS with intact vegetation, high NZMS with vegetation removed, and high NZMS with intact vegetation) in the Portneuf River during summer 2012. *Points* show means $\pm$ SE ($n = 3$).

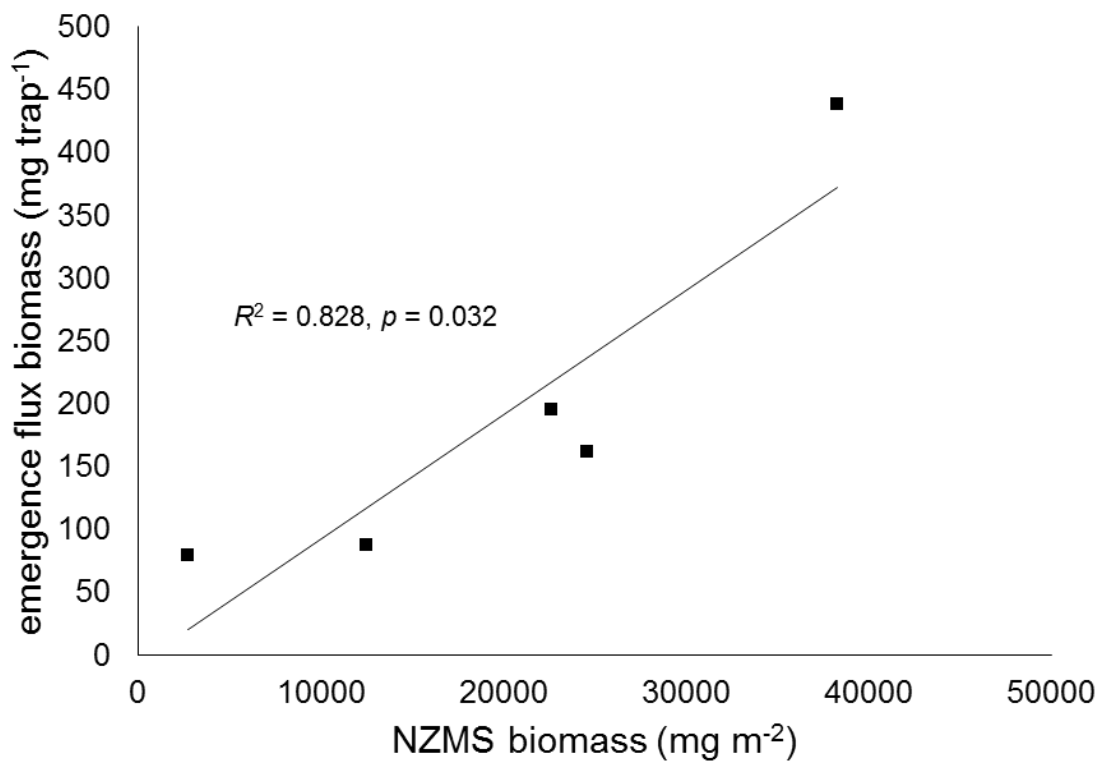


Figure 5. Relationship between mean emergence flux and mean New Zealand mudsnail density at sites 1, 5, 7, 11, and 12 on the Portneuf River in 2012.

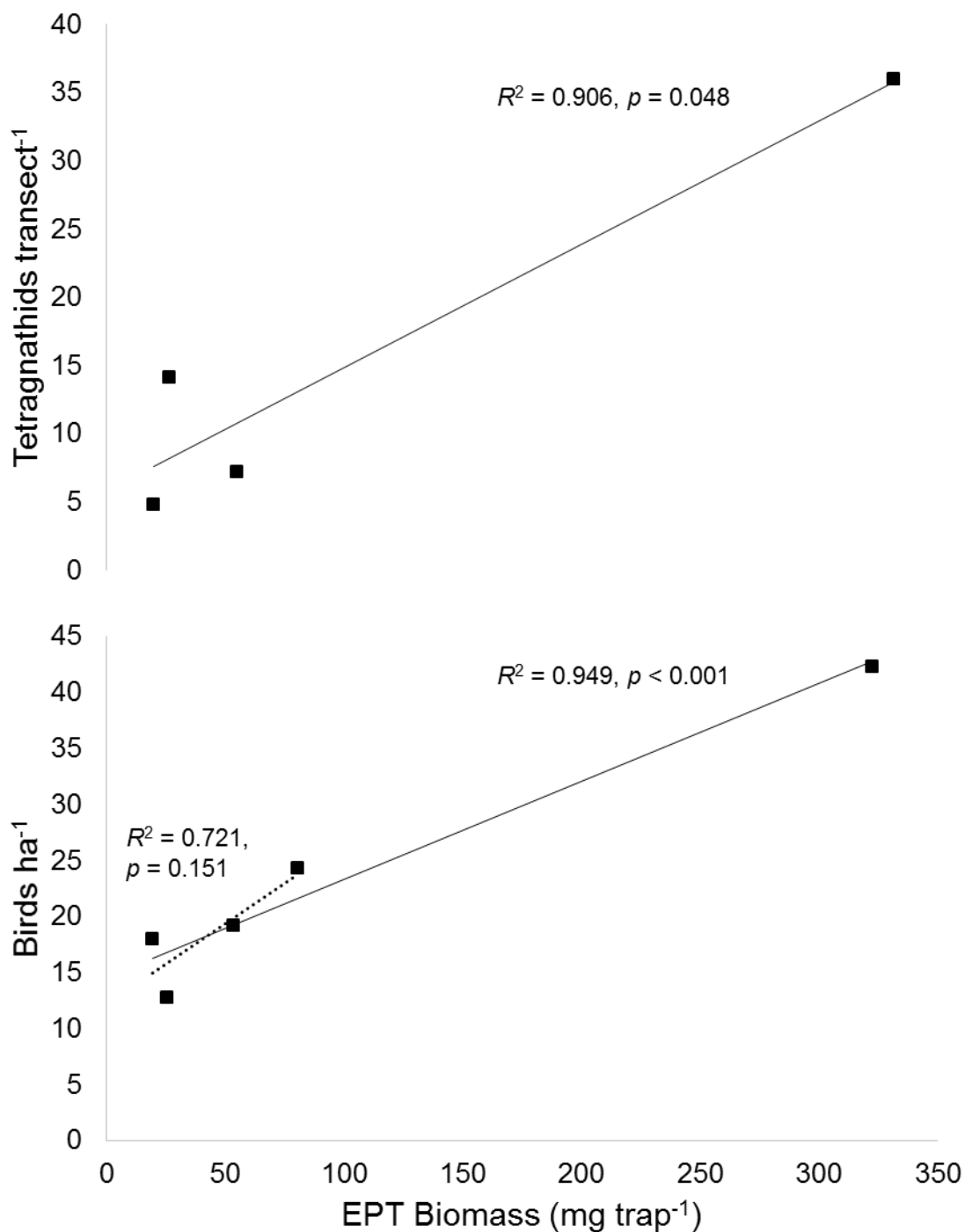


Figure 6. Top: Relationship between tetragnathid spider densities and mean EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa at sites 5, 7, 11, and 12; Bottom: Relationship between mean insectivorous bird densities and mean EPT taxa at sites 1, 5, 7, 11, and 12 on the Portneuf River in 2012.

For: *Ecology*

OF OLIVES AND CARP: INTERACTIVE EFFECTS OF AN AQUATIC AND A
TERRESTRIAL INVADER ON THE STREAM-RIPARIAN ECOSYSTEM

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angustifolia, invasional meltdown, nutrient dynamics

Abstract

Multiple invasive species may interact, influencing one another and generating synergistic effects on food webs and ecosystem processes. We investigated the interaction between two non-native species widespread in the western USA: common carp (*Cyprinus carpio*) and Russian olive (*Elaeagnus angustifolia*), an invasive riparian tree associated with di-nitrogen fixation. Deep Creek, Idaho was an International Biological Program site in the early 1970's; at that time carp were rare and Russian olive was absent. Subsequently, Russian olive was introduced and now forms a dense stand, increasing allochthonous inputs and nitrogen-rich benthic organic matter. Since 1971, carp density has increased ~4X (an increase our bioenergetic analysis suggests could not have been sustained by pre-Russian olive resources) and ~40% of gut contents consist of olives. A small-scale, short-term experimental removal of these subsidized carp caused ~3-fold increases in organic matter and chlorophyll-a concentration, suggesting they may limit algae and macrophyte biomass. Moreover, carp that consumed nitrogen-rich olives excreted 2-3X more nitrogen (ammonium, total dissolved nitrogen, and total nitrogen) compared to those that had not, which may amplify recycling and export from streams invaded by both species. This scenario is characteristic of an 'invasional meltdown,' with attendant changes in food webs and ecosystem processes.

Introduction

Many ecosystems now host multiple non-native species, and in some environments the number of non-native species outnumbers native taxa (Johnson et al. 2009). However, studies of non-native invasive species effects on one another are relatively few, and multiple invaders maybe involved in a variety of complex interactions that could lead to profound consequences for native communities and ecosystems (Simberloff and Von Holle 1999). Invaders may negatively affect one another through competition and/or predation, positively influence one another through facilitative interactions, or have no effect on each other (Simberloff and Von Holle 1999, Johnson et al. 2009). Depending on the interaction, their combined influence on native communities and ecological impacts may be diminished (Ross et al. 2004) or amplified. The latter, in addition to ecosystem impacts, could result in continual establishment and spread (Ricciardi 2001, Grosholz 2005). The scenario of potential facilitation and possible increased magnitude of impact are characteristic of an 'invasional meltdown' (Simberloff and Von Holle 1999). An absolute 'invasional meltdown,' where interspecific facilitation leads to an accelerating increase in the number of introduced species and their impact, has yet to be conclusively observed in natural systems (Simberloff 2006).

Biological invasions are numerous in freshwater systems and include a wide variety of invaders that can develop enormous populations, and many studies have demonstrated that invasions can strongly alter native populations, community structure, and ecosystem processes (Strayer 2010). However, there

is a need for studies that address both consequences for community structure and ecosystem processes, as well as the direct vs. indirect effects. Invading primary consumers (e.g., mollusks) have been shown to dominate nutrient cycling and can ingest so much photosynthetic biomass that they significantly affect the quantity and composition of primary producers (Hall et al 2003). Changing the amount and quality of primary production, as in the case of zebra mussels (*Dreissena polymorpha*), can affect nearly every part of an ecosystem (Strayer et al. 1999, Strayer 2009). Likewise, the spread of introduced, non-native salmonid fishes severely alters stream communities through direct predation on and competition with native species, as well as imparting indirect effects on food webs (Flecker and Townsend 1994, Simon and Townsend 2003).

Biological invasions can interrupt resource flows, like the cycling of nutrients, and have far reaching effects on interconnected ecosystems (Hall et al. 2003), including impacts that can propagate across the land-water interface (Baxter et al. 2004, Benjamin et al. 2011). Recently, the framework and approaches of ecological stoichiometry have become a focus for improved understanding of ecosystem nutrient dynamics and the influence of organisms on these processes (Sturner and Elser 2002, Sitters et al. 2015). Invasive species may directly affect nutrient storage and cycling through growth, and excretion or egestion (Capps and Flecker 2013, Capps et al. 2015). Stoichiometry theory predicts that nutrient recycling by a consumer depends upon the imbalance between the nutrient content of the consumer and its food. All else being held equal, a consumer will excrete or egest more of a nutrient if the proportion of that

nutrient in its food is higher than its stoichiometric requirements. Moreover, the ratios of nutrients that are recycled should also behave according to stoichiometric principles. For example, a consumer with a low body nitrogen:phosphorus (N:P) ratio should excrete or egest nutrients at a higher N:P ratio than a consumer with high body N:P, if the two consumers eat the same food (Sterner and Elser 2002). Species invasions may influence nutrient storage and recycling by shifting the stoichiometry between consumers and their resources (Capps et al. 2015), but this has only begun to be investigated.

One of the most common woody riparian species in the western United States is Russian olive, *Elaeagnus angustifolia* (Friedman et al. 2005). Russian olive is an invasive, non-native riparian tree that was introduced to this region in late 1800s (Katz and Shafroth 2003). It was intentionally planted as an ornamental, for windbreaks, erosion control and wildlife enhancement, but escaped cultivation throughout Idaho in the 1950s (Christiansen 1963, Knopf and Olson 1984). Factors that contribute to the establishment and spread of Russian olive include its large seed size and seed longevity, horticultural cultivation, and altered flow regimes (i.e., damming and irrigation water withdrawals) (Katz and Shafroth 2003). It is a deciduous tree that adds a large flux of allochthonous litter to streams along which it invades, and this material is slow to decompose (Royer et al. 1999) and thus reduces stream ecosystem efficiency (Mineau et al. 2012). Russian olive also has a high capacity to fix N_2 through microbial association and has been shown to change in-stream nutrient dynamics (Mineau et al. 2011).

Russian olive has the potential to interact with other invasive species in stream-riparian ecosystems, but this possibility has not yet been studied/evaluated.

Another invasive, non-native species common to streams along which Russian olive are spreading is the common carp (*Cyprinus carpio*). The common carp is one of the most pervasive non-native fish in the Western US and one of the most destructive invasive fish species to natural ecosystems (Zambrano et al. 2006). Millions of dollars are spent annually by natural resource agencies to control common carp populations in the United States (Cole 2005). They were brought to the US in 1831 (DeKay 1842) and were first introduced to Bear Lake and Oneida Counties, Idaho in 1882 (American Fisheries Society 2013). Carp are highly fecund; a single female is capable of laying over a million eggs in a year (Bajer and Sorensen 2010). In the Midwestern US, common carp have been found to reach especially high densities (up to 1,000 kg ha⁻¹), likely making up the majority of the fish biomass in the region (Bajer and Sorensen 2010). In Europe, invasive carp appear to achieve only about one-tenth of the superabundance observed in the US, causing many to speculate as to why some regions are better suited than others for this highly invasive fish (Crivelli 1983). Studies identifying processes that might explain their restricted pattern of superabundance are generally lacking (Bajer and Sorensen 2010).

Carp can influence water quality and nutrient dynamics and have been shown to reduce aquatic macrophytes and macroinvertebrate abundance (Parkos et al. 2003, Matsuzaki et al. 2007). Through their benthic feeding, carp affect bottom-up processes, modifying nutrient and turbidity concentrations and

primary producer abundance and diversity, but may also exert top-down effects by preying upon invertebrates or changing the foraging condition for other fishes (Weber and Brown 2009). Additionally, carp can have strong direct and indirect impacts on nutrient dynamics and littoral community structure through their bioturbation and excretion (Matsuzaki et al. 2007). Such impacts have been well documented. However, the effects of carp may also be mediated or amplified by interactions with other invaders, including Russian olive, but this has not been investigated.

Here we investigate the interactive effects of a terrestrial (Russian olive) and an aquatic (common carp) invader on stream ecosystems. Further, we explore of how ecological stoichiometry of invasive, non-native consumers and their resources may influence critical ecosystem processes, such as the cycling of N and P in streams. Our study was conducted through a combination of comparative and experimental studies in southeast Idaho, at Deep Creek. Multiple reaches of Deep Creek were extensively studied as a representative cool-desert stream during the International Biological Program (IBP) in the early 1970's (Minshall et al. 1972, Minshall et al. 1973, Minshall 1978). The same reaches were recently studied after Russian olive invaded (Mineau et al. 2011, Mineau et al. 2012). We hypothesized that the invasion of Russian olive facilitated the invasion of the common carp, and that the synergistic impacts of these subsidized carp include consequences for in-stream primary producers, organic matter standing stocks, and nutrient dynamics. Our specific objectives were to: (1) quantify the spread of Russian olive at the study site; (2) examine

and compare carp abundance pre- and post-Russian olive invasion; (3) evaluate the contribution of Russian olive to the diets and population size of carp; (4) determine the effects of carp that are subsidized by Russian olive on stream macrophytes, periphyton, and organic matter standing stocks; and (5) investigate the influence of carp consuming Russian olive on the nutrient content and stoichiometry of carp excretion.

Methods

Study Site

Deep Creek, of southeast Idaho, is a spring fed stream located in the sagebrush steppe ecoregion in the northern Great Basin watershed (42.11°N, 112.67°W; elevation 1457 m). It is typical of most streams in the Intermountain west that are spring influenced. Its characteristics include a common assemblage of species, as well as a legacy of water withdrawal for irrigation, and land use (particularly cattle grazing). Multiple sites were studied during the IBP studies in the 1970s, but at one site (IBP “site 2”), Russian olive was introduced for bank stabilization in the 1980s and now forms a dense stand (Figure 1). Mean annual water temperature at this site is 18°C and the mean annual discharge is 0.635 m³ sec⁻¹, including periods of low flow in the summer during water withdrawals. Patterns of water use have remained consistent throughout the decades between past and present studies at the site. We focused on this site because of its degree of Russian olive invasion and because previous work considering the effects of Russian olive (e.g., increases in allochthonous inputs and benthic organic matter, and changes in nutrient dynamics) was also conducted at this site (Mineau et al. 2011, Mineau et al. 2012). Fish population data were collected during the IBP and our general observation of an increase in carp subsequent invasion of Russian olive was part of the motivation for this study.

Russian olive at Deep Creek

To assess spread of Russian olive, and fluctuations in its dispersal over time, historical coverage of the total area of Russian olive imagery (1992, from Google Earth) was compared with recent digital imagery for the county (2011) from the National Agriculture Imagery Program (NAIP). Riparian vegetation along a 4500 m transect was digitized using ArcGIS. After polygons from the 2011 NAIP image were digitized, the 1992 image was georeferenced and also digitized. To assess the differences between the digitized polygons, total shape areas and average shape areas were quantified and presence/absence of Russian olive along the transect was calculated.

Carp population

The current abundance and size structure of carp in the Deep Creek study section was estimated using a combination of electrofishing and visual (underwater and bank-side) surveys, and these estimates were compared to historic data collected during the IBP. Fish were sampled using a minimum three-pass removal procedure with a backpack electrofisher (LR24; Smith-Root, Vancouver, Washington). Sample reaches (same locations as those sampled during the IBP) were approximately 100 m in length and were blocked at the upstream and downstream ends using 4.8-mm mesh nets. Captured fish were measured (total length and fork length; nearest mm), weighed (mass; g), and then returned to the stream. The IBP studies employed this method, but only documented abundance. Therefore density was used as the basis of comparison

between IBP studies and the present. Electrofishing surveys encompassed only short reaches and we were interested in a broader assessment of the population throughout the study section and throughout the year. Because nearly all carp were large adults and water clarity was high, we were able to complement electrofishing surveys with weekly bank side counts during both years of our study in order to increase the frequency and the coverage of counts with minimal in-stream disturbance. Bank side point counts were conducted along 18 randomly located 20-m reaches of the stream for a 5-minute period by at least 2 observers. Further, in spring 2014 we conducted monthly underwater surveys via mask and snorkel (Li and Li 2006) for 3 randomly located 100-m reaches of the stream. To reduce double-counted or missed fish, counts were conducted by two snorkelers, one fixed at the upstream end of the reach and one who started at the downstream end and worked upstream. Comparisons for carp population estimates were made between all 3 techniques (when surveys overlapped) and did not differ. Biomass of carp, both current and historic, was estimated by multiplying population estimates by the mean weight of carp collected. Carp during the IBP studies were described as predominantly large adults, similar in size to the current resident carp (G. W. Minshall, Idaho State University, personal communication), so we used the current mean weight to estimate the historic population biomass.

Carp diets and stable isotope analyses

To quantify the diets of common carp, individuals were collected from Deep Creek seasonally during electrofishing surveys in 2013 and 2014 (63 total guts). Fish guts were dissected in the field, immediately placed on ice, and transported to the lab for analysis. Diet analysis was conducted using methods similar to Higgins et al. (2006). The fore-most 1/5 of gut contents were processed using a dissecting microscope and contents sorted into the following categories: Russian olive material, terrestrial plant material, aquatic plant material, detritus of unknown origin (hereafter “amorphous detritus”), and aquatic macroinvertebrates. Materials from each category were dried at 60°C for 48 hours, and weighed to obtain dry mass (g) and determine percentage contributions of each to gut contents.

To supplement gut content data and determine the extent to which Russian olive material was assimilated by carp, we conducted stable isotope analyses. In spring 2014, we collected carp muscle tissue, as well as samples of the major items present in carp guts for analyses. Carp muscle tissue was collected from fish collected for gut content analysis, Russian olive material (olives and leaves) was collected from trees, the dominant macrophyte (*Potamogeton spp.*; syn. *Stuckenia spp.*) from the streambed, and macroinvertebrates were collected using a kick net and sorted visually in the field. Filamentous algae (*Cladophora spp.*) and other periphyton were rarely observed in carp guts, but because we wanted to include them as potential basal resources in our analyses, we used values obtained as part of a previous, recent

study conducted at Deep Creek (Mineau 2011). All tissue samples were dried at 60°C for 48 hours, then cooled in a desiccator. Once dried, samples were homogenized using a mortar and pestle and were weighed and encapsulated for isotopic analysis of ^{13}C and ^{15}N on an isotope ratio mass spectrometer (ECS 4010 elemental analyzer, Idaho State University Interdisciplinary Laboratory for Elemental and Isotope Analysis). We used the mixing model software IsoSource (Phillips & Gregg 2003) to calculate the relative assimilation by carp of C and N from different sources using the isotope data with tolerance set at 0.05, and assumed a literature based (Post 2002) trophic transfer shift of +0.4 per mil for C and +3.4 per mil for N. Because there was poor differentiation between $\delta^{13}\text{C}$ values of basal resources (particularly Russian olive and *Cladophora*), we re-ran the mixing model software using $\delta^2\text{H}$ (deuterium) to evaluate resource use (Doucette et al. 2007). $\delta^2\text{H}$ values for all of the basal resources were also obtained from Mineau (2011).

Bioenergetic analysis of carp

We estimated the demand for food by the carp present in Deep Creek and compared it to the available resources pre- and post-Russian olive invasion at the site. We used published values of carp bioenergetics (Huisman 1976, Lupatsch et al. 1998) to determine the organic matter requirements of individual carp at Deep Creek. Scaling these values, we calculated the demand for the estimated population at Deep Creek and compared this to the availability of resources before and after Russian olive invasion. Availability of resources was

calculated from organic matter production and standing stock estimates for Deep Creek during the IBP and post-Russian olive invasion, after Mineau et al. (2012). Further, daily consumption of Russian olive material by carp was calculated (using methods from Bajkov 1935) and compared to resource availability. Daily consumption (D) was calculated as:

$$D = A \left(\frac{24}{n} \right)$$

Daily food consumption by fish in the field was estimated from the average amount of food (A) in the guts at the time of sampling (which we measured) and the number of hours (n) necessary for complete gastric evacuation (which we estimated, based upon published values). We used these estimates to compare and evaluate whether or not the current carp population's demand could be met by resources available prior to Russian olive invasion.

Manipulative experiment

To investigate the impacts of subsidized carp on stream ecosystem organic matter resources we conducted a carp exclusion experiment. The experiment lasted for a two-month period during spring 2013, when carp densities were highest. We sampled on both day 30 (mid point) and day 60 (end point). The experiment was modeled after a similar study that investigated the ecological role of *Prochilodus*, a detritivore that dominated fish assemblage biomass, in a tropical stream (Flecker 1996). Like *Prochilodus*, carp in Deep Creek could be selectively excluded from portions of the stream because it was the case that nearly all of the carp present were large adults. In order to quantify the effects of

carp, the experiment was set up with a carp exclusion treatment ($n = 7$) and an open cage control ($n = 7$). Cages were 2 x 2 m in dimension, constructed of poultry wire (25 mm mesh size) and supported by fence posts (Figure 2). Cages were built without floors so that the bottom of each cage was natural stream and control cages were open on the downstream side to allow visitation of carp. Sampling events involved depth and velocity measurements and estimation of substrate composition and macrophyte cover within each cage. In each cage, three locations were randomly selected for a core sample in which we collected periphyton and benthic organic matter. First, a stovepipe corer was inserted carefully onto the stream bottom. We gently stirred the water to suspend only the surficial organic matter in the corer without greatly disturbing the larger mineral sediment (Hall et al. 2011). A small sample was collected and filtered (glass fiber filters; 1.6 μm particle retention size) for periphyton and placed on ice in the field and later frozen until laboratory analysis of ash free dry mass (AFDM) and chlorophyll-a following standard methods (APHA 1995). After the periphyton sample was taken, all enclosed core material was removed with a cup to a depth of ~10 cm into the substrata and placed into a bucket. Material within the bucket was stirred and elutriated through a 250 μm sieve until no particulate organic material remained in the bucket. All matter retained on the 250 μm sieve was rinsed into a plastic bag and preserved with ~70% ethanol. Substrata composition and sample volume were estimated for each core sample based on materials in the bucket. We also sampled very fine particulate organic matter (VFPOM, < 250 μm). A ~250 mL subsample from the initial elutriated stovepipe

core sample was collected in a cup as it passed through the 250 μm sieve. Total volume of the sample was recorded, and the contents within the bucket and sieve discarded. All VFPOm samples were placed in a cooler on ice to reduce decomposition until they were processed.

In the laboratory, we rinsed samples through stacked 1 mm and 250 μm sieves to separate coarse (>1 mm) fractions from fine (<1 mm, >250 μm). Coarse fractions were examined in a sorting tray under a Fiber-Lite® MI-150 high intensity illuminator and all macroinvertebrates were removed. We sorted coarse particular organic materials (CPOM) into Russian olive material, aquatic vegetation, leaves, sticks, bark, seeds, and amorphous detritus. Any unrecognizable material was placed into a miscellaneous CPOM category. CPOM and FPOM subsamples were dried at 60°C for 48 hours. Samples were then cooled in a desiccator, weighed to the nearest 0.001 g, and ashed in a muffle furnace at 500°C for >4 hours. Samples were returned to the desiccator, cooled, reweighed to estimate ash-free dry mass (AFDM), and corrected for area sampled to yield g AFDM m^{-2} .

For VFPOm, within 24 hours of field collection, we re-suspended samples using distilled water and filtered 10-250 mL of the suspension through pre-ashed and weighed glass fiber filters (1.6 μm). Filtered VFPOm samples were processed using the same method as FPOM and CPOM. Filters were rewetted with distilled water, returned to the drying oven for 24 hours, and then reweighed to estimate AFDM. Values were corrected for original volume collected and area sampled by the corer to yield g AFDM m^{-2} .

Carp excretion

To address the hypothesis that carp subsidized by Russian olive influence nutrient recycling, we measured rates of their excretion and egestion of nitrogen and phosphorus. Carp were collected from Deep Creek via electrofishing during three sample periods in early, mid, and late spring of 2013 and 2014. We compared excretion by individual carp with variation in the quantity of Russian olive in their guts. Additionally, in early and late Spring 2013 (to coincide with Deep Creek sampling), a small number of carp ($n = 10$) were collected at a nearby site from a similar, spring influenced stream, the Portneuf River. This site has no Russian olive, so it provided a comparison of excretion by carp that had likely never consumed Russian olive versus those at Deep Creek. All excretion experiments were conducted following methods similar to those of Schaus et al. (1997). In brief, carp were first placed in a holding tank for observation (~20 minutes) to allow for recovery after their capture. Next, carp were transferred to a cooler with 5 gallons (19.9 L) of filtered, bottled water whose conductivity was adjusted to match in-stream conductivity by addition of small amounts of salt. 'Before' samples were taken after the carp had been in the cooler for 5 min to allow for a short adjustment period and to account for any nutrients present, that may not have been the result of excretion. 'After' samples were collected 30 min after the 'before' sample. Both filtered (0.45 μm) and non-filtered samples were collected (to measure rates of excretion and egestion, respectively) and analyzed for the following: filtered - NH_4 , SRP, TDN, and TDP; non-filtered - total N, and

total P (SmartChem® 200 Discrete Analyzer, Idaho State University Center for Ecological Research and Education). To account for any effect of the coolers themselves, control samples were also collected from coolers without carp ($n = 5$).

Statistical Analyses

Pre- and post-Russian olive invasion carp populations and carp exclusion experiment data were compared with one-way analysis of variance (ANOVA). Regressions were used to examine the relationship between carp excretion of nutrients and the amount of Russian olive in their guts. Comparisons of nutrient excretion by carp that did versus did not consume Russian olive were analyzed with ANOVA, and analysis of co-variance (ANCOVA) was also conducted with excreted nutrients as the dependent variable and date, temperature, and fish size as separate covariates. When appropriate, data were log transformed to reduce heteroscedasticity. Analyses were conducted using PROC GLM in SAS (version 5; SAS Institute, Cary, NC, USA). Because p -values describe a “continuous measure of evidence” and are influenced by small sample size (Gelman 2013), we used a graded approach to describe our certainty that results differed from what would be expected by chance alone. Based upon this rationale, p -values <0.05 were considered significant, and p -values between 0.05 and 0.1 were considered marginally significant.

Results

Spread of Russian olive and carp invasion

Russian olive cover from 2011 at Deep Creek was ~3X greater than its total area of coverage in 1992 (Figure 3). The total area of the polygons digitized from the 1992 image (Google Earth) was 22109 m², the average polygon area was ~221 m, and Russian olive was found along 3584 m of the study section. The total area of the polygons digitized from the 2011 NAIP image was 66521 m², the average polygon area was ~426 m, and Russian olive was found along 4477 m of the section. Since 1992, we estimate the area of Russian olive cover has increased, on average, ~2323 m² year⁻¹. The average size of Russian olive stands in 2011 were ~2X larger than those in 1992, indicating that both cover and size of continuous stands are increasing.

Subsequent the Russian olive invasion, carp density has increased ~4X, from 0.03 ± 0.02 (carp m⁻² $\pm$ 1 SE) in the early 1970s to 0.10 ± 0.02 at present ($F_{[1,12]} = 7.82$, $p = 0.016$) (Figure 4). Numbers were highest during the late winter/early spring, and were lowest during the summer period of lower flow due to irrigation withdrawal. Carp in Deep Creek were predominantly adults, and size of carp varied little. The mean length ($\pm$ 1 SE) was 49.5 ± 0.7 cm TL, and average weight ($\pm$ 1 SE) was 1.5 ± 0.1 kg.

Diet analysis

Carp gut contents consisted, on average of ~40% Russian olive material (Figure 5). On average, guts contained 2500 ± 600 mg AFDM ($\pm$ 1 SE) of Russian olive

material (almost exclusively the olives themselves). Amorphous detritus was the second most dominant item, also making up ~40%, followed by aquatic vegetation (~20%), aquatic macroinvertebrates (~5%), terrestrial vegetation (<1%), and rock/shell fragments (<1%). *Cladophora* did not show up in the diets of the carp we analyzed. The median of plausible mixing model outcomes indicated that, on average $28 \pm 3\%$ (± 1 SE) of C in tissue of Deep Creek carp was derived from Russian olive. However, carbon isotope signatures of Russian olive and *Cladophora spp.* were not strongly separated, and the model estimate for *Cladophora spp.* was $47 \pm 7\%$, followed by periphyton ($20 \pm 3\%$), and *Potamogeton spp.* ($6 \pm 1\%$) as contributors to C in carp tissue. When we conducted analysis of deuterium, we found similar contribution of Russian olive to carp tissues; based upon the median plausible outcomes, deuterium in carp tissues was derived from $30 \pm 3\%$ Russian olive, $22 \pm 1\%$ *Cladophora spp.*, $29 \pm 5\%$ *Potamogeton spp.*, and $19 \pm 4\%$ periphyton. As expected, isotopic N values reflected trophic level fractionation (see Figure 6).

Bioenergetic analysis of carp

Using values from the literature, we determined that one adult carp at Deep Creek requires between $2.0 \text{ g AFDM day}^{-1}$ (Lupatsch et al. 1998) and $3.2 \text{ g AFDM day}^{-1}$ (Huisman 1976). Conservatively, we estimated the demand for the entire population at Deep Creek: $1200 \pm 200 \text{ g AFDM day}^{-1}$ to sustain the carp population in the early 1970s and $3500 \pm 800 \text{ g AFDM day}^{-1}$ to sustain the current population.

Based upon energy and organic matter budgets created for Deep Creek pre- and post-Russian olive invasion (Minshall 1978, Mineau et al. 2012) we estimated the availability of resources to be 1800 g AFDM day⁻¹ in the early 1970s and 12200 g AFDM day⁻¹ today for the total stream segment for which the carp population was estimated. Nearly all of the difference was attributable to Russian olive inputs, of which ~30% are olives, totaling 3100 g AFDM day⁻¹. Our calculations suggest that the demand required to sustain the current population of carp is ~2X greater than the available resources in the early 1970s, and that only with addition of resources like the Russian olive inputs would the current availability of resources be sufficient to support today's carp population.

Daily consumption (*D*) of Russian olive material by a carp, using the average amount of RO found in carp guts at Deep Creek (*A* = 2515 mg AFDM) and a gastric evacuation rate (*n*) of 37 hrs (Kevern 1966, Donner 2011) totaled 1.6 g AFDM day⁻¹ of Russian olive. Therefore, the daily consumption of Russian olive for the entire carp population at Deep Creek is approximately 2800 g AFDM day⁻¹, and falls within our estimation of available resources.

Effects of subsidized carp

Over the course of our short-term experimental exclusion of carp, physical characteristics of exclusions and controls did not differ. Average depth of exclusions was 21.8 ± 2.6 cm, and controls 23.3 ± 2.5 cm. Average velocity for exclusions was 0.13 ± 0.4 m s⁻¹ and controls 0.14 ± 0.5 m s⁻¹. Substrate

composition for exclusions was made up of $76.5 \pm 11.1\%$ fine particles (sand/silt) and controls $75.7 \pm 14.6\%$.

Carp exclusion caused a 3-fold increase in macrophyte biomass by the completion of the experiment ($F_{[1,12]} = 5.41$, $p = 0.038$). Exclusion of carp also resulted in $\sim 3X$ higher periphyton chlorophyll-a ($F_{[1,12]} = 6.91$, $p = 0.022$) (Figure 7). Benthic organic matter biomass did not differ between treatment and control, though it was, on average, greater in carp exclusions (Table 1). Russian olive made up $\sim 30\%$ of the CPOM portion of the benthic organic matter in both exclusions and controls, providing evidence that during the experiment, carp apparently did not “root” for olives in the benthos. The VFPOM portion was $\sim 2X$ greater in carp exclusions compared to controls with a significant treatment and time effect ($F_{[1,12]} = 5.52$, $p = 0.037$) (Figure 7).

Carp excretion

We observed a significant positive correlation between the amount Russian olive in the guts of carp and the amount of different forms of nitrogen they excreted and egested (Figure 8). Among measured variable, the strongest relationship was between the amount of Russian olive in the guts and total nitrogen (TN; $R^2 = 0.495$, $p < 0.001$), followed by total dissolved nitrogen (TDN; $R^2 = 0.268$, $p = 0.026$). The association between Russian olive and ammonium (NH_4) excretion was marginally significant ($R^2 = 0.136$, $p = 0.063$).

Carp from Deep Creek that ate Russian olive had 2-3X higher recycling rates for all forms of N than did those from the nearby Portneuf River that had not

(NH₄: $F_{[1,18]} = 8.85$, $p = 0.008$; TDN: $F_{[1,18]} = 8.71$, $p = 0.009$; TN: $F_{[1,18]} = 12.23$, $p = 0.003$; by ANOVA). However, we observed no differences in the recycling rates of orthophosphate (soluble reactive phosphorus, SRP), total dissolved phosphorus (TDP), or total phosphorus (TP) (Figure 9). Thus, the N:P ratio of recycled material was generally higher for carp that had consumed Russian olive. The TN:TP ratio was significantly higher for carp that consumed Russian olive ($F_{[1,18]} = 9.64$, $p = 0.008$), and differences in the TDN:TDP ratio were marginally significant ($F_{[1,18]} = 3.21$, $p = 0.092$), whereas the NH₄:SRP ratio was not significantly different (Figure 9). We detected no significant effect of carp size or water temperature on excretion. Though the slopes of the regression lines for the different dates were similar, the Y intercepts were significantly different ($F_{[1,17]} = 42.81$, $p < 0.001$; ANCOVA), such that excretion values tended to be higher at later dates in the spring.

Discussion

The invasion of Russian olive and subsequent facilitation of non-native carp is characteristic of an “invasional meltdown” (Simberloff and Von Holle 1999), and the combination of these two interacting invaders appears to be driving changes in food webs and ecosystem processes for our study site. In Deep Creek, Idaho, a representative cold desert stream and site of long term studies (Minshall 1978, Mineau et al. 2012) we found that the carp population increased ~4X with Russian olive invasion. Russian olive made up nearly 40% of carp diets and sustained ~1/3 of their production. In turn, these subsidized carp caused 2-3-fold reductions in chlorophyll-a, benthic organic matter, and aquatic macrophytes, and carp that consumed Russian olive recycled up to 2X more nitrogen than those that did not. Similarly, a previous study demonstrated that the combined effects of rusty crayfish (*Orconectes rusticus*) and the Chinese mystery snail (*Bellamya chinensis*) had greater consequences for native snail communities compared to when they occurred alone (Johnson et al. 2009). Though few studies have directly investigated the possibility, it seems that multiple invasive species are likely to be involved in a variety of complex interactions and their facilitation and combined effects may differ from that of a single invader, leading to consequences for native communities and ecosystem processes (Simberloff and Von Holle 1999, Ricciardi 2001, Grosholz 2005).

Our findings show that carp are being subsidized by Russian olive inputs to streams. We observed carp consuming large amounts of Russian olives and stable isotope analyses suggest that they assimilate this Russian olive material.

The isotope signatures and mixing model results indicate that filamentous algae (*Cladophora spp.*) is a main contributor of C to carp but there was not good isotopic separation between *Cladophora* and Russian olive C. We did not find filamentous algae in the diets of the carp we sampled. Though, of course, C from this source may reach carp via invertebrate prey. On the other hand, when we used deuterium to determine relative contributions, we found Russian olive to be much more important for carp. *Potamogeton spp.* was also more important compared to its plausible contribution of C. We cannot be certain of interpretation of either C or H isotope results alone, and further, we do not know the diet composition of carp prior to Russian olive invasion. We speculate that, historically, carp in Deep Creek would have relied more on consumption of aquatic macrophytes. Our calculations of carp demand versus resource availability, as well as estimates of the daily consumption of Russian olive, indicates that Russian olive sustains the current carp population. Overall, we judge that the current carp population is being subsidized by Russian olive inputs based on the combination of evidence from gut content analysis, isotope analysis, and our bioenergetic calculations comparing carp demand versus resource availability. Stronger evidence could be derived from a large scale and long term experimental manipulation (e.g., Russian olive removal) and, indeed, such an experiment is planned for this site in the future. Similar studies could be conducted at several streams throughout the western United States where Russian olive and carp invasions are widespread (Schade and Bonar 2005, Nagler et al. 2011).

Deep Creek is similar to many streams in the Intermountain west that host multiple invasive, non-native species, and it provides a rare opportunity for assessing effects of multiple invaders in that it has been studied historically as non-natives have invaded. Other non-native species present in Deep Creek include New Zealand mudsnails (*Potamopyrgus antipodarum*), largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), and rainbow trout (*Oncorhynchus mykiss*), though of these only bass are numerous at present. This study did not address these additional non-native species, but we expect that other important interactions may be occurring that include them. For example, the New Zealand mudsnail, another common invader of the west (Kerans et al. 2005), was also found in the guts of carp during our study and has been documented in the diets of carp in other settings (e.g., Donner 2011). Our findings here highlight the need for similar studies elsewhere.

Our short term experimental exclusion of carp demonstrated that carp subsidized by Russian olive can cause reductions in aquatic primary producers and alter stream organic matter dynamics. Previous investigations have shown that carp in other settings have similar effects, with a range of consequences for other animals (e.g., macroinvertebrates) (Parkos et al. 2003, Matsuzaki et al. 2007, Weber and Brown 2009). We did not investigate the full range of potential effects of subsidized carp owing to limited temporal and spatial scales of our experiment. For example, initially we planned to evaluate impacts of carp on the invertebrate assemblage, but a preliminary assessment suggested the scale of the experiment was inadequate to properly investigate such a response.

Macrophyte responses might also have been much stronger if the experiment had lasted longer. Macrophytes were apparently more abundant during the IBP studies (Minshall et al. 1973), and, indeed, there is much more luxuriant macrophyte growth in Deep Creek upstream of an irrigation diversion where Russian olive occurs but carp do not (K. K. Heinrich and C. V. Baxter, personal observation). However, during this study we did not quantify the current biomass of aquatic macrophytes present at Deep Creek. Mineau et al. (2011) reported that primary production rates at Deep Creek have remained similar since Russian olive invasion, and because periphyton chlorophyll-a concentration was quickest to respond to carp exclusion, we suspect that this primary production has shifted from being macrophytes to periphyton driven. The experiment did not allow us to evaluate the consequences for other animals present at Deep Creek, like the non-natives mentioned above, but also the remaining native fish species, the speckled dace (*Rhinichthys osculus*) and the native pilose crayfish (*Pacifastacus gambelli*). Speckled dace does not appear to eat Russian olive material, and has for unknown reasons experienced a substantial population decrease since the IBP studies (Minshall et al. 1973, Heinrich and Baxter, unpublished data). The pilose crayfish may consume Russian olive material, but it is currently rare at Deep Creek (K. K. Heinrich, personal observation). Previous studies have demonstrated that crayfish are displaced by carp via habitat depletion and alteration in behavior (Hinojosa-Garro and Zambrano 2004). Therefore, it seems likely that subsidized carp are having indirect effects on organisms like these as well, but this remains to be investigated.

Our study findings extend the investigation of the role of ecological stoichiometry in regulating critical ecosystem processes, such as nutrient cycling, to the context of invasive species, as we measured 2-3X more nitrogen recycled from carp that ate Russian olive compared to carp that did not. Previous studies have considered how species introductions influence ecosystem dynamics, but few have quantified how these changes affect nutrient dynamics in freshwater ecosystems (see Capps and Flecker 2013, Capps et al. 2015). The nutrient recycling by a consumer depends on the imbalance between its nutrient content and its food (Sterner and Elser 2002). In this case, we studied an invasive consumer and a novel, invasive food source. Fish excretion, in general, has consequences for ecosystem nutrient dynamics but has received relatively little research attention until recently (Vanni et al. 1997, Vanni 2002, McIntyre et al. 2008, McIntyre and Flecker 2010). Owing to its strong association with microbial di-nitrogen fixation, Russian olive has already been shown to subsidize stream ecosystems with nitrogen (Mineau et al. 2011), and we found that carp consuming Russian olive recycle more nitrogen within the stream. Therefore, the synergistic effects of these two invaders may lead to added or altered stoichiometric imbalances that then propagate through the ecosystem, and perhaps to downstream habitats as well. As part of previous study at Deep Creek, Mineau et al. (2011) estimated uptake at the study section as $0.02 \text{ mg m}^{-2} \text{ min}^{-1}$ and $<0.01 \text{ mg m}^{-2} \text{ min}^{-1}$ for NH_4 and SRP, respectively. If we calculate the mean excretion by carp ($\text{mg m}^{-2} \text{ min}^{-1}$) for the entire study section at Deep Creek we find that carp excretion far exceeds the rate of ecosystem uptake (~4000X

more for NH_4 , and ~400X more for SRP). Mineau et al. (2011) observed that concentrations of nitrogen increased from up to downstream along a cumulative gradient of Russian olive exposure, and we might expect a similar response to carp distribution as well. McIntyre et al. (2008) showed that spatial distribution of fish could generate hotspots of nutrient recycling in a tropical stream. Similarly, carp in Deep Creek typically aggregate in pools, so N concentrations are likely highest just downstream of such aggregations.

Numerous studies have shown fish can influence the spiraling and export of nutrients in streams (Vanni 2002, Hood et al. 2005, McIntyre et al. 2008). Fish with relatively high phosphorus will excrete more nitrogen compared to fish with less phosphorus demand (Sterner and George 2000). Because carp are P-rich, they have higher demand for phosphorus compared to the native speckled dace present in streams like Deep Creek, and because their Russian olive food source is nitrogen rich, they are recycling even more nitrogen. This may amplify the spiraling and export of nitrogen from streams invaded by both species. Our study of carp excretion was initially intended to include a controlled feeding experiment to more precisely evaluate impacts of Russian olive on this process. However, that experiment failed because carp stopped eating when held in mesocosms and we were concerned that excretion values would not be realistic or representative under such conditions. Therefore, we compared fish within Deep Creek that had consumed variable amounts of Russian olive and those from a segment of the Portneuf River where Russian olive were not present. Factors that are known to contribute most to differences in fish excretion rates include

diet, size, and temperature (Schindler and Eby 1997). The gut contents of carp from the Portneuf River were primarily composed of aquatic plant material and noticeably more macroinvertebrates than were observed in diets of Deep Creek carp; the latter would be expected to result in excretion of relatively more nitrogen. Therefore, we considered the differences we observed in N recycling rates to be a conservative estimate of the effects of Russian olive consumption. Additionally, fish excretion is positively related to temperature and size of fish (Schaus et al. 1997, Vanni 2002). Larger fish excrete more, but at a lower surface area to volume ratio (Gido 2002). We controlled for size by measuring excretion from fish of similar size, and rates were scaled by weight. During the first round of measurements Deep Creek was $\sim 8^{\circ}\text{C}$ warmer than the Portneuf River, but temperature was similar during the other sampling periods. Regardless, excretion values for carp consuming Russian olive were $\sim 2\text{X}$ higher whether temperature was similar or different.

The majority of studies that explore stoichiometric mechanisms underpinning consequences of species loss or addition focus on measures of the recycling of inorganic forms of nitrogen and phosphorus. Both NH_4 and SRP undergo quick uptake by microbes in streams including primary producers like algae (Hall et al. 2002). However, the dissolved organic forms of N and P are also important in streams, but require further processing to be assimilated as they spiral downstream (Newbold et al. 1981). If we had only measured the inorganic forms recycled by carp, we might have missed key patterns. For instance, carp recycling of TDN was more than double that of NH_4 , and

differences between those that ate Russian olive versus those that did not were stronger for TN than for NH₄. Moreover, the differences in N:P ratio recycled by carp that did versus did not consume Russian olive were significantly different for TN and TP, were marginally significant for TDN and TDP, and were not significant for NH₄ and SRP.

Our study showed Russian olive can facilitate carp, but a reciprocal relationship may occur as well; carp may facilitate the spread of Russian olive, and/or other invaders. Indeed, fish are often overlooked as seed dispersers (Correa et al. 2007), though the fossil record suggests they were the first vertebrate seed dispersers (Tiffney 1986). For instance, fruit-eating *Colossoma* and *Piaractus* are very effective seed dispersers in South America (Anderson et al. 2009). Further, the common carp is known to ingest hard-coated seeds of an aquatic macrophyte, *Sagittaria emersum*, which pass through its gut, resulting in improved germination (Pollux et al. 2006). With this possibility in mind, we conducted a pilot study whereby we planted Russian olive seeds that had passed through the guts of carp, and we found that those seeds were still viable. Thus, there is potential for carp to aid in dispersal and increased germination of Russian olive, and these possibilities deserve investigation. Moreover, carp may facilitate other invaders. As mentioned above, carp consume New Zealand mudsnails and these snails often survive consumption by fish (Vinson and Baker 2008), such that consumption may contribute to their spread. Furthermore, owing to their high fecundity, carp may serve to subsidize invasive piscivores (e.g., largemouth bass and yellow perch), contributing to their spread and driving

indirect effects on remnant native fishes. Future studies are warranted along these lines.

Susceptibility to invasion and to interactive effects (i.e., meltdown) may be mediated by legacies of land and water use, and associated changes of habitat and communities that have occurred in the past (Vitousek et al. 1997; Didham et al. 2005, 2007). In the Intermountain west, grazing and water withdrawal causes habitat degradation which then sets the stage for loss of native biodiversity and spread of invasive species. Russian olives were planted throughout the west as windbreaks and to control bankside erosion, and water withdrawals (which drive loss of native riparian trees and shrubs) may have initially facilitated its spread (Christiansen 1963, Knopf and Olson 1984). Historically, native fishes in Deep Creek and other streams like it would have included large bodied cyprinids and suckers (e.g., Utah chub, *Gila atraria* and Utah sucker, *Catostomus ardens*) that could have eaten Russian olive, but these are no longer present and this loss could have created an open resource opportunity for carp. Any management solutions to invasive species or their interactions should take into account these legacies.

Invasive species management and eradication efforts are expensive and labor intensive (Pimentel et al. 2005). Russian olive removal and carp eradication efforts (e.g., “carp derbies”) are becoming pervasive activities around the west, but are not connected to one another conceptually. Restoration efforts may yield an array of developmental pathways and habitat performances, but only those that occur within constraints imposed by potential capacity which in many cases

has been altered from its historic character (Ebersole et al. 1997). Removal of Russian olive may not be an effective form of restoration if native species cannot return or if interactions exist with other invaders. Very little is known regarding the effectiveness or responses to Russian olive removal as restoration (Gaddis and Sher 2012). Similarly, removal of carp may be ineffective if management does not address underlying problems that set the stage for invasion to begin with. Further, understanding synergistic consequences of invaders, such as the increased nitrogen fluxes we have described accompanying Russian olive and carp invasion, should inform adaptive management of multiple invasive species. For example, large-scale removals could be designed and treated as experiments, with monitoring that extends not only to desired native species, but also other non-natives. Demonstrating tangible, but unforeseen, and wide-reaching effects of invasive species, such as consequences to in-stream communities and ecosystem processes, will provide information critical for the understanding and application of adaptive management for multiple invasive species.

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Table 1. Mean benthic organic matter ($\pm$ SE) standing stocks in carp exclusion and control cages at Deep Creek. CPOM = coarse particulate organic matter (>1 mm); RO = Russian olive; FPOM = fine particulate organic matter (<1 mm >250 μ m); VFPOM = very fine particulate organic matter (<250 μ m >1.6 μ m); BOM = benthic organic matter. Values are g ash-free dry mass (AFDM) m^{-2} .

	Exclusion	Control
Total CPOM	373.4 (61.7)	246.3 (57.4)
<i>RO material</i>	104.6 (40.6)	71.5 (15.9)
FPOM	150.8 (22.3)	121.2 (28.9)
VFPOM*	277.6 (48.8)	149.1 (24.7)
Total BOM	792.9 (121.2)	525.5 (88.1)

* Indicates difference ($p < 0.05$) between exclusion and control.



Figure 1. Deep Creek, Idaho, USA (UTM coordinates: 362143 4663403), prior to Russian olive invasion (1970, top) and after (2014, bottom). 1970 Photo credit: G. Wayne Minshall.



Figure 2. Photo showing the two treatments at the start of the carp exclusion experiment. The control has the downstream end open so that carp have access.

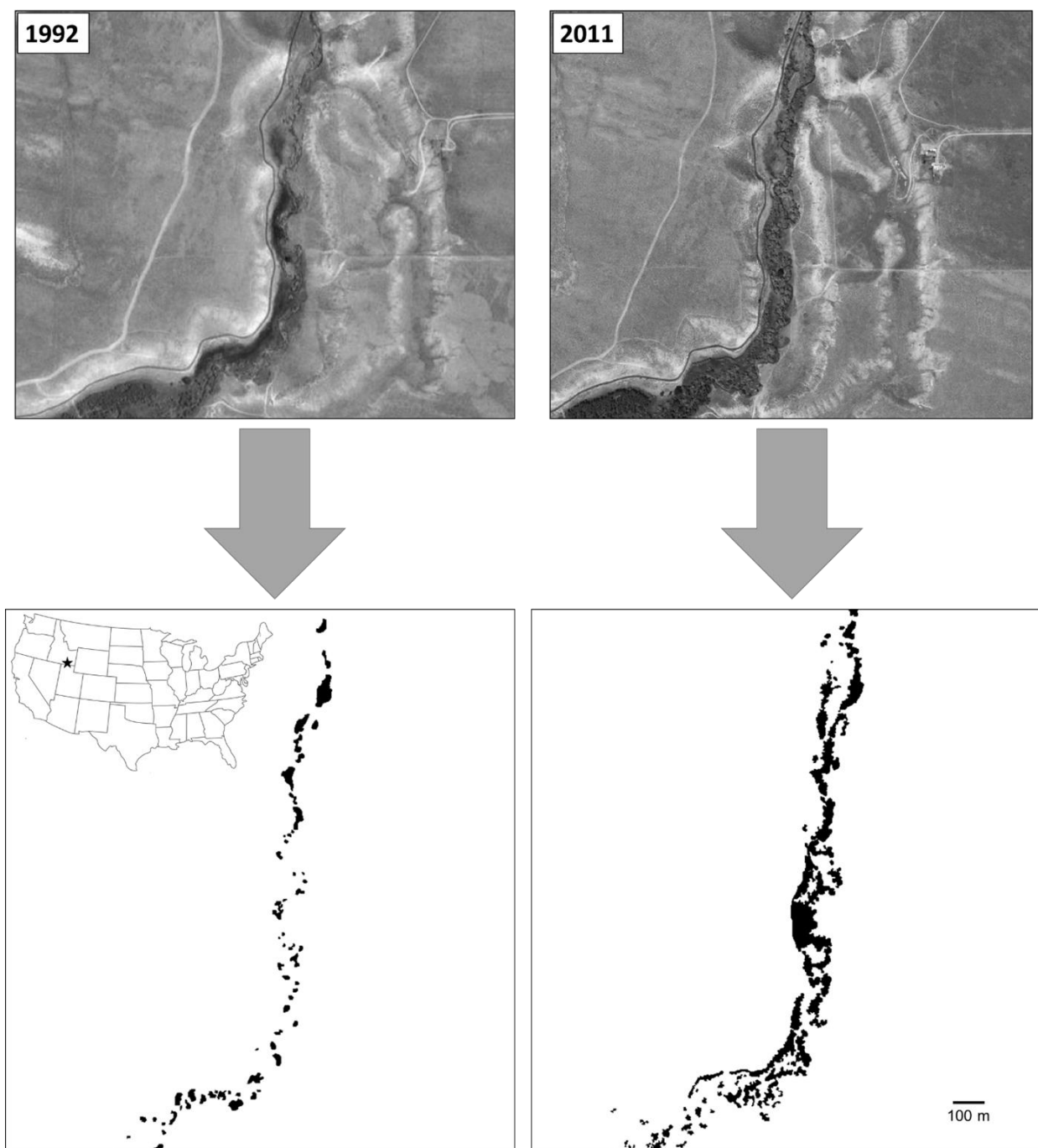


Figure 3. 1992 and 2011 images with associated polygons digitized using ArcGIS to show the total area of Russian olive cover.

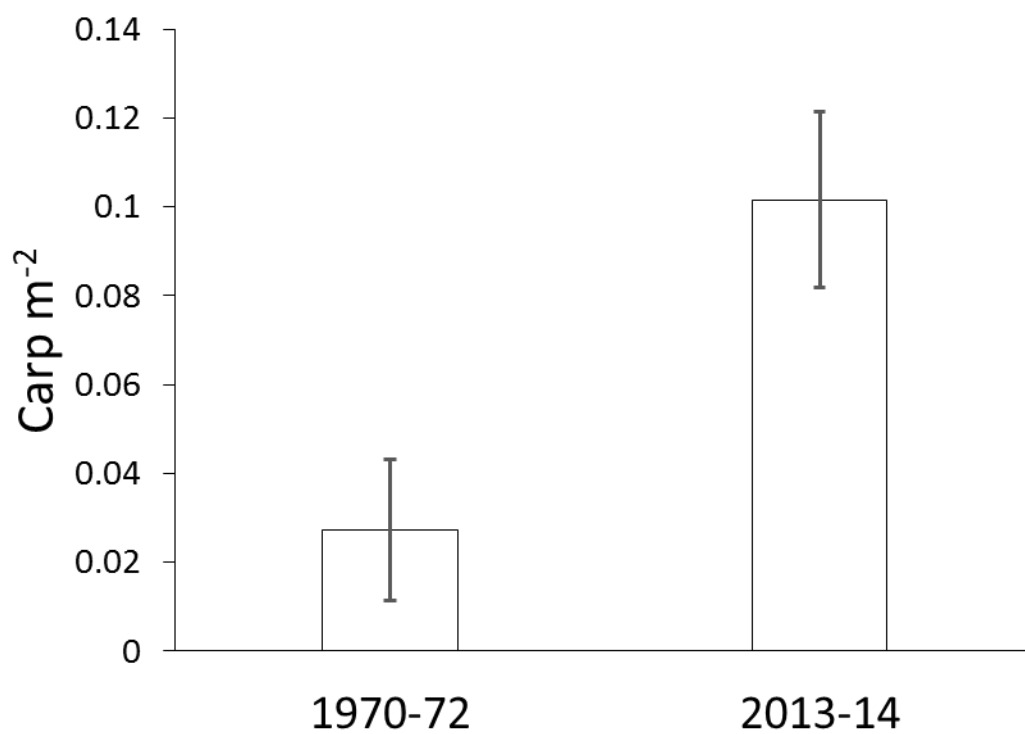


Figure 4. Carp density m⁻² (± 1 SE) at Deep Creek during the International Biological Program (1970-72) and 2013-14 ($F_{[1,12]} = 7.82$, $p = 0.016$).

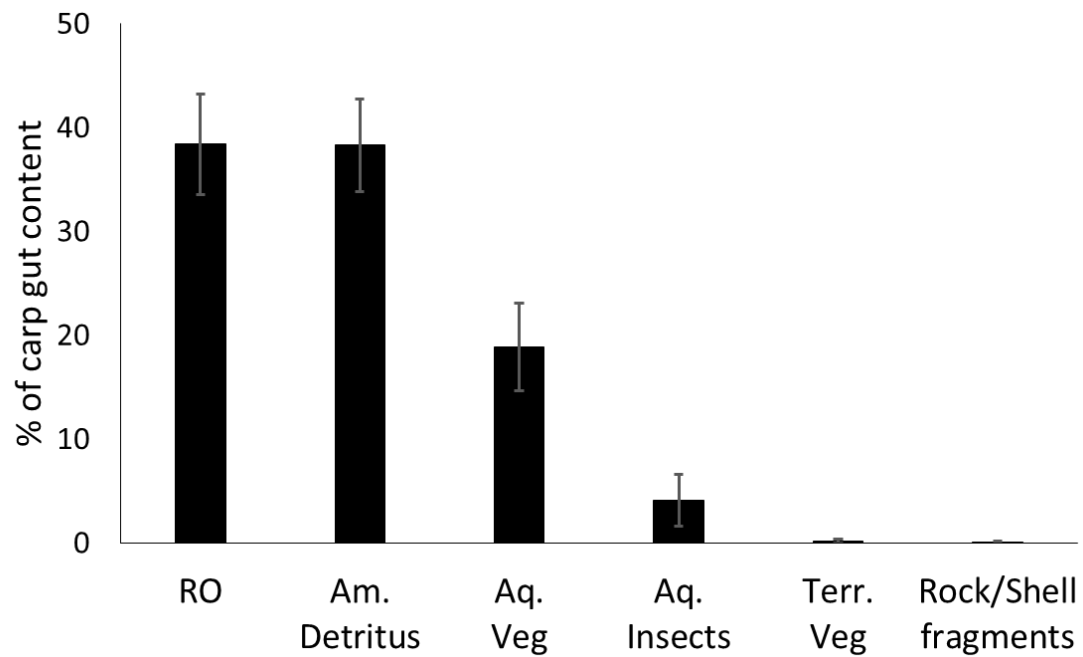


Figure 5. Mean gut content composition ($\% \pm 1$ SE) of dominant diet items from Deep Creek carp.

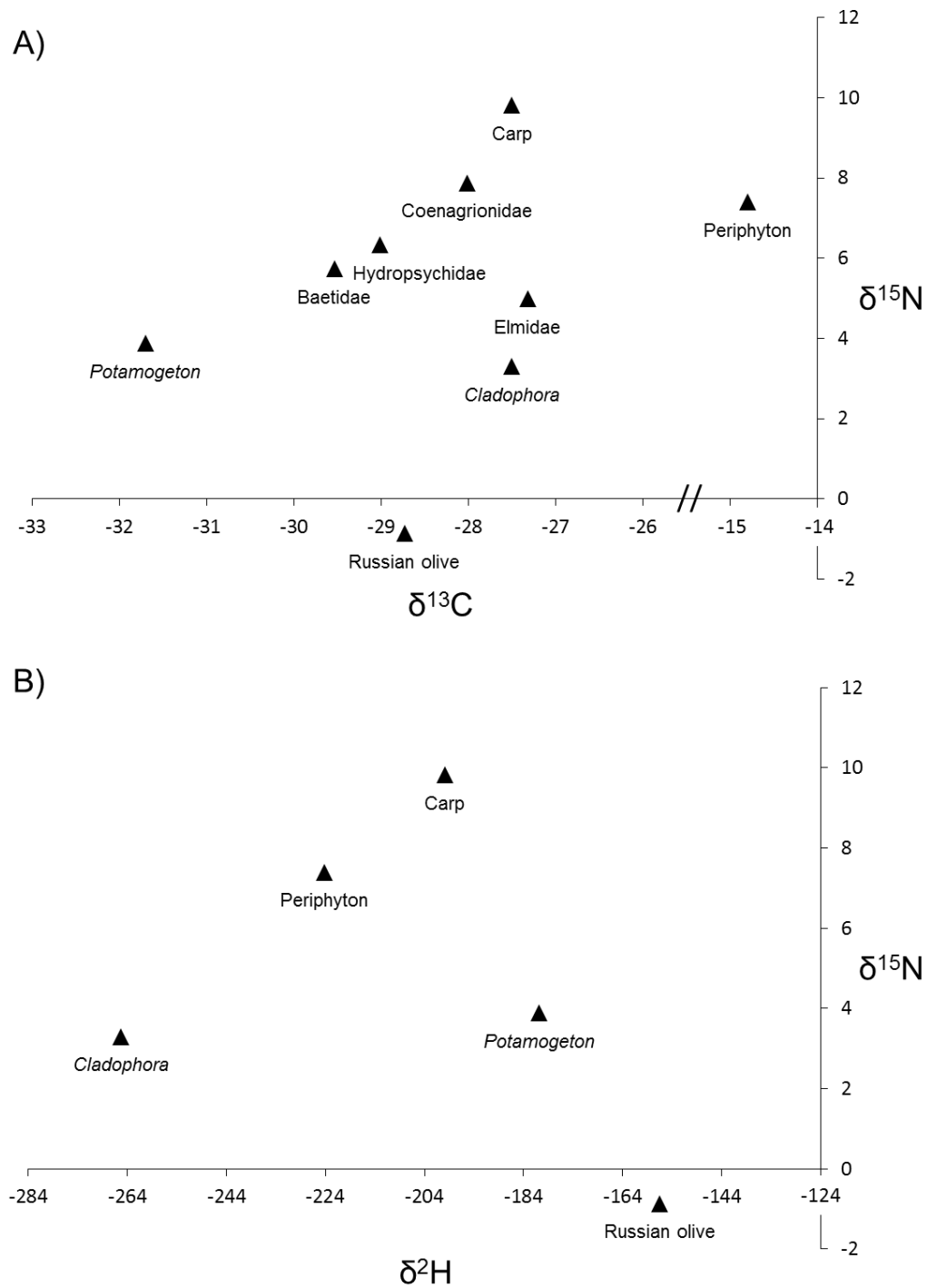


Figure 6. Isotopic signatures A) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of dominant resources and consumers of the Deep Creek food web; B) $\delta^2\text{H}$ and $\delta^{15}\text{N}$ of dominant resources of the Deep Creek food web. *Cladophora* and periphyton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and all resource and carp $\delta^2\text{H}$ values obtained from Mineau (2011).

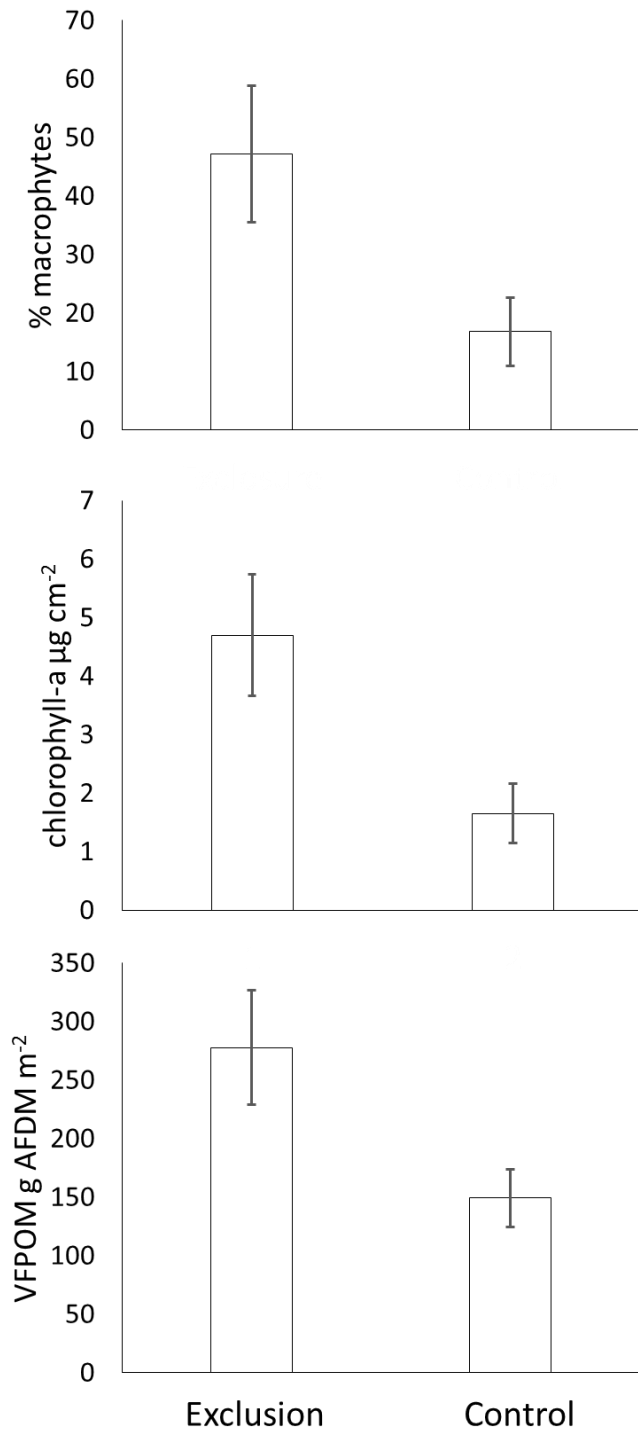


Figure 7. Results from the carp exclusion experiment showing the effects of subsidized carp on macrophyte cover ($F_{[1,12]} = 5.41$, $p = 0.038$), chlorophyll-*a* concentration ($F_{[1,12]} = 6.91$, $p = 0.022$) and VFPOM ($F_{[1,12]} = 5.52$, $p = 0.037$). Error bars are ± 1 SE.

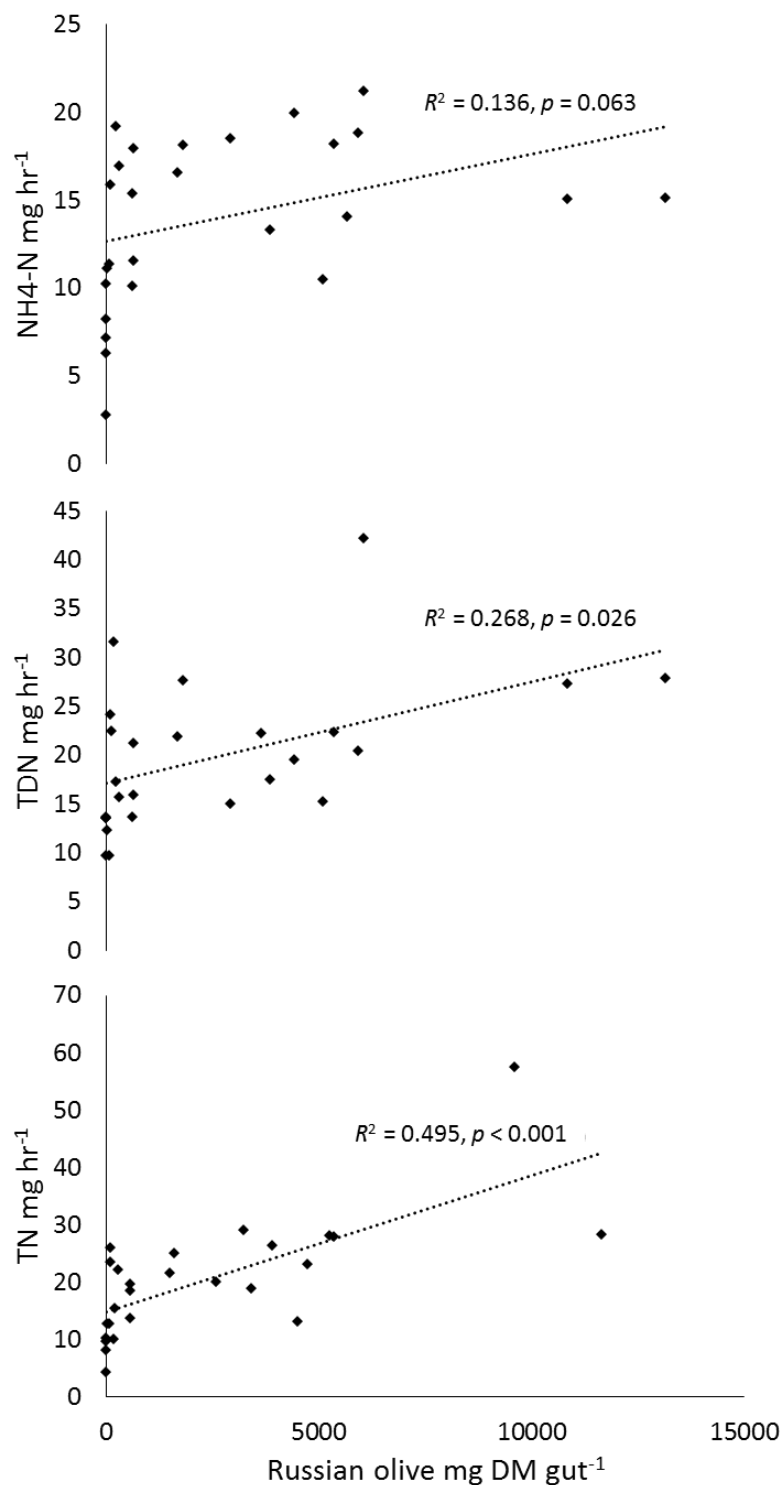


Figure 8. Relationship between the amount Russian olive (mg dry mass [DM] gut⁻¹) in the gut of carp and the amount of nitrogen excreted (mg hr⁻¹) by each. Top: Ammonium (NH₄) excretion; Middle: total dissolved nitrogen (TDN) excretion; and Bottom: total nitrogen (TN) excretion.

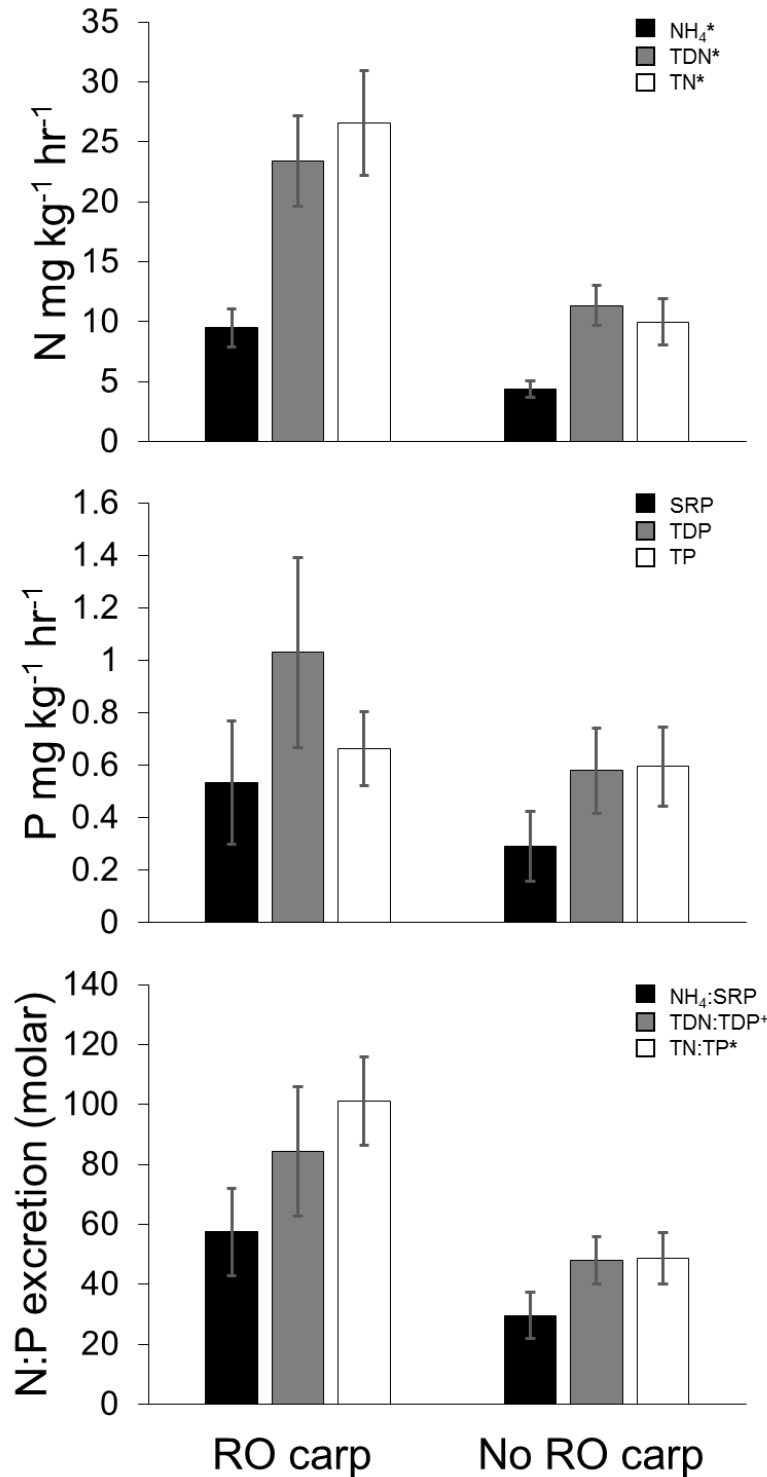


Figure 9. Comparison between carp that consumed Russian olive (RO) and those that did not for excretion of NH_4 ($F_{[1,18]} = 8.85$, $p = 0.008$), TDN ($F_{[1,18]} = 8.71$, $p = 0.009$), TN ($F_{[1,18]} = 12.23$, $p = 0.003$), SRP (n.s.), TDP (n.s.), TP (n.s.) and N:P molar ratios (NH₄:SRP, n.s.; TDN:TDP, $F_{[1,18]} = 3.21$, $p = 0.092$; TN:TP, $F_{[1,18]} = 9.64$, $p = 0.008$). Errors bars are ± 1 SE.

For: *The Science Teacher*

WATER-LAND LINKAGES: AN INQUIRY BASED LESSON ON EMERGENCE
OF ADULT AQUATIC INSECTS AND THEIR IMPORTANCE TO TERRESTRIAL
PREDATORS

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Abstract

Traditionally, exploration of ecosystems has been restricted to connections within conventionally defined ecosystem boundaries (i.e., within a stream, within a forest). Further, investigations that have treated linked habitats highlight unidirectional inputs. We employ a constructivist approach to explore the interactions and connections that propagate across aquatic and terrestrial habitat boundaries, where traditionally, ecologists have emphasized directional inputs from land to water. A new body of ecological understanding has characterized reciprocal interactions and draws attention to fluxes from water to land, including the emergence of adult aquatic insects that serve as prey for terrestrial predators. We present a guide for an inquiry-based outreach activity and collaboration between stream ecologists and students. The specific focal question is: “What is the role of insect emergence in connecting aquatic and terrestrial habitats as single ecosystems?” First, a pre-lesson is delivered in the classroom; second, a field investigation is carried out to highlight differences between prior knowledge and new knowledge. Specifically, insect emergence is studied using floating traps distributed along a stream reach. Insects are collected, identified, photographed, and released. Observations of riparian insectivores (e.g., birds and spiders) are documented and counts recorded. Finally, a discussion of the insect and predator assemblage is generated to apply new knowledge.

Introduction

Ecologists have long recognized the role of terrestrially derived inputs of plant material and invertebrates to streams, but more recently focus has been directed on the flux of aquatic insects in the opposite direction, from streams to riparian zones (Baxter et al. 2005). Nearly all aquatic insects “emerge” as winged adults and through this life stage represent an important link between streams and adjacent riparian habitats, facilitating flow of energy and nutrients from aquatic to terrestrial food webs. As such, adult aquatic insects can serve as important prey resources (commonly referred to as “subsidies”) for a wide range of riparian predators including lizards, birds, bats, spiders, and other terrestrial insectivores (Baxter et al. 2005). Some estimates indicate that only ~3% of the biomass of emerging aquatic insects returns to the stream, when these insects lay eggs, because the majority is consumed by riparian predators (e.g., Jackson and Fisher 1986). In temperate zones, emergence typically peaks in the early summer but still occurs throughout the rest of the year (Nakano and Murakami 2001). Some predators aggregate near streams and forage on these prey during periods of high emergence, while others rely on the lower subsidy from autumn through spring when terrestrial prey are rare (Jackson and Fisher 1986, Nakano and Murakami 2001, Sabo and Power 2002).

Populations of riparian spiders rely heavily upon emerging adult aquatic insects as prey and can closely track availability of this resource (Marzcak and Richardson 2007). Specifically, the family Tetragnathidae are relatively mobile and often build horizontal webs that target emerging insects. Tight linkages

between birds and stream insects have also been documented. Aquatic insects can make up the highest proportion of diets of insectivorous birds during the autumn through spring defoliation season, when terrestrial prey are scarce (Iwata et al. 2003). As a result, riparian birds are now recognized as indicators of stream ecosystem health and biotic integrity (Bryce et al. 2002). Using spider counts and avian communities to assess stream integrity and habitat quality can be less labor-intensive than using invertebrates or fish, and is especially important in communicating results to the public. Further, this creates an opportunity to teach about ecological connectivity.

Constructivist learning theory states that humans actively construct knowledge based on their experiences and interactions. Further, by this model, student learning is shaped by pre-existing knowledge that was constructed through previous experiences (Texley and Wild 2003). Required components of this inquiry-based approach include: 1) eliciting prior knowledge, 2) construction of new and better understanding, and 3) a reflection on learning (Shields 2006). Here, we outline an investigation of insect emergence that includes the required elements of the constructivist lesson and meets Next Generation Science Standards (NGSS) of the Disciplinary Core Idea “LS2A: Interdependent Relationships in Ecosystems.” The main goal of this lesson is to stimulate student understanding of ecosystem connectivity. At its completion students will be able to: 1) identify common adult aquatic insects to the order-level, 2) discuss the life cycles of aquatic insects, 3) observe riparian predators (e.g., spiders and birds) and identify common riparian spiders to family-level, 4) understand how

the connection between aquatic and terrestrial habitats via aquatic insect prey links them as a single ecosystem, and 5) understand the importance of aquatic insects, spiders, and birds as indicators of a healthy stream-riparian ecosystem. As a complementary lesson, this investigation could be paired with another inquiry-based field and laboratory activity that focuses on the flow of resources in the reverse direction, the fluxes of leaves from land to water and the role they play in streams (Hopkins and Smith 2011).

Pre-lesson

Begin with leading a discussion to elicit prior knowledge from the students, asking informal questions: Why are streams/rivers important? What organisms live in streams? How are the stream and land connected? As an assignment, provide a base map/image of a stream and surrounding riparian zone and ask students or groups of students to create a diagram of an aquatic-terrestrial food web. We have provided an example of a food web (Figure 1), but do not show this image to the students until the post-lesson. The assignment will serve as a diagnostic assessment, and will aid in identifying preconceptions. Most students will not recognize emerging insects as an integral part of the web of life connecting water and land.

We recommend playing the “stream food web” excerpt from *Riverwebs*, an award-winning documentary film by Freshwaters Illustrated (<http://freshwatersillustrated.org/link/RiverWebs>). Freshwaters Illustrated’s video “About NABS” may also suffice and can be found on Youtube (https://www.youtube.com/watch?v=dChyTggP_cU). After watching the video, introduce or review the Linnaean classification system: Kingdom-Phylum-Class-Order-Family-Genus-Species. Have the class fill in each category for humans (*Homo sapiens*), then have students attempt to classify common stream insects from Kingdom to Class (Animalia, Arthropoda, Insecta). Provide a handout of the common adult aquatic insects (Figure 2). Next, define emergence and lead into the life cycles of aquatic insects. Emergence events are often referred to as “hatches,” a phrase that may lead to misconceptions because what is occurring

is not an egg to larval transition, but rather a larva (or pupa) to adult transition. These insects go through a complex life cycle known as metamorphosis. The orders Ephemeroptera, Odonata, Plecoptera, and Hemiptera are hemimetabolous and undergo incomplete metamorphosis. Hemimetabolous insect wings develop externally and the immature stages (called instars) are clearly insect like. Orders Coleoptera, Megaloptera, Neuroptera, Trichoptera, Lepidoptera, Diptera, and Hymenoptera are holometabolous and undergo complete metamorphosis, which includes an important pupal stage, not found in hemimetabolous insects. Holometabolous larvae develop wings internally, do not resemble adults (less insect-like), and are often grub or worm-like. As most students will be familiar with the life cycle of a butterfly, this is a good example to use for complete metamorphosis. These topics and activities are easily adjusted based on the amount of time available and the grade level of the classroom. A main point to communicate is that the adult life stage of these aquatic insects is an important food source for both aquatic and terrestrial predators.

Field exercise

The main objective of the field outing is to bring students to an aquatic-terrestrial interface and allow them to explore the habitat and visualize how aquatic insects emerge and how they interact with terrestrial predators. Students will collect emergent aquatic insects, and count and observe riparian insectivores. Use the data sheet provided (Figure 3).

Aquatic insect emergence can be sampled using floating emergence traps (250- μ m mesh) for a period of 3 to 5 days. Emergence traps can be purchased through BugDorm (MegaView Science Co., Taichung, Taiwan) or trap designs can be copied from Malison et al. (2010) and slight modifications can be made to withstand higher flows in larger streams (Heinrich et al. 2014). Emergence traps are lightweight, easy to handle, and inexpensive to make. A typical design includes a frame constructed from PVC with the base wrapped with buoyant swimming pool “noodles” to allow the traps to float, and a mesh “tent” to cover the frame to collect emerging insects (Figure 4). The mesh cover can be made from mosquito netting, available at most outdoor stores, or mesh fabric from a fabric store. Aquatic insect emergence can also be measured using sticky traps (see Cary Institute of Ecosystem Studies Yes-Net Lesson 6) constructed from wire, and 100 cm² acetate cylinders attached to posts and painted with a sticky substance made of castor oil, waxes, and resins (Tanglefoot, Contech Inc., Victoria, B.C.). Both trap types are established methods that have been used to measure emergence in a variety of ecosystems. We recommend the floating traps, because students will be able to collect and observe live insects. If

available, contact a local university or science center that conducts aquatic research for the possibility of borrowing equipment and additional resources. They may also be able to provide expert advice on where to conduct this activity locally and the timing of known emergence events.

For sampling events, tether floating traps to a fence post or rebar pounded into the streambed and leave them set for 24-48 hours. Trap placement is important. Traps placed mid-channel and traps set along the stream bank may collect different insect taxa (Malison et al. 2010). Emergence can vary with distance from stream edge and between pool and riffle habitats. We recommend encompassing multiple habitats and positions within the stream.

Have students collect the adult insects from the traps using an aspirator (Carolina Biological Supply, Burlington, NC, U.S.A.) and identify insects to order-level using the handout from the pre-lesson (Figure 1). Students should also be on the look-out for riparian spiders and birds (documenting counts on data sheets). Focus on spiders belonging to the family Tetragnathidae. An identification key for the most dominant riparian spiders along streams in temperate zones can be used in the field (Figure 5). Ideally, counts would occur an hour after nightfall for spiders and just after sunrise for birds; but these time periods will likely be difficult to meet with a class of students. Searching for spiders during the daytime may be more challenging, but to overcome this difficulty, students can effectively “beat” riparian vegetation with a net (this may also reveal adult insects on the vegetation). Students can also quickly and effectively search for webs in the vegetation, and webs will commonly have adult

aquatic insects ensnared in them. Recognition of how dense spider webs are along and over streams can be a good representation of their abundance.

Post-lesson

Lead a discussion where students share what they found/discovered. Have them report the number of insects they counted and from what taxonomic orders, as well as the number of Tetragnathid spiders and birds counted. Have specimens or pictures of the insects, spiders, and birds so that students can recall what they observed. Focus on members of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) because they are widely regarded as indicators of healthy aquatic habitats (Barbour et al. 1999). Additionally, explain that other taxa (e.g., Chironomid midges, Order Diptera) are more tolerant of habitat degradation. Given this new information on how insects can be indicators of stream quality, have students make inferences and draw conclusions about the health of the stream being investigated. Remind the students of the link between emergence and riparian predators. Ask how might changes in-stream lead to changes in the terrestrial habitat, and discuss how changes in stream habitat for insects can result in changes in land animals (i.e., spiders and birds). Encourage students to think about how they might design their own studies using insect emergence, observing EPT taxa abundance and diversity, Tetragnathid spider density, or bird abundance and diversity.

Lastly, the aquatic-terrestrial food web diagram should be completed a second time so that students can reflect on how their thinking has changed and teachers can assess student learning. Examples of 'before' and 'after' food web diagrams are included (Figure 6). Students should now understand that ecosystems are not enclosed within distinct boundaries and that they are

connected and interact via flows of energy and resources. If learning objectives were met, the majority of food webs should show greater complexity and include aquatic insects.

Conclusion

The interface between aquatic and terrestrial habitats is bridged by many flows of materials and organisms. This complex web continues to be studied by scientists and this path of inquiry demonstrates how scientists can investigate complexity in a more holistic fashion, by making such connection the focus of study rather than by reducing or simplifying it.

This activity could also be expanded in a number of ways to achieve additional outcomes. For example, more directed focus on insect life cycles would fulfill NGSS Disciplinary Core Idea LS1B: “Growth and Development of Organisms.” Further, emergence sampling could be conducted at the same site for several consecutive years or a class could sample at different times of the year so that students could make comparisons and identify temporal patterns. Comparisons between streams that vary in water quality (i.e., degraded/polluted vs. healthy stream) or emergence sampled from many sites along a single stream can reveal spatial patterns. This would satisfy NGSS Disciplinary Core Idea LS2C: “Ecosystem Dynamics, Functioning, and Resilience.”

New knowledge can be effectively distilled through novel teaching tools, as this investigation demonstrates. The data are largely qualitative, but the lesson encourages students to work collaboratively and use established field methods that aquatic ecologists are using in their own scientific investigations.

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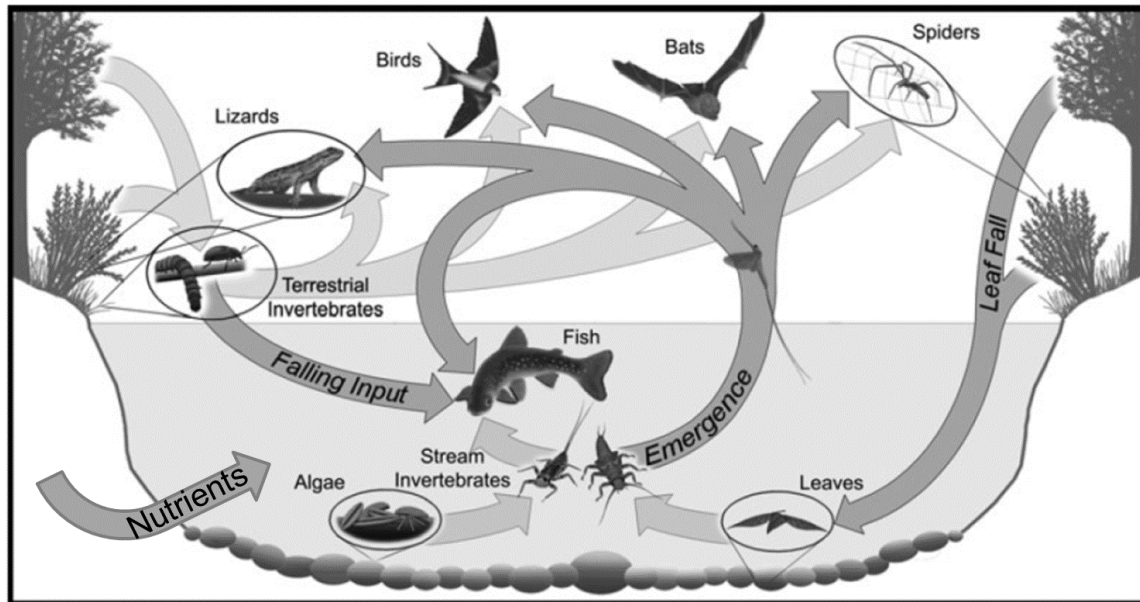


Figure 1. Diagram showing linked aquatic-terrestrial linked food web. Figure modified from Baxter et al. (2005).

AQUATIC INSECT ORDERS

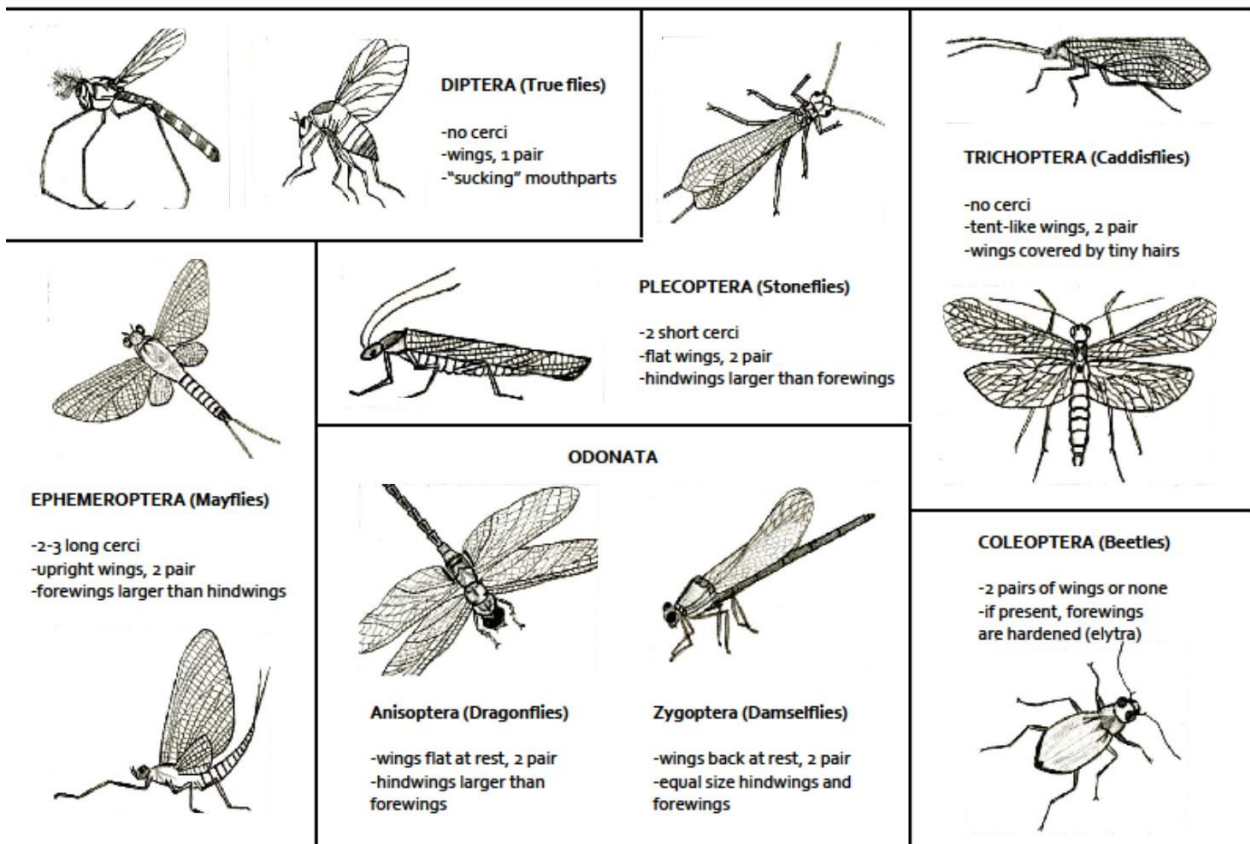


Figure 2. Handout depicting adult forms of common orders of aquatic insects, highlighting distinguishing characteristics.

Field Datasheet

Stream: _____

Date: _____

Personnel: _____

Start time: _____ End time: _____

Temperature: _____ °F or °C (circle one)

Cloud cover: _____ %

Wind speed (circle one):

Calm - no wind

Light breeze - leaves rustle, wind felt on face

Gentle breeze - leaves and twigs in motion

Moderate breeze - wind raises dust and loose paper

Strong breeze - trees sway, wavelets form on water

Insect emergence

Total number counted: _____

Ephemeroptera:	Plecoptera:	Trichoptera:	Diptera:	Odonata:	Other:
				Anisoptera Zygoptera	

Riparian spiders

Total number counted: _____

Tetragnathidae:	Araneidae:	Lycosidae:	Linyphiidae:	Other:

Birds

At site (< 50 m):	near site (> 50 m):	fly-overs:

Additional notes/observations:

Figure 3. Example data sheet for use in the field portion of proposed activity.



Figure 4. A floating emergence trap used for sampling aquatic insect emergence.

SPIDERS IN THE RIPARIAN ZONE

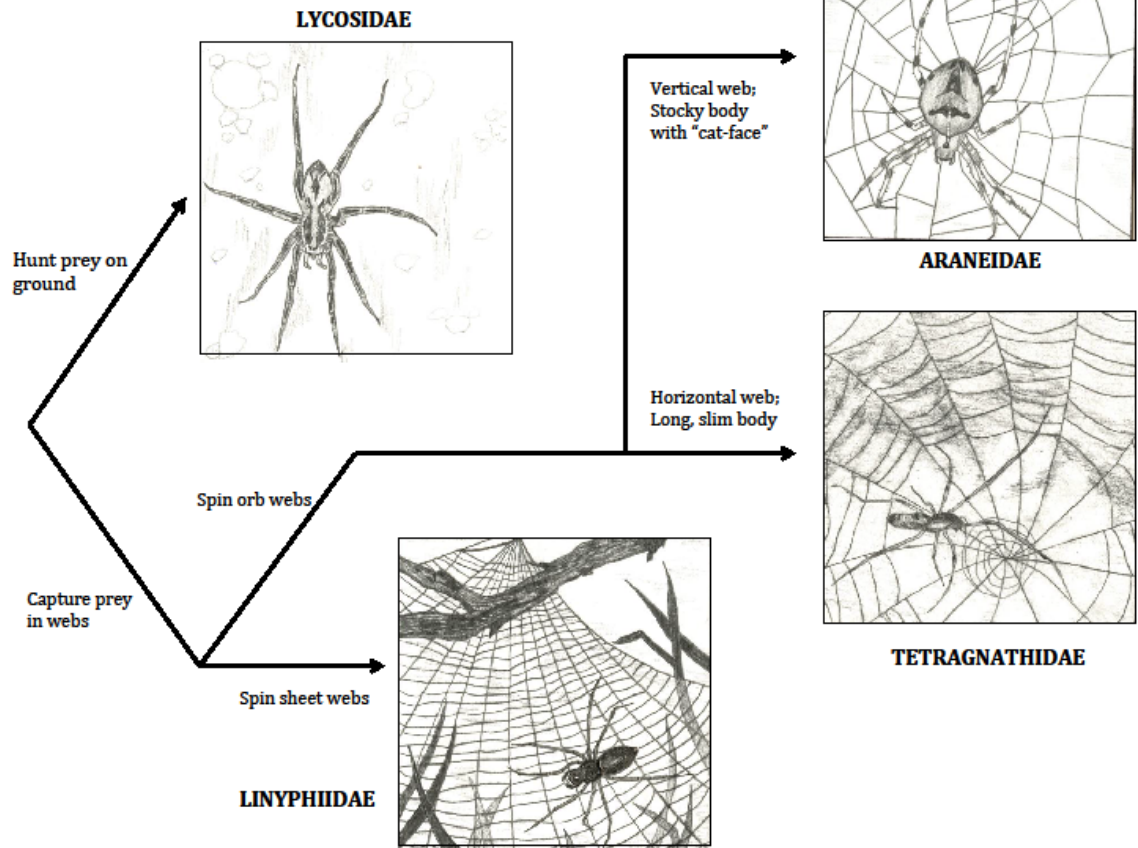


Figure 5. Identification key for the dominant families of spiders found along streams in temperate zones.

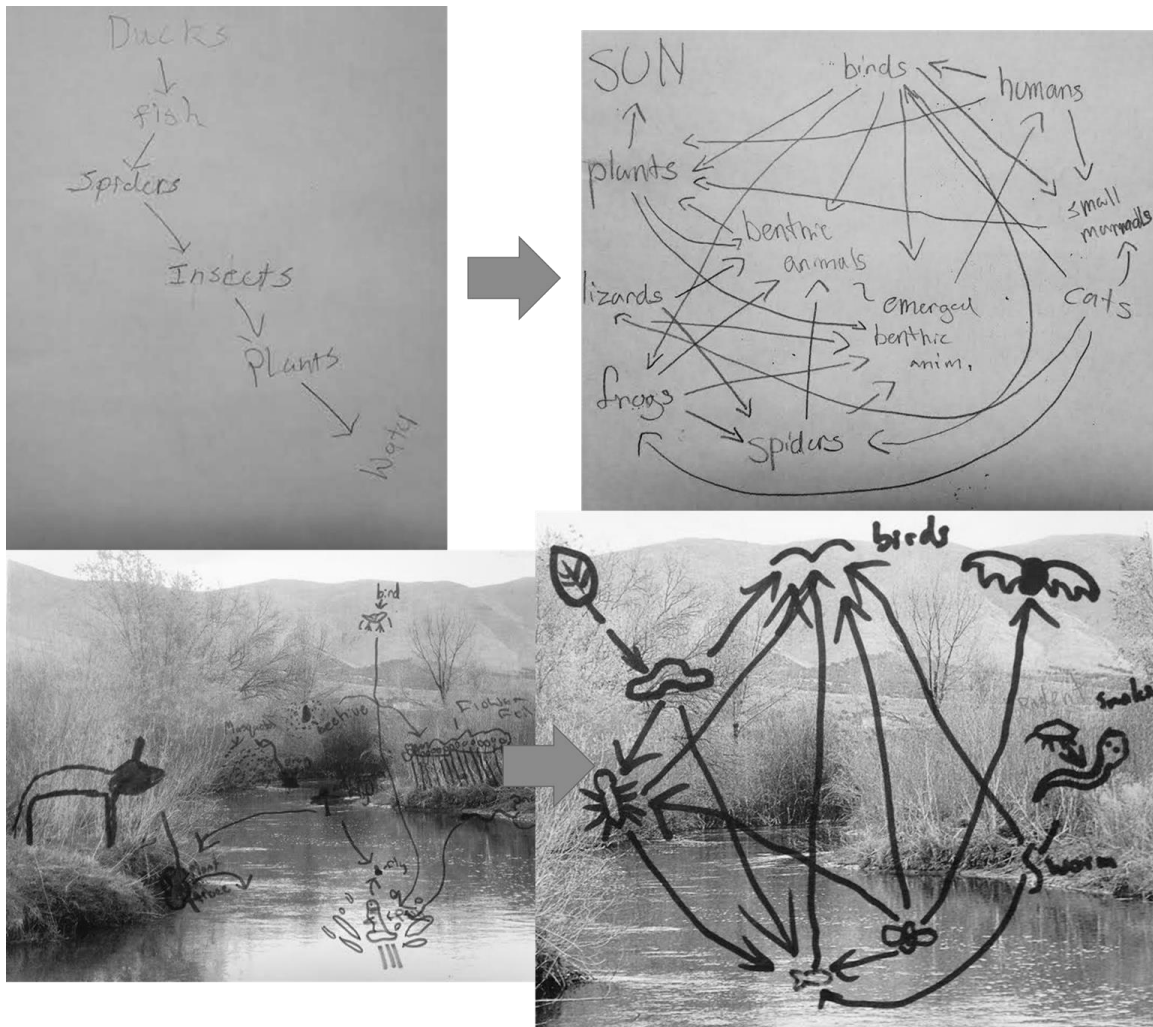


Figure 6. Examples of food webs before (left) and after (right) the activity. The food webs drawn prior to the lesson are simple. If the student does connect the stream with its riparian zone, the arrows usually point from land to water. After the completion of the lesson, food webs are more complex and include arrows pointing from land to water and vice versa.

VITA

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