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Ecological Importance of Invader Source Population and Disturbance in Aquatic Invasions

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Ecological Importance of Invader Source Population and Disturbance in Aquatic Invasions

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

by

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Virginia Commonwealth University
Bachelor of Science in Environmental Studies, 2013

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Invasive species are a global problem and their effects on recipient ecosystems may be mediated by disturbance and intraspecific variation. Crayfish can substantially influence stream structure and function, and invasive crayfish often have differential impacts than native crayfish in aquatic ecosystems. Since species traits often vary across a distribution, it is possible that invasive crayfish from different source populations may have distinct impacts on recipient ecosystem structure and function. In the Ozark Highlands of Arkansas and Missouri, USA, invasive *O. neglectus* (the Ringed Crayfish) may be leading to the displacement of native *O. eupunctus* (the Coldwater Crayfish). The objective of this thesis is to assess variation in the impacts of *O. eupunctus* and *O. neglectus* from multiple source populations. First, we experimentally examine the effects of crayfish source population and drought on stream structure and function and crayfish growth and survival. We found growth rates differed between *O. eupunctus* and *O. neglectus*, and also between *O. neglectus* from different source populations. In addition, we found *O. neglectus* from different source populations differentially affected stream structure, and *O. eupunctus* and *O. neglectus* differentially affected stream function. Drought and crayfish source population had interactive effects on macroinvertebrate richness, but no other variables. Important aspects of stream structure and function were also influenced by drought. In the second study, we assessed variation in morphology and traits related to invasiveness for *O. eupunctus* and *O. neglectus* from multiple source populations. We examined variation in chelae size in order to investigate potential competitive ability for *O. eupunctus* and *O. neglectus*. We found *O. eupunctus* had larger chelae than *O. neglectus* indicating that displacement of *O. eupunctus* by *O. neglectus* may not be related to competitive dominance. In addition, we found *O. neglectus* morphology varied across source populations. The results of these studies indicate

that morphology and impacts of invaders from different source populations may vary, and disturbance and invasive species may have compounding effects on recipient ecosystems. Therefore, abiotic disturbance and the source population of an invasive species are important considerations when addressing and predicting invasion impacts.

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Dedication

This thesis is dedicated to my mother, Cindy Graham. Thanks for raising me to not be afraid of dirt and bugs, and teaching me how to put a worm on a hook. I wouldn't be where I am now without you. This one's for you. Sincerely, thank you.

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List of Published Papers

Chapter 1: Graham NE, Magoulick DD (In preparation) Effects of Invasive Species Source Population and Drought on Crayfish Populations and Ecosystem Structure and Function. *Biol Invasions*.

Chapter 2: Graham NE, Magoulick DD (In preparation) Assessing Spatial Variation in Morphology and Traits Related to Invasiveness in an Introduced Crayfish Species. *J Crust Biol*.

Introduction

Invasive species are a global problem that can have negative consequences on native biota, ecosystem structure and function, and human health, and can lead to direct or indirect economic losses. Invaders can lead to biotic homogenization (Kolar and Lodge 2000), habitat alteration (Matsuzaki et al. 2009; Gallardo et al. 2016), disease transmission and altered food yields and storage (McMichael and Bouma 2000), and can cost up to billions of dollars annually (Pimentel et al. 2000). Aquatic environments are particularly susceptible to establishment of invaders (Lodge et al. 1998) and invasive species are considered an anthropogenic impact of primary importance in many freshwater ecosystems (Naiman et al. 1995). A vast number of nonindigenous species are introduced into freshwater systems through anthropogenic vectors, and globalization of commerce, waterway engineering, changes in land-use, and intentional stocking can facilitate the spread and intensify the impacts of aquatic invaders (Kolar and Lodge 2000). Furthermore, the impacts of aquatic invasive species may be particularly devastating due to strong trophic links in freshwater systems (Gallardo et al. 2016). The introduction or removal of freshwater species can lead to drastic alterations in trophic dynamics through ‘top-down’ or ‘bottom-up’ cascades (Pace et al. 1999).

Crayfish are omnivores that are important components of freshwater ecosystems due to their substantial influence on aquatic production (Whitledge and Rabeni 1997), local habitat characteristics (Brown and Lawson 2010) and multiple trophic levels (Momot 1995). Nearly half of the native crayfish in North American are imperiled, and invasive crayfish are a significant threat to native crayfish diversity (Taylor et al. 1996) and multiple components of freshwater ecosystems. Invasive crayfish tend to have different effects on recipient ecosystems than native crayfish (Twardochleb et al. 2013). Crayfish invasions can lead to declines in native taxa,

hybridization, economic losses for agriculture and water management entities, disease transmission, and substantial changes to aquatic communities (Lodge et al. 2012). Previous studies show invasive crayfish can have strong negative effects on nutrient dynamics and phytoplankton (Matsuzaki et al. 2009), macrophytes (Matsuzaki et al. 2009; Twardochleb et al. 2013), macroinvertebrates (McCarthy et al. 2006; Matsuzaki et al. 2009; Twardochleb et al. 2013), and amphibians and fish (Twardochleb et al. 2013).

Orconectes neglectus (the Ringed Crayfish) is native to portions of the Ozark Interior Highlands and Central Great Plains ecoregions. Invasive populations of this species have become established in Oregon (Bouchard 1997), New York (Daniels et al. 2001), and Arkansas and Missouri (Flinders and Magoulick 2005; Magoulick and DiStefano 2007). The invasive population established in the Spring and Eleven Point river drainages of Arkansas and Missouri, USA, is leading to the apparent displacement of a vulnerable endemic congener, *O. eupunctus* (the Coldwater Crayfish) (Flinders and Magoulick 2005; Magoulick and DiStefano 2007). Common mechanisms of displacement of native crayfish by invasives are competition, predation, transmission of diseases, and interference with reproduction (Lodge et al. 2000). Natural or anthropogenic abiotic disturbances can also mediate the impacts and spread of invasive species (D'Antonio 2000).

The Ozark Highlands are subjected to frequent disturbance by drought, and this may play an important role in facilitating crayfish invasions. Riffle and run habitats can often dry completely during seasonal stream drying in the Ozark Highlands, leading to isolated pool habitats (Homan et al 2005). Drought can affect stream biota in many ways, including limiting dispersal capabilities (Chester et al 2015), altering community structure (Boulton 2003), and modifying habitat use (DiStefano et al. 2009). In addition, drought can affect important aspects

of stream structure (Rolls et al. 2012; Timoner et al. 2014) and function (Datry et al. 2011, Acuña et al. 2015). Furthermore, drought may alter biotic interactions (Gasith and Resh 1999) and has been implicated as a factor facilitating the establishment and dispersal of *O. neglectus* in the Spring and Eleven Point river drainages of Arkansas and Missouri, USA, due to differential tolerances to desiccation in *O. neglectus* and *O. eupunctus* (Larson et al. 2009).

Previous studies have examined the comparative effects of *O. neglectus* and *O. eupunctus*, and these two species appear to be largely ecologically redundant in terms of their trophic roles (Magoulick and Piercey 2016) and effects on stream structure and function (Magoulick 2014). However, intraspecific variation in invasive species can lead to differential effects on recipient ecosystems (Lankau 2011) and therefore potential ecological redundancy of invaders with natives may be variable. In addition, Larson and Olden (2010) found morphological and life history traits of invasive crayfish from adjacent drainages (extralimital invaders) may differ from traits of invasive crayfish from distant drainages (extraregional invaders). Furthermore, Fetzner (2017) found morphological differences in *O. eupunctus* within and across drainages in Arkansas and Missouri, USA. Therefore, traits of *O. neglectus* may also vary within and across drainages. Spatial variation of traits of an invasive species can lead to varying impacts on recipient communities (Phillips and Shine 2006) and therefore source population of an invasive species may mediate its invasion impacts.

A comprehensive understanding of the factors that influence the spread and impacts of aquatic invasive species is necessary in order to better inform management decisions and prevent ecological and economic losses. The objective of this thesis is to assess variation in the impacts of native *O. eupunctus* and invasive *O. neglectus* from multiple source populations. This thesis is comprised of two manuscripts focused on developing a better understanding of how invader

source population may influence recipient ecosystems. In the first manuscript, I experimentally examine the comparative effects of *O. eupunctus* and *O. neglectus* on stream structure and function, with a concentration on invader source population and potential compounding effects of drought. In the second manuscript, I examine potential differences in crayfish morphology and traits associated with invasiveness for *O. eupunctus* and *O. neglectus* from five different source populations. These manuscripts are assembled in this thesis in order to meet the degree requirements of the University of Arkansas Graduate School.

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Effects of Invasive Species Source Population and Drought on Crayfish Populations and Ecosystem Structure and Function

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Abstract

Source population of an invader may alter ecosystem effects on recipient communities. However, little is known about the relative impacts of extraregional versus extralimital invasions. Invasive crayfish threaten native endemic crayfish in the Ozark Highlands, an area where drought is a prominent disturbance. We conducted a fully factorial mesocosm experiment examining the effects of crayfish source population (native *O. eupunctus*, extralimital *O. neglectus*, and extraregional *O. neglectus*) and drought (drying and control) on crayfish growth and survival, leaf decomposition, chlorophyll a, periphyton ash-free dry mass, autotrophic index, sediment levels, net primary production, macroinvertebrate richness and Chironomidae abundance. Analysis of variance and negative binomial regressions were used to examine the effects of treatments on response variables. There was no significant interaction between crayfish source population and drought, except for macroinvertebrate richness. Macroinvertebrate richness was greater in control than drying treatments except for in the extralimital *O. neglectus* treatment. Crayfish growth, chlorophyll a content, and leaf decomposition differed across source populations. Carapace growth was greatest in extralimital *O. neglectus* treatments and mass growth was least in *O. eupunctus* treatments. *Orconectes eupunctus* and extralimital *O. neglectus* reduced chlorophyll a more than extraregional *O. neglectus*. Leaf decomposition was lower in *O. eupunctus* treatments than in either *O. neglectus* treatment. Drought reduced chlorophyll a, autotrophic index, sediment levels, Chironomidae abundance, and net primary production. Results indicate extraregional and extralimital invaders may have different effects on ecosystems. Drought can have strong effects on ecosystem processes, but these effects may be context dependent.

Keywords: source population, invasive species, ecosystem impacts, crayfish, drought

Introduction

Effects of extraregional invasive species have been well studied for a variety of taxa (e.g. Hickman et al. 2010; Kirsch and Dzialowski 2012; Koenig et al. 2013; Cooke 2016; Willson 2017). An invasion is termed extraregional if the invader crosses major terrestrial or drainage boundaries (Hobbs et al. 1989). On the other hand, extralimital invaders are introduced from adjacent regions or drainages (Riggert et al. 1999). Few studies focus on the impacts of extralimital invasions (e.g. McKinney 2005; Magoulick and DiStefano 2007; Spear and Chown 2008). However, these invasions are relatively more common (McKinney 2005). In addition, extralimital invasions have been found to have a more homogenizing effect on recipient communities than extraregional invasions (McKinney 2005; Spear and Chown 2008). Therefore, ecological effects of extralimital versus extraregional invaders may differ, and invasion scale is an important consideration when assessing potential invasion impacts.

Aquatic systems are particularly vulnerable to establishment of nonindigenous species due to the relative ease of dispersal of organisms in aquatic environments and multiple available introduction vectors (Lodge et al. 1998). Nonindigenous aquatic species may be introduced extraregionally or extraliminally through ballast water (Hutchings 1992; Carlton and Geller 1993), aquaculture and aquarium shipments (Courtenay and Stauffer 1990; Carlton 1992), or live wells and bait bucket introductions (Ludwig and Leitch 1996). Aquatic invaders have consistent negative impacts on the abundance of many aquatic communities and alter ecosystem structure and function in multiple ways (Gallardo et al. 2016). Invasive species in aquatic ecosystems have been shown to impact food web structure and function (Miehls et al. 2009). They can alter the food web through strong ‘top-down’ (Ordóñez et al. 2010) or ‘bottom-up’ (Ward and Ricciardi

2007) trophic effects. In addition, exotic species that are ecosystem engineers can lead to changes in local habitat conditions (Crooks 2002).

Lotic crayfish are ecosystem engineers (Brown and Lawson 2010) that impact multiple aquatic trophic levels (Momot 1995), substantially influence aquatic production through the processing of coarse particulate organic matter (Whitledge and Rabeni 1997), and serve as prey for more than 200 species (DiStefano 2005). Of 571 crayfish species and subspecies worldwide, 77 percent are native to North America (Taylor 2002). Approximately 50 percent of North American crayfish are considered imperiled, due in part to invasive crayfish (Taylor et al. 1996). Displacement of native crayfish by invaders is often attributed to predation, competition, transmission of diseases and interference with reproduction (Lodge et al. 2000). However, the role of abiotic disturbances in mediating the distributions and ecological impacts of invasive crayfish has received recent attention.

Abiotic disturbances can facilitate the establishment and spread of invasive species, as well as alter their ecological impacts (Hobbs and Huenneke 1992; D'Antonio 2000; Facon et al. 2006). Stream drying is a frequent disturbance in the Ozark Highlands of Missouri and Arkansas, USA, an area with an array of diverse and endemic crayfish (Tisseuil et al. 2013). One such endemic crayfish, *Orconectes eupunctus* (Coldwater Crayfish), is being extirpated from areas within its range following the invasion of *Orconectes neglectus* (Ringed Crayfish) from an adjacent watershed (Flinders and Magoulick 2005; Magoulick and DiStefano 2007).

Neither adult (Rabalais and Magoulick 2006) nor juvenile (Larson and Magoulick 2009) competition with *O. neglectus* appears to be responsible for the displacement of *O. eupunctus*. However, *O. neglectus* demonstrated a higher tolerance to desiccation than *O. eupunctus* and thus stream drying may have played a role in facilitating the establishment of *O. neglectus* in the

Spring and Eleven Point river drainages (Larson et al. 2009). In addition, *O. eupunctus* and *O. neglectus* appear to be largely ecologically redundant in terms of trophic niche (Magoulick and Piercey 2016) and effects on stream structure and function (Magoulick 2014).

In a trait analysis of crayfish native to Missouri and Kentucky, Larson and Olden (2010) found traits of extralimital invasive crayfish from adjacent watersheds differ from traits of extraregional invasive crayfish from distant states or watersheds. Thus, extralimital invasions may vary from extraregional invasions with regard to displacement of native species and ecological redundancy (Magoulick 2014). Previous research has examined the comparative ecological impacts of native versus invasive crayfish (e.g. Usio et al. 2006; McCarthy et al. 2006; Magoulick 2014). However, few studies have investigated the relative ecological impacts due to extralimital invasions versus extraregional invasions.

The native distribution of *Orconectes neglectus* is disjunct (Schainost 2011), and this species is invasive extralimitaly (Flinders and Magoulick 2005; Magoulick and DiStefano 2007) and extraregionally (Bouchard 1977; Daniels et al. 2001). This offers a unique opportunity to examine the relative effects of extralimital versus extraregional invasions within a single species. Our objective was to examine the effects of invasive species source population and drought on crayfish populations and stream structure and function. We used stream mesocosms to experimentally investigate effects of stream drying and source population (native *O. eupunctus*, extralimital *O. neglectus*, and extraregional *O. neglectus*) on ecosystem structure and function. We hypothesized that extralimital *O. neglectus* would have stronger effects on ecosystem structure and function than extraregional *O. neglectus*.

Materials and Methods

Experimental Design and Setup

We performed a fully factorial mesocosm experiment with crayfish source population (native *O. eupunctus*, extralimital AR *O. neglectus*, and extraregional KS *O. neglectus*) and drought (simulated drying and control) as factors. Each treatment had five replicates. Response variables included crayfish growth and survival, leaf decomposition, chlorophyll a concentration, periphyton ash-free dry mass (AFDM), autotrophic index, sediment level, net primary production (NPP), macroinvertebrate richness and Chironomidae abundance.

Oval tanks (416 L) were used as mesocosms and were arranged in a four by eight grid within a climate-controlled greenhouse. Treatments were assigned to mesocosms systematically with a random start. A mix of gravel, pebble, and cobble was distributed into the mesocosms to create distinct riffle and pool habitats and mesocosms were then filled with dechlorinated tap water to a depth of 50 cm. The riffle habitat was 38 cm long and 10 cm deep. A slope connected this to the pool habitat that was 38 cm long and 40 cm deep. Fluval 205 canister filters (Rolf C. Hagen Inc., Montreal, Canada) filtered and circulated water within the mesocosms. Intake hoses were anchored in the pool habitat and output hoses were anchored in the riffle habitat.

Mesocosms were inoculated on August 16, 2015, using cobble collected from Dye Creek (35.94189, -94.18368) in the White River drainage. Cobble was kept in buckets containing stream water that was collected on site for approximately two hours prior to being distributed into mesocosms. Five randomly selected stream cobbles were placed in pool habitat and four cobbles were placed in riffle habitat of each mesocosm. In order to measure leaf decomposition, leaf bags (32cm by 22cm with ~2.5mm mesh (Volm Companies, Antigo, Wisconsin)) were constructed containing three grams of dried sugar maple (*Acer saccharum*) leaves per bag. Two seven cm long slits were cut into each leaf bag to allow for crayfish access. On August 27, 2015 two leaf bags were placed in the riffle and two leaf bags were placed in the pool of each tank.

Additionally, two 11cm by 11cm unglazed ceramic tiles were positioned in both the riffle and pool habitats on the same day to serve as sampling devices for periphyton and macroinvertebrates.

Crayfish Collection

Form II male crayfish were collected August 17-30, 2015 from the South Fork of the Republican River in Kansas (39.77833, -101.80685); Sugar Orchard Creek (36.29117, -92.91911) and Huzzah Creek (36.23183, -92.99002) in the White River drainage in Arkansas; and the Spring River (36.22472, -91.25081) and South Fork of the Spring River (36.3554, -91.6324; 36.3673, -91.8110; 36.4067, -91.8172) in Arkansas. *Orconectes neglectus* were collected from the Republican River drainage (extraregional) (hereafter KS *O. neglectus*) and White River drainage (extralimital) (hereafter AR *O. neglectus*), and *O. eupunctus* were collected from the Spring River drainage. All crayfish were collected using a kick seine (3 mm mesh, 1.8 m length x 1.1 m height) and transported on ice to holding tanks inside the greenhouse until the start of the experiment.

Experimental Execution and Data Collection

On September 4, 2015, six crayfish were weighed and measured, then placed into each of the appropriate mesocosm tanks. Initial crayfish carapace lengths ranged from 10mm to 34mm, and initial weights ranged from 0.3g to 16.7g. Mean initial size for *O. eupunctus* was 21.9mm and 4.2g, for KS *O. neglectus* was 20.6mm and 2.8g, and for AR *O. neglectus* was 18.9mm and 3.1g. Densities were less than five individuals per square meter and within the range of natural crayfish densities for the Spring River drainage (Flinders and Magoulick 2005). Fiberglass mesh (1mm by 1mm) was placed over each of the tanks and secured with binder clips to prevent crayfish escape. Water withdrawals from the simulated drought treatments began on October 17,

2015. The water level in each of the 15 tanks within the drought treatment group was reduced by five cm each day, until the water level was reduced by 35 cm and was five cm above the level of the substrate in the pool habitat (October, 23, 2015). Canister filters in the drought treatments were turned off on October 19, 2015, when the water level fell below the substrate level in the riffle habitat. The water level was maintained at 15 cm (five cm above the substrate in the pool habitat) for the remainder of the experiment. On November 21, 2015, dissolved oxygen and temperature in each tank were measured from daybreak until nightfall every two hours with a DS 5X Hydrolab sonde in order to estimate NPP. On November 22, 2015 leaf packs and tiles were removed from the mesocosms and placed in individually labeled resealable plastic bags in a freezer for storage. Crayfish were recovered from tanks, and weighed and measured. The mean increase in crayfish carapace length and weight, and survival per tank were recorded.

In the laboratory, leaves were removed from leaf bags and dried, desiccated and weighed. They were then placed in a muffle furnace at 500 C for two hours. The resulting ash was wetted with distilled water, dried, desiccated and weighed in order to calculate AFDM. Ceramic tiles were scrubbed with a nylon brush and rinsed with water in order to slough off periphyton and macroinvertebrates. The subsequent slurry solution was measured in a graduated cylinder and two eight ml subsamples were filtered onto pre-ashed (500 C for two hours) and weighed 47 mm type A/E Pall (Pall Corporation, Port Washington, New York) glass fiber filters. Ethanol (95%) was added to the remaining slurry solution and this was preserved for macroinvertebrate collection. Each subsample filter was then placed into a pre-labeled centrifuge tube and stored in a freezer for 24 to 72 hours prior to extraction. Twenty four hours prior to chlorophyll a analysis, each tube was filled with 13 ml of 95% ethanol and placed in darkness at room temperature to allow for extraction. Three ml of extract was pipetted into a 1 cm glass cuvette and a

spectrophotometer was used to measure light absorbed at 664 nm and 750 nm for an ethanol blank and each subsample. 0.1 N HCl was then added to each subsample and blank, and after a 90-second adjustment period, light absorbance was measured at 665 nm and 750 nm (EPA Method 446.0). The entire extract was then poured into a pre-ashed and weighed labeled tin and dried, desiccated and weighed. Tins and filters were then placed in a muffle furnace at 500 C for 2 hours, rewetted, dried, desiccated and weighed for periphyton AFDM. Inorganic content on the filters was considered sediment. Chlorophyll a concentration was calculated for each subsample. Averages of periphyton AFDM, sediment, and chlorophyll a values from the two subsamples were used to calculate total mesocosm values. Autotrophic index was calculated as the ratio of chlorophyll a concentration to periphyton AFDM. The preserved periphyton slurry was filtered through a 500 micron sieve and macroinvertebrates were collected and identified to the lowest possible taxonomic level and counted. Net primary production was calculated using the methods of Bott (1996). Due to a lack of data on nighttime dissolved oxygen values, we used the average re-aeration coefficients for drying and control tanks from Magoulick (2014). This experiment was conducted in a similar location using similar tanks, and the re-aeration coefficients for this experiment were determined using a night-time regression of dissolved oxygen exchange following the methods of Wiley et al. (1990) and Young and Huryn (1996).

Data Analysis

Effects of crayfish source population and drought on individual response variables (crayfish growth (g), crayfish carapace growth (mm), crayfish survival, leaf AFDM, chlorophyll a concentration, periphyton AFDM, autotrophic index, sediment level, macroinvertebrate richness, and NPP) were assessed using two-way analysis of variance (ANOVA). If a significant interaction between source population and drought was found, one-way ANOVAs were

performed examining the effects of each factor on the response variable individually. A negative binomial regression was used to analyze the effects of treatments on Chironomidae abundance because this data did not meet the assumptions of an ANOVA. Response variables were analyzed separately for the riffle and pool habitats when habitat specific data was available. Data and residuals were inspected graphically to ensure they met assumptions of the test. A significance level of $\alpha=0.05$ was used for all analyses. When treatments were determined significant in the ANOVAs, Tukey's HSD post-hoc test was used to determine which levels of the treatment were significantly different. All analyses were conducted using program R (R Core Team 2016).

Results

Crayfish Growth and Survival

There was no interaction between crayfish source population and drought for crayfish mass growth (g), crayfish carapace growth (mm) and crayfish survival. Drought did not have a significant effect on any of these response variables (ANOVA $p=0.616$, ANOVA $p=0.627$, and ANOVA $p=0.257$ respectively) and source population did not have a significant effect on crayfish survival (ANOVA $p=0.548$) (Fig. 1). However, crayfish source populations differed significantly in mass growth (g) (ANOVA $p=0.029$) and carapace length growth (mm) (ANOVA $p<0.001$) with AR *O. neglectus* gaining significantly more mass than native *O. eupunctus* (Tukey's KS *O. neglectus*: *O. eupunctus* $p=0.247$, Tukey's AR *O. neglectus*: *O. eupunctus* $p=0.022$, Tukey's AR *O. neglectus*: KS *O. neglectus* $p=0.452$). In addition, AR *O. neglectus* had significantly greater carapace length growth than KS *O. neglectus* and native *O. eupunctus*, which did not differ from each other (Tukey's KS *O. neglectus*: *O. eupunctus* $p=0.435$, Tukey's

AR *O. neglectus*: *O. eupunctus* $p < 0.001$, Tukey's AR *O. neglectus*: KS *O. neglectus* $p = 0.008$) (Fig. 1).

Chlorophyll a, Autotrophic Index, and Periphyton AFDM

There was no interaction between source population and drought for chlorophyll a, autotrophic index, and periphyton AFDM in either habitat (ANOVA $p > 0.05$). Drought significantly reduced chlorophyll a and autotrophic index in riffle habitat (ANOVA $p < 0.001$), but not in pool habitat (ANOVA $p \geq 0.367$) (Fig. 2). Periphyton AFDM was not significantly affected by drought in either habitat (riffle habitat ANOVA $p = 0.161$, pool habitat ANOVA $p = 0.485$) (Fig. 2). Crayfish source population had a significant effect on chlorophyll a concentration in the pool habitat (ANOVA $p = 0.048$), but not the riffle habitat (ANOVA $p = 0.179$). In pool habitat, chlorophyll a concentration was greatest in KS *O. neglectus* treatments (Fig. 2), although pairwise differences were not significant due to Tukey's experimentwise error rate correction (Tukey's KS *O. neglectus*: *O. eupunctus* $p = 0.064$, Tukey's AR *O. neglectus*: *O. eupunctus* $p = 0.974$, Tukey's AR *O. neglectus*: KS *O. neglectus* $p = 0.100$). Crayfish treatments did not significantly affect periphyton AFDM (riffle ANOVA $p = 0.074$, pool ANOVA $p = 0.122$) or autotrophic index (riffle ANOVA $p = 0.581$, pool ANOVA $p = 0.102$) in either habitat (Fig. 2).

Chironomidae Abundance and Sediment Level

Crayfish source population and drought did not show an interaction for sediment levels and Chironomidae abundance in either habitat. Chironomidae (Diptera) abundance accounted for 97% of the total macroinvertebrate abundance for all tanks (2018:2076). Drought significantly reduced Chironomidae abundance (riffle negative binomial regression $p < 0.001$, pool negative binomial regression $p = 0.176$) and sediment level (riffle ANOVA $p < 0.001$, pool ANOVA $p = 0.645$) in riffle habitats only (Fig. 3). Crayfish source population did not significantly affect

Chironomidae abundance (riffle negative binomial regression $p=0.508$, pool negative binomial regression $p=0.893$) or sediment level (riffle ANOVA $p=0.190$, pool ANOVA $p=0.725$) in either habitat (Fig. 3). However, substantial differences in variation were observed for Chironomidae abundance across crayfish treatment levels. The coefficient of variation for Chironomidae abundance in the native *O. eupunctus* treatment was 228.277, in AR *O. neglectus* was 244.028, and in KS *O. neglectus* was 170.233.

Macroinvertebrate Richness

Macroinvertebrates recovered from tanks included Chironomidae (Diptera), Daphnia (Cledocera), Copepoda: Cyclopoida, Acari: Hydrachnidia, and Mollusca: Gastropoda. There was a significant interaction between crayfish population and drought on macroinvertebrate richness in the riffle habitat (ANOVA $p=0.025$) (Fig. 4). Macroinvertebrate richness was significantly reduced by drought in riffle habitat of native *O. eupunctus* (ANOVA $p=0.005$) and KS *O. neglectus* (ANOVA $p=0.046$), but not AR *O. neglectus* (ANOVA $p=0.141$) (Fig. 4). In the pool habitat, drought significantly reduced macroinvertebrate richness (ANOVA $p=0.021$), but richness did not differ among crayfish treatments (ANOVA $p=0.659$) (Fig. 4).

Leaf Decomposition and Net Primary Production

There was no interaction between crayfish source population and drought on NPP or leaf decomposition in either habitat. Drought did not significantly affect leaf decomposition in either habitat (riffle ANOVA $p=0.310$, pool ANOVA $p=0.306$) (Fig. 5). However, drought significantly affected NPP (ANOVA $p=0.002$), with drying tanks having lower NPP than control tanks (Fig. 5). There was a significant effect of crayfish source population on leaf decomposition in the pool habitat only (riffle ANOVA $p=0.078$, pool ANOVA $p=0.003$) (Fig. 5). In the pool habitat, native *O. eupunctus* had significantly greater leaf mass remaining than both AR *O.*

neglectus and KS *O. neglectus*, which did not differ from each other in leaf mass (Tukey's KS *O. neglectus*: *O. eupunctus* $p=0.045$, Tukey's AR *O. neglectus*: *O. eupunctus* $p=0.002$, Tukey's AR *O. neglectus*: KS *O. neglectus* $p=0.402$). Crayfish source population did not significantly affect NPP (ANOVA $p=0.601$) (Fig. 5).

Discussion

We found that crayfish source population and drought affected stream ecosystem structure and function. Invasive AR *O. neglectus* showed greater growth than KS *O. neglectus* or native *O. eupunctus*. Both AR *O. neglectus* and *O. eupunctus* reduced chlorophyll a more than KS *O. neglectus*. Both *O. neglectus* treatments showed greater leaf breakdown than native *O. eupunctus*. Drought reduced chlorophyll a, autotrophic index, sediment levels, Chironomidae abundance and NPP.

Crayfish Growth and Survival

In the present study, AR *O. neglectus* increased in carapace length significantly more than both *O. eupunctus* and KS *O. neglectus*. It is possible that lack of gene flow between *O. neglectus* from the Ozark Interior Highlands and central Great Plains has led to divergent evolution and varying growth rates for populations within this species. The “local adaptation hypothesis” suggests that invasive species may perform poorly in recipient communities because of a lack of adaptation to the local environment and available resources (D'Antonio 2000). However, extralimital invaders from adjacent drainages may be pre-adapted to local conditions and may therefore be more successful invaders. Duncan and Williams (2002) found introduced plants with congeneric natives were more likely to be pre-adapted to recipient communities and thus were more likely to naturalize. Since the mesocosms in this experiment were inoculated with cobble from a local stream and contained substrate characteristic of the Ozark Highlands, it

is possible that AR *O. neglectus* was pre-adapted to these conditions and thus outperformed KS *O. neglectus* in terms of growth. Potential pre-adaptation of extralimital invaders may lead to stronger ecosystem impacts than extraregional invasions and this warrants additional research in order to further understand factors contributing to invasion success.

In addition, we found mass growth rates of AR *O. neglectus* to be greater than that of native *O. eupunctus* and this may contribute to the successful establishment of *O. neglectus* in the Spring River drainage and apparent displacement of *O. eupunctus*. Previous research found growth rates of *O. neglectus* and *O. eupunctus* did not differ (Magoulick 2014). However, crayfish growth can be directly or indirectly affected by multiple factors such as density (Ramalho et al. 2008), temperature (Renai et al. 2007), and food quality (Olsson et al. 2008), and therefore can be context dependent. Moreover, previous research found that growth rates in the congener *O. rusticus* were greater in individuals from invasive populations than native populations (Pintor and Sih 2009; Sargent and Lodge 2014). In a review of trait evolution in nonindigenous populations, Whitney and Gabler (2008) found that introduced populations often develop faster growth rates, greater tolerances to environmental conditions, increased reproductive capabilities, and shorter generation times. Thus, it is possible that growth rates in invasive populations of *O. neglectus* may be greater than in native populations of *O. neglectus*. This is of particular interest since rapid growth rates are common among successful invaders and can often lead to strong invasion impacts (Sakai et al. 2001; Lamerque et al. 2011).

It is somewhat surprising that drought had no effect on crayfish growth and survival. Larson et al. (2009) found that simulated intense drought negatively affected crayfish survival and native *O. eupunctus* was more affected than invasive *O. neglectus*. Conversely, Magoulick (2014) allowed crayfish to access surface water in pool habitats of mesocosms in drought

treatments, and found no effects of drought on crayfish growth or survival. In the present study crayfish always had access to water in the pool habitat, and therefore drought impacts on crayfish growth and survival may vary according to the drying intensity. It is possible that growth and survival of *O. eupunctus* is only affected by complete stream drying and therefore permanent streams may serve as a refuge habitat for this species. This may have important implications for the survival of this vulnerable species and should be addressed further.

Ecosystem Structure

We found a significant effect of crayfish source population on chlorophyll a concentrations in the pool habitat, indicating extralimital and extraregional invaders may differentially affect ecosystem structure. Chlorophyll a concentration was greatest in the pool of KS *O. neglectus* treatments and therefore resource use may differ for KS *O. neglectus* and AR *O. neglectus*. Previous research shows that conspecific omnivorous crayfish may adjust their diet in relation to habitat characteristics and food availability (Johnston et al. 2011; Klose et al. 2012). In addition, local adaptation may lead to functional differences within a species across their range (O'Neil et al. 2014). Evans-White et al. (2001) found the diet of *O. neglectus* from a prairie stream in Kansas consisted mainly of detritus and filamentous green algae. The algal composition in the mesocosms in the present study was typical of an Ozark Highlands stream and therefore may differ from native Kansas algae. Therefore, AR *O. neglectus* and *O. eupunctus* may be better adapted to the present environment, leading to their stronger effects on the algal components of periphyton in the mesocosms.

Conversely, crayfish source population had no effect on periphyton AFDM, autotrophic index, Chironomidae abundance or sediment levels. This is somewhat surprising since previous research found *O. eupunctus* reduced sediment levels and autotrophic index more than *O.*

neglectus (Magoulick 2014). However, crayfish ecosystem effects are context dependent (Creed 1994) and species-specific effects of crayfish on sediment levels have been found to differ across streams (Klose et al. 2012). In addition, although crayfish source population did not significantly affect Chironomidae abundance, there are notable differences in the coefficients of variation across treatment levels. We saw much less variation in Chironomidae abundance occurring in KS *O. neglectus* treatments than in the other treatment levels. Therefore, there may be subtle underlying differences in the effects of the crayfish treatments on Chironomidae abundance.

In addition, we found a significant interaction between drought and crayfish source population on macroinvertebrate richness in the riffle habitat. Macroinvertebrate richness was reduced by drying in the riffle habitat, except in the AR *O. neglectus* treatment. Consumer effects on ecosystems affected by stream drying can be context dependent (Murdock et al. 2010). However it appears that AR *O. neglectus* may impact macroinvertebrate richness in a similar manner in both dry and flowing riffles in a relatively simple mesocosm experiment. This is likely due to direct or indirect effects of crayfish consumption and may warrant further research.

Not surprisingly, drought reduced autotrophic index, chlorophyll a concentrations and Chironomidae abundance. Previous mesocosm experiments saw drought reduce autotrophic index (Magoulick 2014), and Timoner et al. (2014) found between a 60 and 90 percent reduction in the chlorophyll a concentration of biofilm in an intermittent Mediterranean stream during the non-flow phase. In addition, Boulton (2003) found drying of riffle habitats in Australian intermittent streams to have negative effects on multiple macroinvertebrate taxa.

Surprisingly, we found drought significantly reduced sediment levels in the riffle habitat. Reductions in sediment levels due to drought are not well documented in natural streams. In fact, stream drying can cause sediment to settle out from suspension and it is well known that low

flows can lead to sediment accumulation (Rolls et al. 2012). Thus, it is possible that riffle habitats in this study were directly or indirectly affected by the specific artificial drying process used, and this result may not accurately reflect the effects of stream drying on sediment in riffle habitats of natural streams. However, Lynch and Magoulick (2016) found press and pulse drying reduced sediment levels in the shallow habitat of similar mesocosms, and this result was attributed to minimal time available for sediment to accumulate before water fell below the substrate level in the riffle habitat.

Ecosystem Function

Crayfish source population significantly affected leaf decomposition. *Orconectes eupunctus* broke down significantly less leaves than both *O. neglectus* treatments, indicating that these two species are not ecologically redundant in terms of their effects on ecosystem function. In contrast, Magoulick (2014), found *O. eupunctus* and *O. neglectus* to be largely ecologically redundant. However, the present experiment was conducted for approximately twice as long as Magoulick (2014) and occurred during a different season and year. Temporal differences between these two studies may have directly or indirectly led to varying outcomes. Therefore, ecological redundancy between natives and invasives may be context dependent and this is an important consideration when quantifying invasion impacts.

Unexpectedly, drought did not significantly impact leaf decomposition in the present study. Previous studies found stream drying decreased leaf breakdown in temporary streams (Datry et al. 2011) and mesocosms (Schlief and Mutz 2009; Magoulick 2014). However, decomposition of leaves may vary in relation to the sampling protocol used. Magoulick (2014) used plastic fasteners to hold together natural packs of leaves in order to analyze leaf decomposition. Previous studies show that leaf decomposition rates in natural leaf packs are

faster than in mesh leaf bags in stream riffle habitats (Cummins et al. 1980). Furthermore, Schlieff and Mutz (2009) found leaf breakdown rates were decreased by flow reduction when leaves were contained in coarse mesh leaf bags (5mm) but not in fine mesh leaf bags (0.33 mm). Therefore, although slits were cut into leaf bags in the current study to allow for crayfish access, mesh presence and diameter may have affected leaf decomposition rates.

In addition, drought significantly reduced NPP in the present study. This is not surprising as drought may have negative impacts on algal communities (Timoner et al. 2014) and secondary production (Ledger et al. 2011), and may therefore alter energy flow in streams. Previous studies have found drought to reduce GPP (Magoulick 2014) and long durations of non-flow periods promote heterotrophy in streams (Acuña et al. 2015). Therefore, drought may have strong impacts on food web structure and ecosystem processes, particularly during periods of complete drying.

Although we found a strong influence of crayfish source population and drought on stream structure and function, there are limitations to this study. It was conducted for a relatively short period of time in a laboratory environment in mesocosms. Short-term studies of invasive species impacts may not fully assess invasion consequences (Strayer et al. 2006) and mesocosm environments may differ from the natural world. In order to further understand applications of these findings additional field and laboratory studies would be beneficial.

Conclusion

Differences in growth rates and effects on ecosystem structure between extralimital and extraregional invasive species found in this study may indicate that extralimital invasions are fundamentally different from extraregional invasions. Extralimital invasions may have stronger effects on recipient ecosystems due to invader pre-adaptation and this is an important

consideration when addressing and predicting invasion impacts. In addition, findings from this study indicate that ecological redundancy of native and invasive species may be context dependent. These findings may be applicable to additional taxa, particularly omnivorous invaders. A better understanding of the ecological impacts of invader source population on recipient communities and potential context dependent ecological redundancy is necessary for improving invasive species management and to quantify the effects of invaders across a broad range of spatial scales and environmental conditions.

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Figures

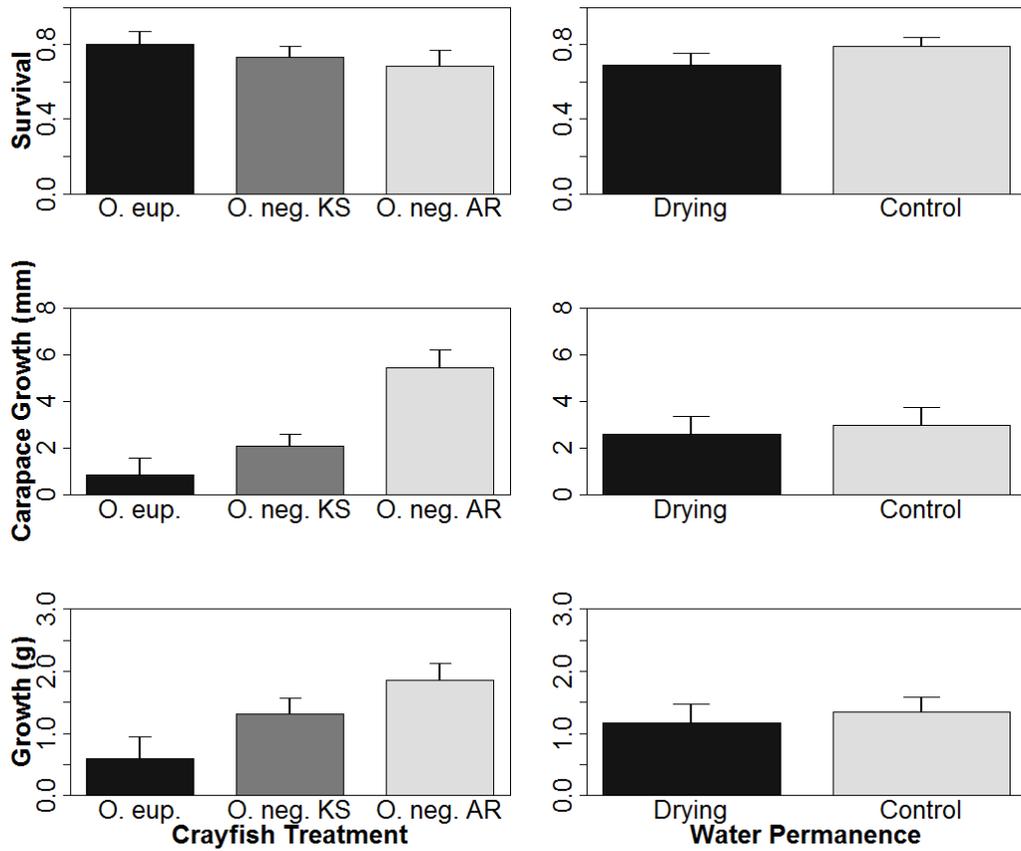


Fig 1 Mean (\pm SE) crayfish growth and survival across treatments. Crayfish treatments are native *O. eupunctus* (*O. eup.*), KS *O. neglectus* (*O. neg. KS*) and AR *O. neglectus* (*O. neg. AR*).

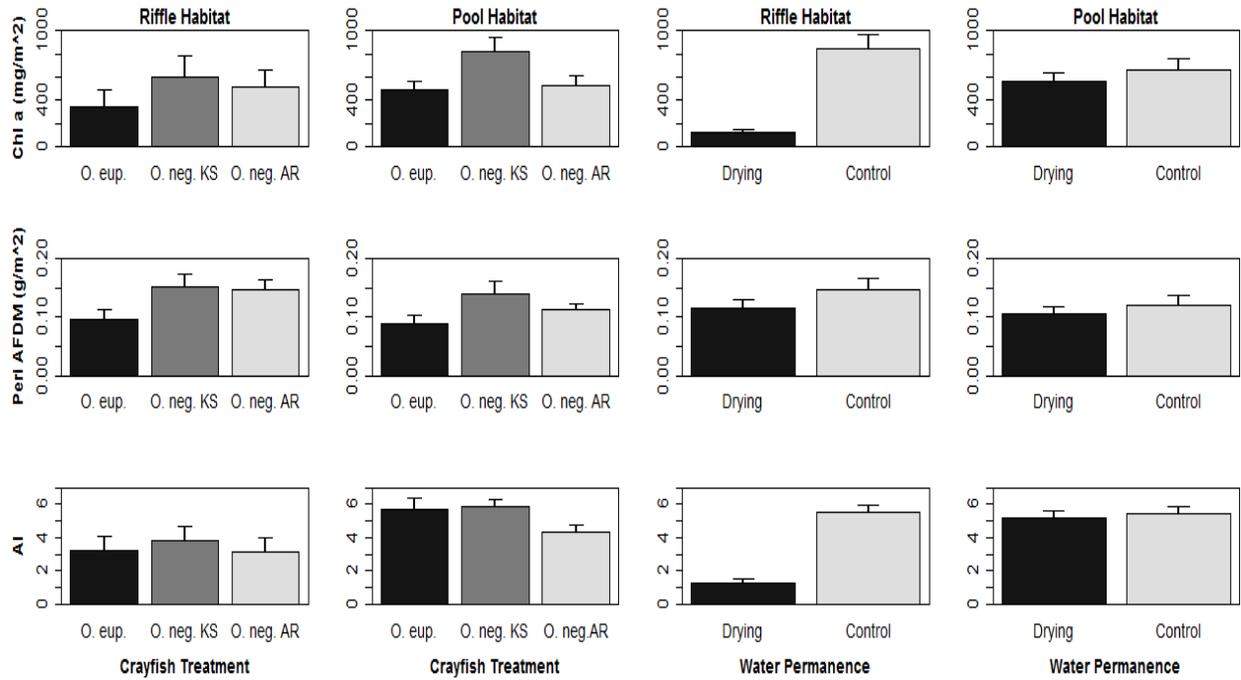


Fig 2 Mean (\pm SE) chlorophyll a concentration (Chl a), periphyton AFDM (Peri AFDM) and autotrophic index (AI) per habitat across treatments. Crayfish treatments are native *O. eupunctus* (O. eup.), KS *O. neglectus* (O. neg. KS) and AR *O. neglectus* (O. neg. AR).

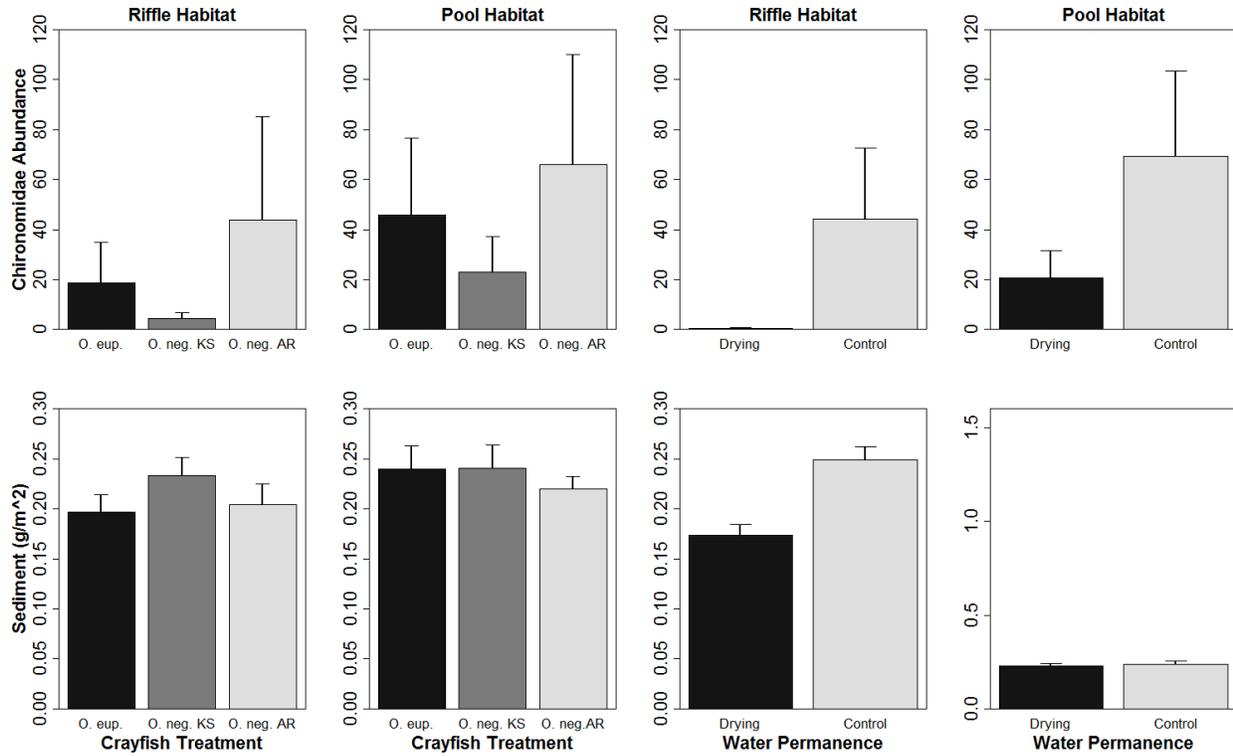


Fig 3 Mean (\pm SE) sediment and Chironomidae abundance per habitat across treatments. Crayfish treatments are native *O. eupunctus* (O. eup.), KS *O. neglectus* (O. neg. KS) and AR *O. neglectus* (O. neg. AR).

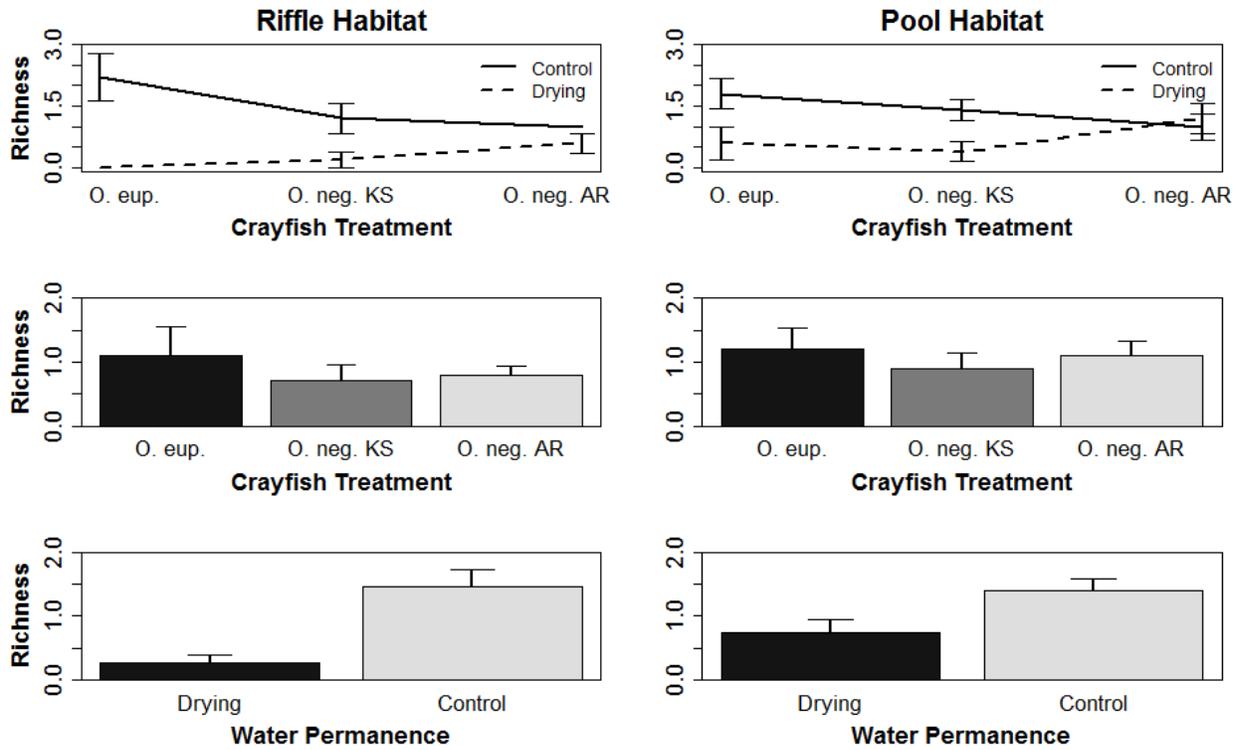


Fig 4 Mean (\pm SE) macroinvertebrate richness per habitat across treatments. Crayfish treatments are native *O. eupunctus* (O. eup.), KS *O. neglectus* (O. neg. KS) and AR *O. neglectus* (O. neg. AR).

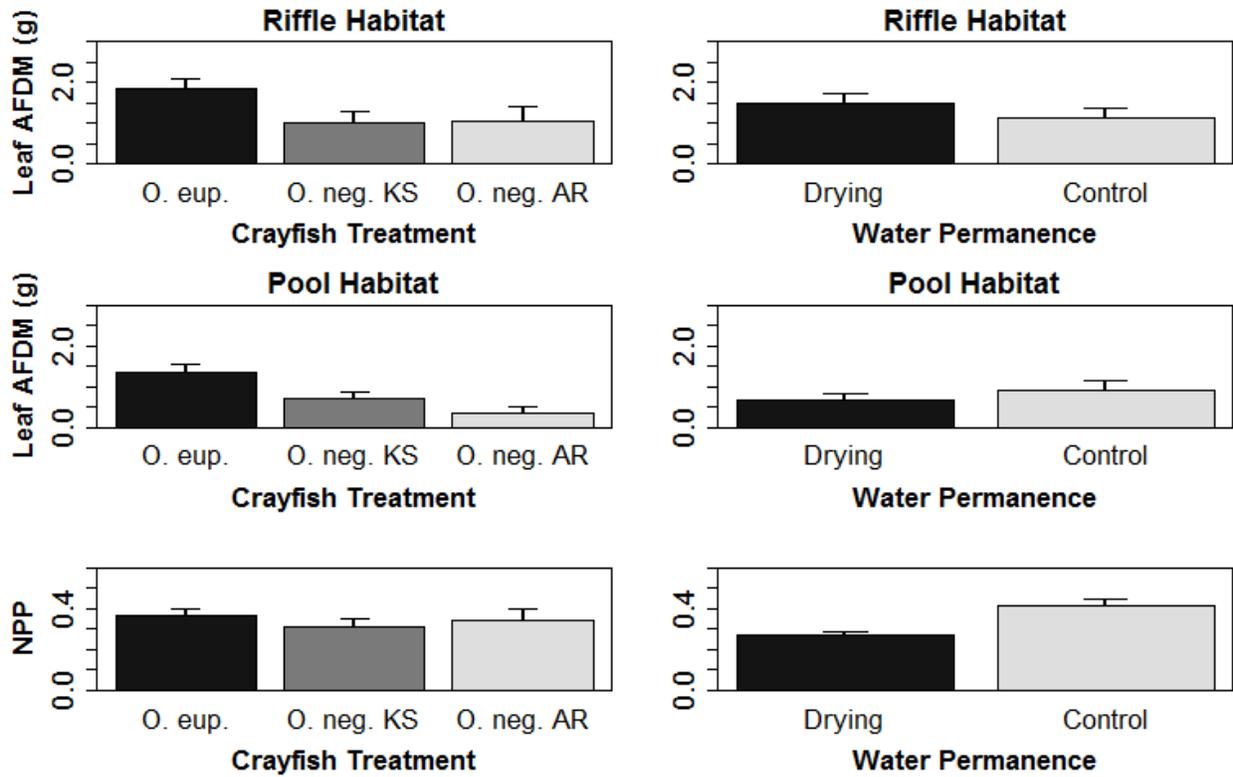


Fig 5 Mean (\pm SE) leaf AFDM per habitat and net primary production (NPP) across treatments. Crayfish treatments are native *O. eupunctus* (O. eup.), KS *O. neglectus* (O. neg. KS) and AR *O. neglectus* (O. neg. AR).

Assessing Spatial Variation in Morphology and Traits Related to Invasiveness in
an Introduced Crayfish Species

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Abstract

Spatial morphological variation in an invasive species may lead to varying impacts on recipient ecosystems in relation to the source population of an invader. We assessed variation in traits associated with competitive dominance and pinching strength, for a native crayfish (*Orconectes eupunctus*) and an invasive crayfish (*O. neglectus*) from multiple source populations. In addition, we examined spatial variation in overall morphology of *O. neglectus*. We hypothesized *O. neglectus* would possess larger chelae than *O. eupunctus*. We also hypothesized *O. neglectus* morphology would vary significantly across source populations. We used multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) to examine differences in chela width to carapace length ratios and chela length to chela width ratios for native *O. eupunctus* and invasive *O. neglectus* from five different source populations. Non-metric multidimensional scaling (NMDS) analysis was used to examine overall morphological variation in *O. neglectus* across source populations. We found significant differences in chela width to carapace length ratio and chela length to chela width ratio across crayfish groups. Surprisingly, we found native *O. eupunctus* had a significantly greater chela width to carapace length ratio than *O. neglectus* from all source populations and a lower chela length to chela width ratio than *O. neglectus* from several source populations. Thus, it is possible that displacement of *O. eupunctus* by *O. neglectus* is not related to competitive dominance. In addition, we found significant morphological variation in *O. neglectus* across source populations. Therefore, ecological impacts on recipient communities that are related to invader morphology may differ in regard to invader source population for this species.

Keywords: spatial variation, invasive species, crayfish, morphology, chela width

Introduction

Multiple studies document spatial variation in species morphology in relation to local environmental characteristics (e.g. Baumgartner 1992; Norberg and Leimar 2002; Woolbright and Steward 2008; Cambell-Tenant et al. 2015; Stevens et al 2016). Morphological variation in invasive species can lead to varying impacts on recipient ecosystems (Phillips and Shine 2006). Therefore, spatial variation in morphology of an invasive species across its native distribution may lead to differential invasion impacts in relation to the source population of the invader.

Aquatic invasive crayfish are a major threat to crayfish biodiversity in the United States (Taylor et al. 1996). Introduced crayfish can lead to displacement of native crayfish species through direct competition or predation, or interference with reproduction and disease transmission (Lodge et al 2000). Since crayfish can substantially influence production (Whitledge and Rabeni 1997), sediment levels (Brown and Lawson 2010) and multiple trophic levels (Momot 1995) in aquatic systems, replacement of native crayfish by invasives may lead to changes in structure and function of recipient ecosystems.

Orconectes neglectus (the Ringed Crayfish) is a widely distributed crayfish in the central United States. An invasive population of this species in the Spring River Drainage of Arkansas and Missouri, USA is leading to the apparent displacement of a vulnerable endemic species, *O. eupunctus* (the Coldwater Crayfish) (Flinders and Magoulick 2005; Magoulick and DiStefano 2007). It is presumed that *O. neglectus* was introduced to this drainage via bait bucket introduction from an adjacent native population in the White River Drainage (Magoulick and DiStefano 2007).

Larson and Olden (2010) found morphological and life history traits of invasive crayfish introduced from adjacent drainages (extralimital invaders) may differ from traits of invasive

crayfish from distant drainages (extraregional invaders). Extraregional invaders were identified by generalist habitat associations, large native ranges, large body size and high fecundity, whereas extralimital invaders were identified by lotic habitat associations and localized native ranges. Traits of *O. neglectus* from extralimital populations may vary from traits of *O. neglectus* from extraregional populations. In addition, Fetzner (2017) found spatial variation in the morphology of *O. eupunctus* within and across drainages in Arkansas and Missouri, USA. Therefore, overall morphology of *O. neglectus* may also vary within and across drainages.

Crayfish chela width to carapace ratio is an important component of crayfish morphology and is considered a measure of pinching strength (Claussen et al. 2008). Crayfish with larger chelae may be competitively dominant (Rutherford et al. 1995) and morphological variation in chela width within an invasive crayfish species may be associated with the ability of an invader to displace native species. Thus, if there is spatial variation in overall morphology and chela width to carapace ratio for *O. neglectus*, invasion impacts of this species may vary with source population.

The objectives of this study were to assess traits related to competitive ability for *O. eupunctus* and *O. neglectus* and to examine spatial variation in the overall morphology of *O. neglectus*. We hypothesize *O. neglectus* will possess relatively wider chelae than *O. eupunctus* since this trait is associated with competitive dominance. We also hypothesize *O. neglectus* morphology will vary significantly across source populations.

Materials and Methods

Specimen Collection

All specimens collected in this study were form II males. Seventeen *O. eupunctus* were collected August 20, 2015 from the Spring River (36.22472, -91.25081) and South Fork of the

Spring River (36.3554, -91.6324; 36.3673, -91.8110; 36.4067, -91.8172) in Arkansas and 19 *O. neglectus* were collected August 29, 2015 from the South Fork of the Republican River in Kansas (39.77833, -101.80685) (hereafter KS *O. neglectus*). Crayfish were preserved in 95% ethanol within one week of collection. On April 6-8, 2017, *O. neglectus* (19, 22, and 20 individuals respectively) were collected from Huzzah Creek (36.232198, -92.990266) (hereafter AR Huzzah *O. neglectus*), Long Creek (36.228365, -93.327581) (hereafter AR Long *O. neglectus*), and Brush Creek (36.133863, -93.951423) (hereafter AR Brush *O. neglectus*) in the White River drainage of Arkansas, and 22 *O. neglectus* were collected from Wildcat Creek (36.159475, -94.309960) (hereafter AR Wildcat *O. neglectus*) in the Illinois River drainage of Arkansas (Fig. 1). Specimens collected in 2017 were immediately preserved in 95% ethanol.

Morphological Measurements

On all specimens 17 morphological characteristics were measured to the nearest 0.1 mm using calipers. Measurements were carapace length, carapace width, carapace depth, post-orbital carapace length, rostrum length, areola length, areola width, chela depth, chela width, chela length, dactyl length, palm length, total gonopod length, central projection length, mesial process length, antennal scale length and antennal scale depth. Chela measurements were obtained from the right chela. All measurements were done on April 8, 2017. Although preservation times differed for specimens, carapace length and chela width of specimens measured in both 2016 and 2017 did not differ. In addition, DiStefano et al. (1994) found carapace length and chela width of crayfish preserved in 100% ethanol did not significantly change after a period of 180 days.

Data Analysis

In order to capture variation in crayfish morphology, we calculated 17 morphological ratios similar to Fetzner 2017. Morphological ratios included carapace length to carapace width

(CL.CW), carapace length to carapace depth (CL.CD), post-orbital carapace length to carapace width (POCL.CW), post-orbital carapace length to carapace depth (POCL.CD), rostrum length to carapace length (R.CL), areola length to carapace length (AL.CL), areola length to areola width (AL.AW), chela length to carapace length (ChL.CL), chela width to carapace length (ChW.CL), chela length to chela width (ChL.ChW), chela length to chela depth (ChL.ChD), palm length to chela length (P.ChL), dactyl length to chela length (D.ChL), central projection length to total gonopod length (CPL.GL), mesial process length to total gonopod length (MPL.GL), and antennal scale length to antennal scale depth (ASL.ASD). We assessed correlations among these variables and discarded a variable if a correlation coefficient $\geq |0.7|$ was determined. We removed ChL.CL, ChL.ChD, POCL.CW, AL.CL, P.ChL and CPL.GL from analyses based on strong correlations with other variables. The remaining 10 variables were used in analyses (Table 1).

Data analysis was conducted using program R (R Core Team 2016). We focused on ChW.CL and ChW.ChL as characters potentially related to competitive ability and used MANOVA to compare across the six crayfish groups (*O. eupunctus*, KS *O. neglectus*, AR Huzzah *O. neglectus*, AR Long *O. neglectus*, AR Brush *O. neglectus*, and AR Wildcat *O. neglectus*). If MANOVA was significant, we used ANOVA to assess individual variation in ChW.CL and ChW.ChL across crayfish groups. A significance level of $\alpha=0.05$ was used for the MANOVA and ANOVAs, and a Tukey's HSD post-hoc test was used to assess pairwise comparisons for variables determined significant in ANOVAs. Prior to analysis, test assumptions were addressed using graphical examination of the data and residuals. Data was found to meet the assumptions of the tests.

Overall morphological variation in *O. neglectus* was examined using non-metric multidimensional scaling (NMDS). We used the *metaMDS* function in the *vegan* package

(Oksanen et al. 2012) in R (R Core Team 2016) to examine the 10 morphological ratios. The ordination was based on Bray-Curtis distance and we plotted the first two NMDS axes. A solution was determined suitable if stress < 0.20 (McCune and Grace 2002). The *vegan envfit* function was used to fit centroids to the ordination and assess if morphological variation differed significantly across groups ($\alpha=0.05$).

Results

There was significant variation across crayfish groups in the combined responses of ChW.CL and ChW.ChL (MANOVA $p<0.001$). In addition, ChW.CL and ChW.ChL individually varied significantly across crayfish groups (ANOVA $p<0.001$). Chela width to carapace length ratio was greatest for *O. eupunctus*, followed by KS *O. neglectus* and AR Long *O. neglectus*, then AR Huzzah *O. neglectus*, AR Wildcat *O. neglectus* and AR. Brush *O. neglectus* (Fig. 2). *Orconectes eupunctus* had a significantly lower chela width to chela length ratio than AR Brush *O. neglectus*, AR Huzzah *O. neglectus*, and AR Wildcat *O. neglectus* (Fig. 3). Chela width to chela length ratio was significantly greater for AR Wildcat *O. neglectus* than for AR Long *O. neglectus* and KS *O. neglectus* (Fig. 3). However, chela width to chela length ratio did not differ significantly across the other crayfish groups (Fig. 3). In addition, we saw significant variation in the overall morphology of *O. neglectus* across collection locations (Pearson's $r^2 p<0.001$) (Fig. 4). Specimens from Huzzah Creek, Wildcat Creek and Brush Creek appeared to be morphologically similar to one another and different from specimens from Kansas and Long Creek, which appeared morphologically similar to one another (centroid position, Fig. 4).

Discussion

We found traits related to pinching strength and overall morphology differed across crayfish species and populations. It is somewhat surprising that we found *O. eupunctus* had a

greater ChW.CL ratio than all *O. neglectus* groups and a lower ChW.ChL than many *O. neglectus* groups since Larson and Olden (2010) found crayfish invading from adjacent drainages have relatively larger chelae than imperiled crayfish, and this trait was associated with invasion success. Given that *O. eupunctus* have larger chelae, it is possible that displacement of *O. eupunctus* by *O. neglectus* is not related to competitive dominance. However, competitive dominance in crayfish is also associated with aggressiveness (Vorburger et al. 1999) and previous studies have found that chelae size is not the only factor influencing the outcomes of aggressive interactions in crayfish (Bizwell and Mattingly 2010).

Nevertheless, previous research suggests that direct competition is not the mechanism of displacement of *O. eupunctus* by *O. neglectus* (Rabalais and Magoulick 2006; Larson and Magoulick 2009). Rabalais and Magoulick (2006) found presence of adult male *O. neglectus* did not reduce the growth or survival of adult male *O. eupunctus*. In addition, in a laboratory study of *O. neglectus* and *O. eupunctus* juveniles, occupancy of limited shelter was determined by individual size rather than species. Thus, factors other than competition are likely responsible for the displacement of *O. eupunctus* by *O. neglectus*. Instead, drought has been implicated as facilitating the replacement of *O. eupunctus* by *O. neglectus* because *O. neglectus* has a higher tolerance to desiccation (Larson et al. 2009) and drought is a frequent disturbance in the Ozark Highlands of Arkansas and Missouri, USA. Therefore, it appears that *O. neglectus* is an opportunistic invader and may be able to replace *O. eupunctus* in areas within its range during seasonal stream drying.

In addition, we found differences in chela size and overall morphology for *O. neglectus* from different source populations. Morphology differed for source populations from different states (KS and AR), from different drainages (Illinois River and White River drainages), and

within a single drainage (White River drainage). If these morphological differences are related to invasion impacts of this invader, *O. neglectus* from different source populations may have varying effects on recipient ecosystems. Phillips and Shine (2006) found spatial variation in the size of the parotoid glands of invasive cane toads, a morphological trait associated with relative toxicity, and thus invasion impacts. In addition, previous studies have found that intraspecific variation in invasive species can lead to differences in the spread rate (Jongegans et al. 2011) and effects (Lankau 2011) of invasions.

The morphological variation found in this study may be related to local environmental characteristics. Previous studies show spatial variation in morphology may be related to land use (Woolbright and Steward 2008) and habitat fragmentation (Norberg and Leimar 2002), and environment regulated natural selection may lead to phenotypic variation within a species (Stevens et al. 2016). Baumgartner (1992) found morphological differences in threespine sticklebacks were related to hydrodynamics and Bruckerhoff and Magoulick (2017) found morphology of central stonerollers varied with flow regime in the Ozark Highlands and Boston Mountains of Arkansas and Missouri, USA. It is possible that the morphological variation in *O. neglectus* seen in this study is related to local hydrology. However, additional research examining environmental differences across sampling locations and correlations with morphology is needed to address this question.

Conclusion

This study suggests that invasion success in crayfish may not be related to morphological characteristics associated with competitive dominance for certain species. This is an important consideration when assessing the ability of an invasive crayfish to displace native species. Physiological and behavioral differences between native and invasive crayfish should be

considered in addition to morphological differences in order to better understand potential impacts of invasive crayfish. In addition, morphology of crayfish can vary within a single drainage, within a single state and across states. Thus, invasive crayfish from different source populations may differentially affect recipient ecosystems, particularly when morphological traits that vary spatially are related to invasion success. This finding may be applicable to additional taxa and should be considered when predicting invasion impacts.

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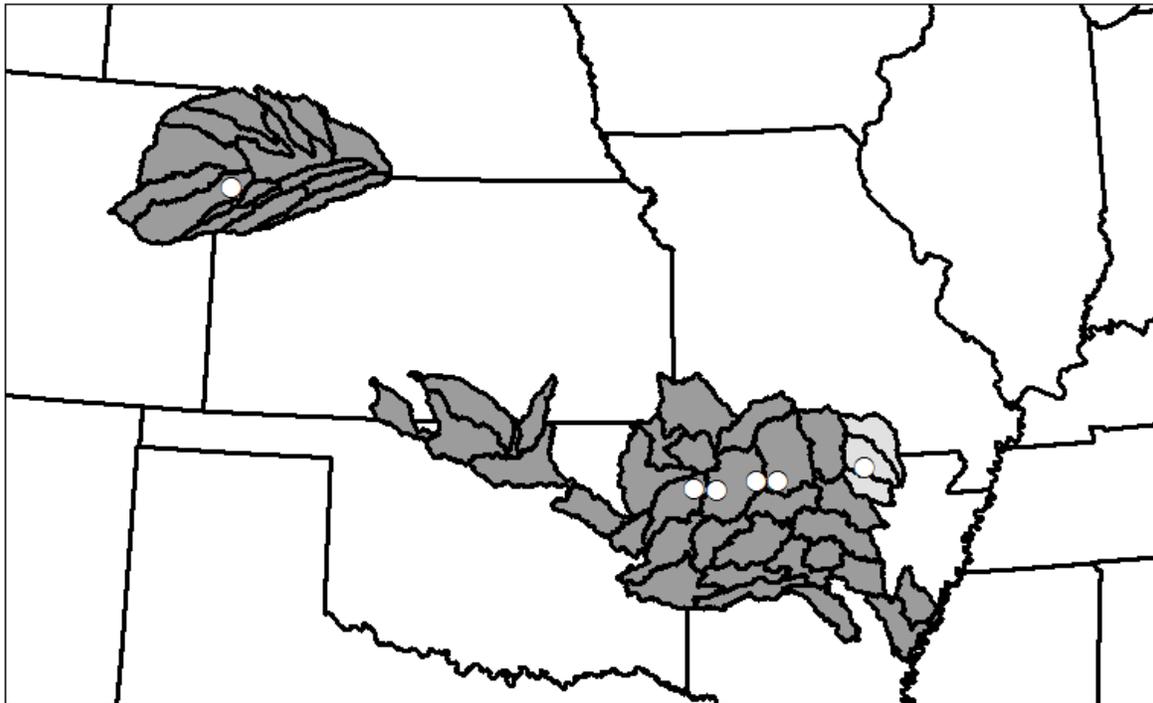
Tables and Figures

Tables

Table 1 Crayfish morphological ratios used in analyses and associated abbreviations

Morphological Ratio	Abbreviation
Carapace length:Carapace width	CL.ChW
Carapace length:Carapace depth	CL.ChD
Post-orbital carapace length:Carapace depth	POCL.CD
Rostrum length:Carapace length	R.CL
Areola length:Areola width	AL.AW
Chela width:Carapace length	ChW.CL
Chela length:Chela width	ChL.ChW
Dactyl length:Chela length	D.ChL
Mesial process length:Total gonopod length	MPL.GL
Antennal scale length:Antennal scale depth	ASL.ASD

Figures



Legend

- sampling locations
- ◻ *O. eupunctus* native
- ◼ *O. neglectus* native

Fig 1 Sampling locations where crayfish specimens were collected. *Orconectes eupunctus* were collected from the Spring River and South Fork of the Spring River in AR and *O. neglectus* were collected from the South Fork of the Republican River in KS, Huzzah Creek in AR, Long Creek in AR, and Brush Creek in AR, and Wildcat Creek in AR.

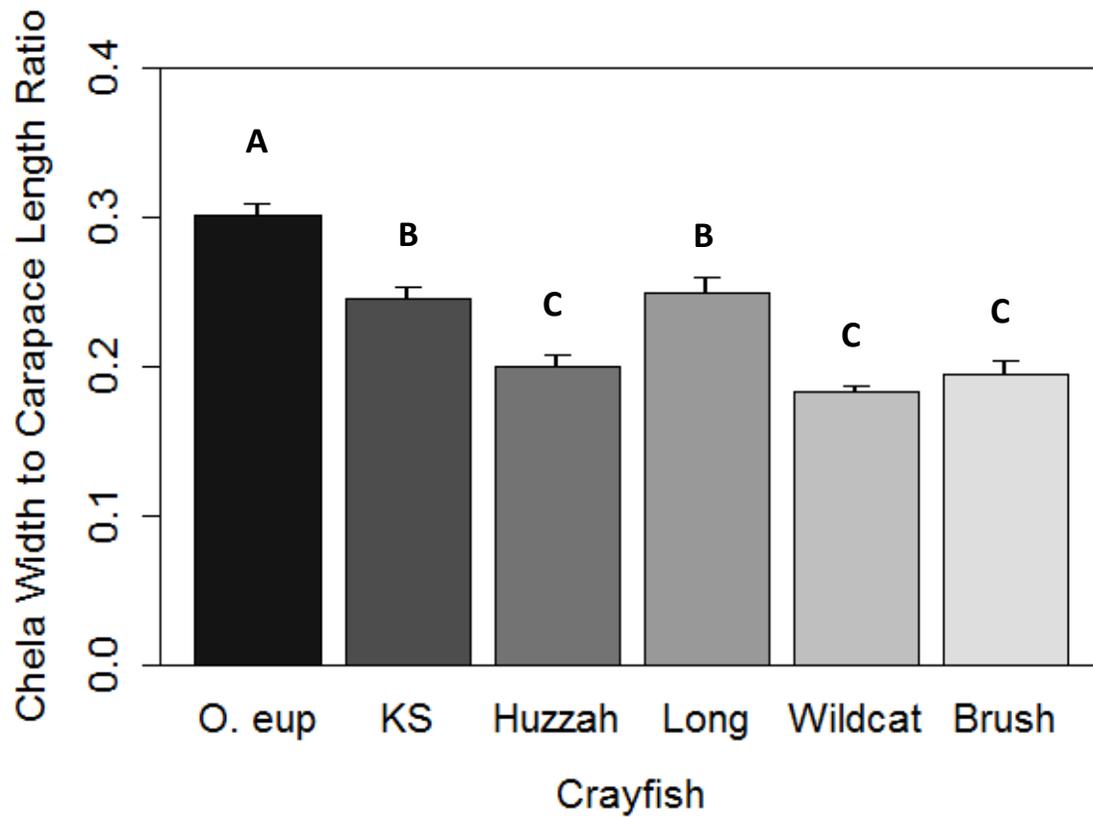


Fig 2 Mean (\pm SE) chela width to carapace length ratio for crayfish groups (O.eup= *O. eupunctus*, KS= KS *O. neglectus*, Huzzah= AR *O. neglectus* Huzzah Creek, Long= AR *O. neglectus* Long Creek, Wildcat= AR *O. neglectus* Wildcat Creek, Brush= AR *O. neglectus* Brush Creek). Letters indicate pairwise groupings in Tukey's HSD post-hoc analysis.

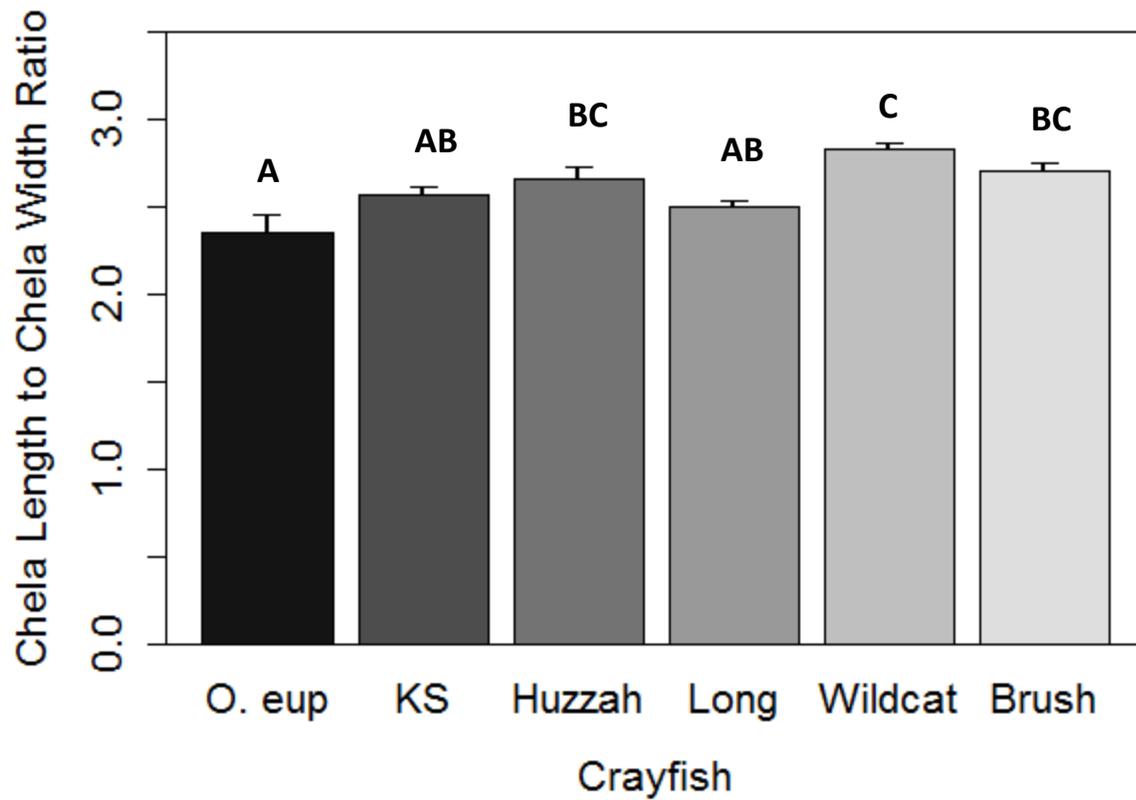


Fig 3 Mean (\pm SE) chela length to chela width ratio for crayfish groups (O.eup= *O. eupunctus*, KS= KS *O. neglectus*, Huzzah= AR *O. neglectus* Huzzah Creek, Long= AR *O. neglectus* Long Creek, Wildcat= AR *O. neglectus* Wildcat Creek, Brush= AR *O. neglectus* Brush Creek). Letters indicate pairwise groupings in Tukey's HSD post-hoc analysis.

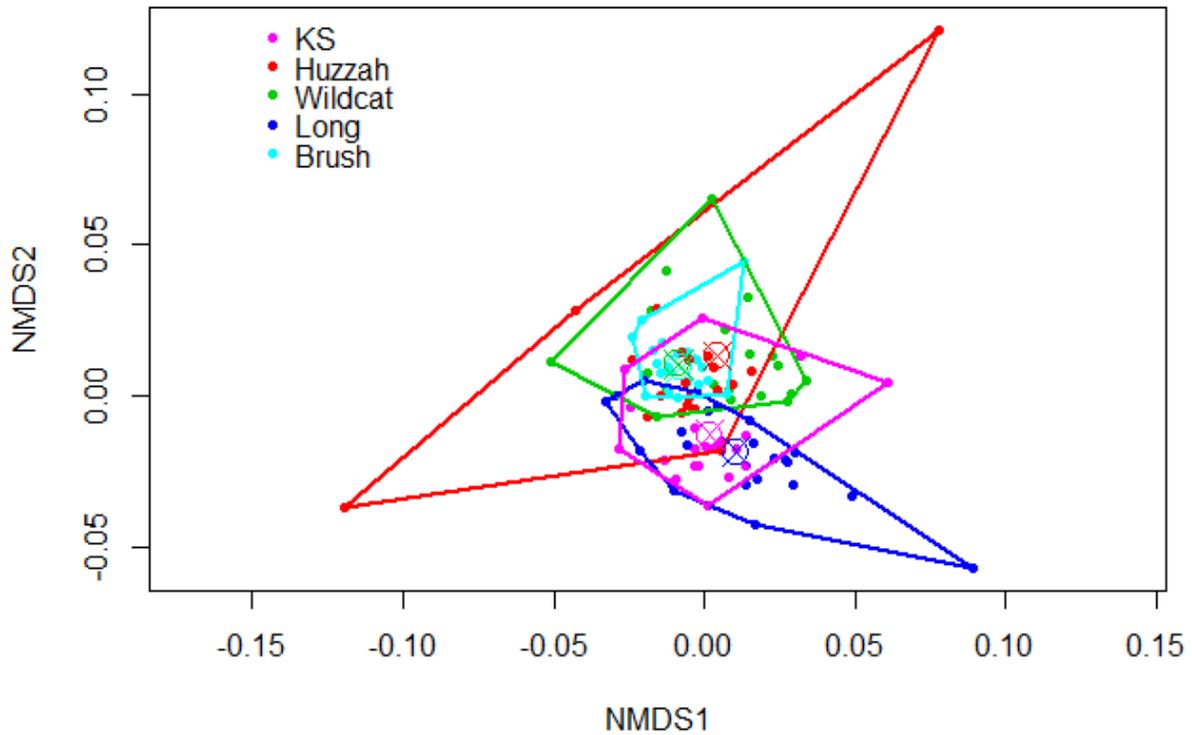


Fig 4 Results from non-metric multidimensional scaling of morphological variation in *O. neglectus* across groups (KS= KS *O. neglectus*, Huzzah= AR *O. neglectus* Huzzah Creek, Long= AR *O. neglectus* Long Creek, Wildcat= AR *O. neglectus* Wildcat Creek, Brush= AR *O. neglectus* Brush Creek). Centroid positions of each group are symbolized by a circled x.

Conclusion

This thesis presents two studies examining how native and invasive crayfish and invasive crayfish from different source populations affect aspects of stream ecosystems. The objective of the first study was to investigate the comparative effects of *O. eupunctus* and *O. neglectus* on stream ecosystems, with a concentration on *O. neglectus* source population and potential compounding effects of drought. We found *O. neglectus* from adjacent (extralimital) source populations had greater growth rates and stronger effects on ecosystem structure than *O. neglectus* from distant (extraregional) source populations indicating extralimital and extraregional invasions may be fundamentally different. In addition, we found *O. neglectus* may have stronger effects on ecosystem function than *O. eupunctus*, demonstrating that these congeners may not be ecologically redundant in Ozark streams. Lastly, we found drought can have strong effects on aspects of stream structure and function, and invasive species presence may interact with these effects.

The objective of the second study was to assess potential differences in traits related to competitive dominance for *O. eupunctus* and *O. neglectus*, and examine variation in morphology of *O. neglectus* from different source populations. We found *O. neglectus* did not possess larger chelae than *O. eupunctus*, suggesting displacement of *O. eupunctus* by *O. neglectus* may not be related to competitive dominance of *O. neglectus*. We also found morphology of *O. neglectus* varied within a single drainage, within a single state and across states. The morphological variation found in this study may be related to potential invasion success and impacts. Overall, these findings suggest that invasive species can have strong impacts on recipient ecosystems, and these impacts may be mediated by abiotic disturbances and factors related to the source population of the invader. This highlights the importance of considering disturbance regulated

effects and source populations of invasive species in order to improve invasive species management and further enable quantification of invasion impacts across broad spatial scales and environmental conditions.